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Scientific Advisory Committee on Nutrition

IRON AND HEALTH

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CONTENTS

INTRODUCTION

Background

1. In their report, *Nutritional Aspects of the Development of Cancer* (Department of Health [DH], 1998), the Committee on Medical Aspects of Food Policy (COMA) highlighted possible links between red and processed meat and colorectal cancer and recommended that "*higher consumers should consider a reduction*" in red and processed meat consumption.

2. Red meat is a rich dietary source of iron; 100 g of cooked red meat contains amounts ranging from 0.8-2.3 mg of iron (Food Standards Agency, 2002). Public reactions to the risk of human exposure to Bovine spongiform encephalopathy (BSE[1]) in cattle have raised concerns regarding the possibility that any reduction in consumption of red meat (beef, lamb, pork and meat products) in the past 20 years might be associated with low iron intakes and, consequently**,** an increased risk of iron deficiency, even though findings from the National Diet and Nutrition Surveys indicate that similar amounts of meat were consumed in 1987 and 2000 (Gregory *et al*, 1990; Hoare *et al*, 2004).

3. Any general recommendation to reduce meat consumption might compromise dietary sources of iron, as well as other micronutrients. This concern was recognised by COMA which recommended that *"the possible associated adverse implications of a reduction in meat consumption on other aspects of health, particularly iron status"* should be the subject of review.

4. Progressive iron deficiency leads to anaemia for which there are reported associations with impairments of: physical performance; cognitive and psychomotor development; immune function; and reproductive efficiency. The thresholds of iron deficiency at which these adverse effects might develop have not been well-characterised and their prevalence in the UK population is not known.

5. UK national surveys have consistently shown that a proportion of the population, particularly young women and children, have low iron intakes relative to recommended reference intakes (Finch *et al*, 1998; Gregory *et al*, 1990; Gregory *et al*, 1995; Gregory *et al*, 2000; Henderson *et al*, 2003), which has raised concerns that they may be at risk of iron deficiency. Additionally, some smaller studies have suggested that children from minority ethnic groups may also be at risk of iron deficiency (see paragraphs 589-590).

6. The possibility that some subgroups of the UK population might be iron deficient, or be at risk of iron deficiency, together with concerns about the potential serious consequences of iron deficiency is a public health issue that requires critical exploration and assessment.

7. Concerns about the possible prevalence and severity of iron deficiency in populations have prompted iron fortification of food products and the use of iron supplements. However, as there are also concerns about possible adverse effects of increased

[1] BSE, commonly known as *mad cow disease,* is a fatal disease that causes progressive neurological degeneration in cattle.

intakes of iron, consideration of the extent and consequences of inadequate iron nutrition in the UK population needs to be sensitive to the balance between iron deficiency and iron excess.

Terms of Reference

8. The Scientific Advisory Committee on Nutrition (SACN) Working Group on Iron was established in 2001, with the following terms of reference:

   To review the dietary intakes of iron in its various forms and the impact of different dietary patterns on the nutritional and health status of the population and to make proposals.

9. It was agreed that consideration of both beneficial and adverse effects of increasing iron intakes was required, including the:

   - Effect of dietary components on iron absorption and utilisation in the body;
   - interaction of infections and inflammation, with iron metabolism and the possibility that this may affect the apparent incidence of iron deficiency.
   - effect of iron deficiency on health and well-being, for example mental and physical development;
   - potential adverse effects of excess iron, including free radical damage and the risk of cardiovascular disease and cancer.

Methodology

10. The SACN *Framework for the Evaluation of Evidence* (SACN, 2002) was used as the basis for identifying and assessing the available evidence. Consideration of the evidence was mainly restricted to cohort studies and randomised controlled trials in humans but cross-sectional studies, case reports, and experimental cell and animal studies were also considered where these informed the interpretation of data.

11. The key issues considered were: iron in the diet; the health consequences of iron deficiency and iron excess; iron intakes and adequacy of iron nutrition in the UK population; and the possible impact of reducing intakes of red and processed meat on the risk of iron and zinc deficiency in the UK.

12. Membership of SACN and the SACN Working Group on Iron is attached as Annex 1.

2. BIOCHEMISTRY & METABOLISM

13. Iron is a transition metal which exists in two biologically relevant oxidation or valency states: the reduced ferrous form (Fe^{2+}) and the oxidised ferric form (Fe^{3+}). The ability of iron to readily accept or donate single electrons means it is an efficient catalyst for electron transfer and free-radical reactions. Although these properties lend themselves to fundamental metabolic processes, the reactivity of iron also means that free iron is potentially toxic and that organisms need to minimise their exposure to it. The role of iron in free radical and oxidative reactions is considered in section 7.

14. Organisms have evolved mechanisms to exploit and control the chemical reactivity of elemental iron, using it to support their metabolism whilst limiting the risk of tissue architectural and functional damage from free iron. This control depends on proteins which are specifically involved in its uptake and transfer from the diet, its distribution around the body and storage in tissues, as well as its delivery to functional sites.

Function

15. Iron, as a component of haemoglobin in erythrocytes (red blood cells), is required for transporting oxygen around the body and, in the form of myoglobin, for the storage and use of oxygen in muscles. The iron in the haem complexes of haemoglobin and myoglobin is stabilised in the ferrous state, and interaction with the adjacent globin protein enables it to bind reversibly to oxygen. This enables oxygen, itself a potentially toxic element, to be safely distributed and stored around the body. In the chemical environment of the lungs, where oxygen concentration and activity is high, haemoglobin binds oxygen while the low concentrations of oxygen in the tissues facilitate its release from haemoglobin. Simultaneously, haemoglobin binds carbon dioxide in the tissues and carries it to the lungs where it is exhaled. The oxygen released in the tissues from haemoglobin is used in oxidative metabolism or stored, for example, in myoglobin.

16. Iron is also present as haem and iron-sulphur complexes in enzymes that are responsible for electron transport and energy generation in mitochondrial respiration and the citric acid cycle, and for ribonucleotide reductase, which is essential for DNA synthesis (Table 2.1).

17. In adults, most body iron is present in haemoglobin (60-70%) in circulating erythrocytes where it is essential for oxygen transport, and in muscle myoglobin (10%). The remaining body iron (20-30%) is found primarily in storage pools located in the liver and reticulo-endothial (macrophage) system as ferritin and haemosiderin. Only about 1% of body iron is incorporated in the range of iron-containing enzymes and less than 0.2% of body iron is in the plasma transport pool where it is bound to transferrin.

18. Iron in the body is constantly recycled between the functional & non-functional pools (see paragraph 21).

Table 2.1: Functional iron-containing proteins in the body (75 kg man)

Iron-containing protein	Function	Location	Iron content (mg)
Haem proteins			
Haemoglobin	Oxygen transport	Red blood cells	3000
Myoglobin	Oxygen storage	Muscle	400
Haem enzymes		All tissues	c 30
Cytochromes a,b,c	Electron transfer Transfer of electrons to molecular oxygen at end of respiratory chain (also requires copper)		
Cytochrome C oxidase			
Cytochrome P450 + b_5	Microsomal mixed function oxidases		
Dcytb	Ferrireductase (duodenal enterocytes)		
Catalase	Hydrogen peroxide breakdown		
Peroxidases	Numerous electron donors		
Sulphite oxidase	Mitochondrial membrane, sulphur metabolism		
	Pyridine metabolism		
Tryptophan 2,3-dioxygenase			
Iodase (iodoperoxidase)	Iodide to iodate		
Non-haem iron enzymes		All tissues	c 30
Ribonucleotide reductase	Ribonucleotides → 2'-deoxyribnucleotides		
(Iron-sulphur proteins)			
Aconitase	Citrate → isocitrate (mitochondria)		
Aldehyde oxidase	RCHO-RCOOH		
Xanthine oxidase	Hypoxanthine – uric acid		
Succinic dehydrogenase	At initial steps of oxidative phosphorylation		
NADH dehydrogenase			
Phenylalanine hydroxylase	Pteridine-dependent		
Tyrosine hydroxylase			
Tryptophan hydroxylase			
Prolyl hydroxylase	Collagen synthesis, need ascorbic acid & α-oxoglutarate		
Lysyl hydroxylase			

Metabolism

19. Iron metabolism can be perceived as a balance between meeting the body's need for iron as an essential nutrient with that of minimising the risk of iron toxicity. Absorption of iron from the gastrointestinal tract is regulated by the systemic need for iron; the risk of tissue damage by free reactive iron is limited by a series of organic molecules which have specific roles in binding free iron, carrying it in the circulation, and delivering it to functional sites or to deposits in which iron that is not immediately needed is deposited in a safe form. This is the basis for the systemic recycling of iron released during tissue and enzyme turnover. A key element of this salvage system is the deposition of iron in ferritin. The principal pool of ferritin is in the liver which serves as a buffer pool of any iron excess to immediate requirements.

20. Control of the body iron burden is by regulation of iron absorption as the body has no means of excreting excess iron. The only way in which iron is lost from the body is adventiously in shed skin, shed epithelia and hair, and in menses.

21. Iron turnover is driven by the formation and destruction of haemoglobin present in erythrocytes. Erythrocytes have a life span of approximately 120 days and are then engulfed and destroyed by the macrophages of the reticulo-endothelial system. The haemoglobin is broken down in the lysosomes, where iron is released from haem by haem oxygenase and transferred to the carrier protein, apotransferrin, in the plasma. Transferrin-bound iron is transported to the erythroblasts in the bone marrow for incorporation into haem for new erythrocytes, or delivered to cells in tissues undergoing growth and development. The macrophages of the reticulo-endothelial system recycle approximately 30 mg/day of iron from senescent erythrocytes. This demonstrates the efficiency of the cyclic conservation and reutilisation of systemic iron.

Iron Losses

22. In healthy individuals obligatory iron losses from the skin and gastrointestinal mucosa are thought to be approximately 1 mg/day in males (Green *et al*, 1968) and slightly more in women of child-bearing age because of additional losses due to menstruation, pregnancy, and lactation.

Absorption

23. The process of absorption comprises the uptake of a nutrient into the intestinal mucosa and its subsequent transfer into the body. Although some nutrients can enter the body by passing between gut mucosal cells, iron uptake and transfer depends on specific cellular carrier mechanisms. The principal, and probably the only, physiological and primary determinant of how much iron is absorbed is the systemic need for iron; this would be to compensate the adventitious losses (see paragraph 20) and for new tissue synthesis (e.g., growth in children, and reproduction in women). Secondary dietary factors affecting iron absorption are considered in section 5.

Molecular control of iron absorption

24. Iron absorption occurs mainly in the proximal small intestine and involves the uptake and transfer of iron across the enterocyte into the systemic circulation. The enterocytes are equipped with iron uptake carrier proteins on their apical surface, which is in contact with the intestinal lumen, and at their basal surface which is in contact with the circulation. Additionally the enterocytes have mechanisms that sense and are responsive to the hepatic mediators of the systemic response to the need for iron, and which control the delivery of iron to the basal transporter.

25. There are two separate mechanisms for the uptake of haem and non-haem iron into the enterocyte.

26. The divalent metal transporter 1 (DMT1) transports inorganic iron, i.e. iron which is not part of the haem molecule, and is specific for ferrous iron. Non-haem iron uptake requires an acid pH, which is provided by gastric hydrochloric acid, to make it more soluble and to produce the protons that are required for its co-transport by DMT1. A haem enzyme, duodenal cytochrome B reductase (DcytB) located on the luminal surface of the enterocytes converts dietary ferric iron to the ferrous state.

27. In the enterocyte, ferrous iron enters a labile or "exchangeable" iron pool from which it can enter three different pathways, depending on the requirements of the body: it may be taken into the local mitochondria for haem synthesis; sequestered into ferritin iron depots (and shed into the gut lumen at the end of the enterocyte's lifespan); or transferred (still in the ferrous state) to the basal transporter (ferroportin 1) for translocation into the body.

28. The mechanism of haem iron absorption remains unclear. The intestinal haem transporter described by Shayeghi *et al* (2005), named haem carrier protein 1 (HCP 1) has now been identified as a folate transporter and its role in haem transport is uncertain (Qiu *et al*, 2006). However, once it has been taken up by the enterocyte the haem molecule is degraded by haem oxygenase to release ferric iron, which is thought to join the enterocytic labile iron pool (Uzel & Conrad, 1998).

29. Studies have also demonstrated that an efficient pathway exists for the intestinal uptake of ferritin, which may be derived from plant and meat based dietary sources. This involves enterocyte uptake via an endocytic pathway followed by lysosomal dissolution of the ferritin core to release the iron (Kalgaonkar and Lonnerdal, 2008a; Kalgaonkar and Lonnerdal, 2008b; San Martin *et al*, 2008)

30. Hephaestin, a ferroxidase found mostly in the basal membrane of enterocytes, is thought to facilitate basolateral iron export from the intestinal epithelial cells by oxidising the ferrous iron back to its ferric form. It is not known whether hephaestin works independently of ferroportin 1, or if the two proteins interact to cause the oxidation (Miret *et al*, 2003). Ceruloplasmin, which is found in plasma, is also a ferroxidase and may be involved in the oxidation of ferrous iron to ferric iron during binding to transferrin.

Regulation of iron absorption

31. Regulation of intestinal iron absorption occurs both at the stage of mucosal uptake and at the stage of transfer to the blood.

32. The absorption of iron by the intestine is sensitive to changes in body iron needs. A large intake of dietary iron, in excess of that required to meet systemic needs, induces the enterocytes to develop a "mucosal block" (Granick, 1946) which prevents excessive absorption by reducing the intestinal transfer of iron for several days (Frazer *et al,* 2003).

33. The principal regulator of iron absorption is hepcidin (Ganz, 2004), a small peptide of 20-25 amino acids encoded by the HAMP gene, which is predominantly expressed in the liver. Transgenic mouse models have shown that hepcidin is the principal down regulator of iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages (Nicolas *et al*, 2002). Hepcidin exerts its effects by directly binding to and degrading the iron exporter ferroportin on the cellular membrane, which prevents iron from leaving the cell (Nemeth *et al*, 2004).

34. Hepatic hepcidin production is increased when iron stores are adequate or high and during inflammation. The released hepcidin, through its effect on ferroportin, then prevents the transfer of iron from the enterocyte to plasma transferrin. The iron that is not transferred is sequestered within the enterocytes and is eventually lost in the gut

lumen when the enterocytes are shed after 1-2 days and lost in the faeces. Similarly, during inflammation or when the systemic iron burden is adequate, hepcidin blocks the release of iron from macrophages. However, when systemic iron requirements are increased or iron stores are low, or both, hepcidin production is decreased, allowing intestinal iron transfer and the release of iron from depots in the macrophages.

35. Defective regulation of hepcidin, or its receptor ferroportin, causes a range of iron overload disorders known as the haemochromatoses (Ganz, 2005). These are characterised by increased iron absorption which leads to excessive iron accumulation and overload. The most common form is associated with hepcidin deficiency.

36. Hepcidin deficiency is a characteristic of mutations in the HAMP gene but most patients with genetic haemochromatosis have changes in the HFE gene, or, rarely in the transferrin receptor 2 (TFR2) or hemojuvelin (HJV) genes, suggesting that these proteins are involved in the regulation of hepcidin synthesis (Nemeth & Ganz, 2006). Juvenile haemochromatosis (JH), the most severe form of haemochromatosis is caused by mutation of the HJV gene (Papanikolaou *et al*, 2004) or the HAMP gene (Roetto *et al*, 2003) indicating that both genes function in the same pathway (Papanikolaou *et al*, 2004). Haemochromatosis caused by mutation of the TFR2 gene also results in severe iron overload (Nemeth *et al*, 2005). Hepcidin deficiency is not as severe in HFE linked haemochromatosis compared to HJV or TFR2 linked haemochromatosis (Nemeth *et al*, 2004) suggesting that HFE modulates the signal from the iron sensor to hepcidin but is not essential for the function of this pathway (Nemeth & Ganz, 2006). As concentrations of TFR2 are regulated by transferrin saturation (Johnson & Enns, 2004), it has been proposed that TFR2 may influence hepcidin expression by acting as a sensor of circulating iron (Fleming & Bacon, 2005).

37. Mutations of the ferroportin gene either cause the protein to be non-functional (i.e. does not export any iron) or unresponsive to hepcidin (leading to excessive iron export from cells) (De Domenico *et al*, 2005). This leads to either iron accumulation in phagocytic cells or in parenchymal cells and, unlike the other forms of genetic haemochromatosis, there is autosomal dominant inheritance.

Plasma Iron Transport

38. Iron is distributed around the body in the circulation where it is bound to a protein, apotransferrin. The iron free apotransferrin can bind one or two atoms of ferric iron to form holotransferrin, which is more usually referred to as transferrin. The subsequent major pathways of iron exchange and whole body iron economy have been delineated from tissue uptake studies using ^{59}Fe bound to transferrin as an intravenous tracer (Finch *et al*, 1970; Cavill & Ricketts, 1980).

39. The uptake of iron by cells is mediated by the binding of holotransferrin (Tf) to transferrin receptors (TfR) on the cell surface which is then internalised by endocytosis. The resulting endosome contains the Tf-TfR complex. Ferrous iron atoms are released and transferred out of the endosome, to the cytoplasm, by a local DMT1. The iron is then either stored as ferritin or used within the cell, e.g. for haemoglobin synthesis in erythroid

precursors. The apotransferrin and the TfR return to the cell surface and the apotransferrin is recycled into the plasma.

40. Transferrin receptors have a greater affinity for fully saturated, diferric transferrin than for monoferric transferrin (Huebers *et al*, 1981; Huebers *et al*, 1985), and do not bind apotransferrin at the neutral pH of plasma. A second transferrin receptor (TFR2) is thought to be involved in the regulation of iron absorption by influencing hepcidin expression (see paragraph 36).

41. Mammalian cells may also acquire iron through transferrin-independent pathways. The transmembrane protein, "*Stimulator of Fe Transport*", facilitates the uptake of both ferrous and ferric iron independently of transferrin and may also have a role in intracellular iron transport (Gutierrez *et al*, 1997; Yu & Wessling-Resnick, 1998; Yu *et al*, 1998). Its significance in the regulation of iron metabolism is presently unclear.

Hepatocyte iron uptake

42. The liver is a major systemic depot of iron. Hepatocytes take up iron from transferrin by the receptor-mediated endocytosis described previously (see paragraph 39), and by the route taken by non-transferrin bound iron (Baker & Morgan, 1994). Iron is released from the hepatocytes in times of increased need.

43. In diseases which cause iron overload (see Table 2.2) the liver continues to accumulate iron, even when iron stores are high, and is therefore vulnerable to developing damage secondary to iron overload.

Iron storage and deposition

44. All cells have the ability to sequester iron either in the soluble complex ferritin or, as its insoluble derivative, haemosiderin. Ferritin is the major intracellular storage protein found in all cells, with the highest concentrations in the liver, spleen and bone marrow. The small amount of apoferritin found circulating in the plasma does not have a known function but is secreted from the newly formed ferritin which undergoes glycosylation.

45. Ferritin binds iron as a ferric oxo-hydroxide [ferrihydrite (Pan *et al*, 2009)] phosphate complex within a protein shell of molecular mass 480 kD. Each molecule can theoretically store up to 4500 atoms of ferric iron but, in practise, it is typically less than 2000 atoms. The protein shell surrounding the iron core is penetrated by six channels through which ferrous iron enters to interact with a ferroxidase at the centre of the molecule (Harrison and Arosio, 1996). Iron is able to exit after it has been reduced. This iron depot is readily accessible for haemoglobin synthesis.

46. Serum ferritin concentrations are normally within the range 15-300 µg/L. They are lower in children than adults; from puberty to middle age, mean concentrations are higher in men than in women (Worwood, 1982). Good correlations have been found between serum ferritin concentrations and storage iron mobilised by quantitative phlebotomy, stainable iron in bone marrow biopsies, and the concentration of both non-haem iron and ferritin in the bone marrow. This suggests a close relationship between the total

amount of storage iron and serum ferritin concentration in normal individuals (Walters *et al*, 1973).

47. Haemosiderin is produced by lysosomal denaturation of ferritin. As the protein shells degrade the iron cores aggregate. Haemosiderin iron is found in lysosomes and cytosol and as it is less soluble than ferritin iron, it is less easily mobilised than ferritin iron.

Cellular iron homeostasis

48. Synthesis of several of the proteins involved in iron metabolism is regulated at the level of RNA translation by two cytoplasmic iron regulatory proteins: IRP1 and IRP2 (Cairo & Pietrangelo, 2000). These proteins are capable of binding to mRNAs that contain stem-loop structures, known as iron responsive elements (IREs). IRP1 contains an iron-sulphur (4Fe-4S) cluster and has a low affinity for the IRE when intracellular iron is abundant. When iron is scarce, however, the iron-sulphur cluster is no longer present and IRP1 binds to the IRE with high affinity. Activation of IRP2 requires accumulation of the protein after new synthesis. Degradation takes place in the presence of iron. Studies in cell lines have shown that IRP1 is the major contributor to iron regulating activity but at physiological tissue oxygen concentrations IRP2 is the dominant regulator (Meyron-Holtz *et al*, 2004).

Response to iron depletion

49. Increased needs for iron are met initially by increased release of iron from ferritin stores. Both haem and non-haem iron absorption show an inverse relationship to serum ferritin concentrations which reflect iron stores (see section 4) (Lynch *et al*, 1989): absorption of dietary iron increases as iron stores decrease.

50. The suggested threshold below which intestinal uptake and transfer responds to iron depletion in humans is at serum ferritin concentrations of approximately 60 μg/L (Hallberg *et al*, 1997). If absorption is not adequate, tissue iron stores are slowly depleted and the amount available for recycling and redistribution to tissues is decreased and this is reflected in a reduction in circulating transferrin saturation. As a result, the delivery of iron to functional sites decreases and iron-dependent functions, such as erythropoiesis, become impaired, leading to a decrease in haemoglobin concentration and the development of anaemia (see section 6). At a cellular level, ferritin synthesis is inhibited while transferrin receptor synthesis is increased. Apotransferrin synthesis by the liver is also increased by iron depletion. Concentrations of other iron containing proteins such as myoglobin, cytochromes and iron-sulphur proteins, are also decreased (Dallman *et al*, 1982).

Inborn errors of iron metabolism

51. A number of sequence variations affect the genes coding for proteins involved in iron metabolism. Identification of these gene mutations has improved understanding of iron metabolism.

52. While some of these genetic changes need be present on just one chromosome to cause disease (autosomal dominant or x-linked), the majority need to be present in two

corresponding chromosomes (autosomal recessive). In the autosomal recessive diseases it is not always clear whether heterozygotes (i.e. individuals with one normal and one aberrant gene) have altered iron metabolism that will affect their iron requirements or predispose them to excessively accumulate iron (see paragraph 55).

Gene mutations affecting proteins involved in iron absorption

HFE (Type 1/Hereditary/Genetic Haemochromatosis)

53. Hereditary or Genetic Haemochromatosis is one of the most common single gene disorders found in populations of North-European origin. It is an autosomal recessive disease caused by mutation of the gene coding for the HFE protein (Feder *et al*, 1996). It results in excessive absorption of dietary iron, causing high levels of iron to accumulate in the body. This can cause organ damage, leading to clinical manifestations including diabetes, arthritis, and cirrhosis of the liver (Bothwell and Macphail, 1998)

54. Two mutations of this gene, C282Y and H63D, have been identified. In the UK, over 90% of patients with hereditary haemochromatosis are homozygous for C282Y. The highest allele frequency of C282Y (10%) is found in Ireland and Brittany, followed by the UK (around 8%) and Scandinavia, and the lowest in Southern Italy (0.5%) (Merryweather-Clarke *et al*, 1997). The mutation is virtually absent in populations of non-European origin. The clinical penetrance of homozygosity for C282Y is very variable; the majority of people with this genotype never become ill as a result of iron overload (Beutler *et al*, 2002; Asberg *et al*, 2002; McCune *et al*, 2006). The H63D variant is more widespread in the general population and has a less defined role in predisposing towards iron loading. Compound heterozygotes account for 4% of patients (UK Haemochromatosis Consortium, 1997), though most individuals with this genotype do not develop iron overload (Jackson *et al*, 2001).

55. Less than a quarter of heterozygotes for the C282Y mutation show mild increases in serum ferritin concentration or transferrin saturation but do not have clinical features of iron overload (Bulaj *et al*, 1996). Although it has been suggested that heterozygotes may also have poorer control of iron absorption (Lynch *et al*, 1989), two studies which compared individuals heterozygous for HFE mutations found no differences in iron absorption between heterozygotes and wild-type controls (Hunt & Zeng, 2004; Roe *et al*, 2005).

56. A third variant, S65C, is less frequent than C282Y and H63D but may be associated with mild forms of haemochromatosis (Mura *et al*, 1999; Wallace *et al*, 2002).

57. Other types of genetic haemochromatosis are outlined in Table 2.2.

Table 2.2: Classification of genetic haemochromatoses

Type	Mutated protein	Mode of transmission	Phenotype	Mechanism	Severity	Relative incidence in populations of European origin
1	HFE	Recessive	Parenchymal iron overload	Hepcidin deficiency	Highly variable	Common (1 in 100-1 in 1000)
2A Juvenile haemochromatosis	Hemojuvelin	Recessive	Parenchymal iron overload. Early onset (2nd or 3rd decades)	Hepcidin deficiency	Severe	Rare
2B Juvenile haemochromatosis	Hepcidin	Recessive	Parenchymal iron overload. Early onset (2nd or 3rd decades)	Hepcidin deficiency	Severe	Rare
3	Transferrin receptor 2	Recessive	Parenchymal iron overload	Hepcidin deficiency	Severe	Rare
4A (Ferroportin disease)	Ferroportin 1	Dominant	Reticuloendothelial iron overload	Functional deficiency of ferroportin	Variable	Rare
4B (Ferroportin disease)	Ferroportin 1	Dominant	Both parenchymal and reticuloendothelial iron overload	Ferroportin shows defective binding of hepcidin	Variable	Rare

58. "African Iron Overload" is a disorder caused by an unidentified genetic defect in iron metabolism combined with increased exposure to iron. The condition is associated with a propensity to accumulate iron by a different mechanism to those found in the haemochromatoses. The source of iron is contamination from drinks (e.g. beer) or food prepared or stored in ungalvanised steel containers or iron cooking pots. In contrast to the haemochromatoses, the excess iron is in both the hepatocytes and Kupfner cells, and both heterozygotes and homozygotes appear to be affected (Andrews, 1999). Its occurrence amongst those of sub-Saharan heritage in the UK is unknown.

Molecular mechanisms causing iron overload

59. In types 1, 2 and 3 haemochromatosis, HFE modulates iron transport through regulation of hepcidin (Beutler, 2006). Hepcidin is directly responsible for regulating both iron release from intestinal epithelial cells and from macrophages through binding to the iron export protein, ferroportin 1. Mutations in HFE, HAMP (hepcidin), HJV (hemojuvelin) and the transferrin receptor 2 gene lead, through changes in signal induction cascades, to reduced synthesis of hepcidin in the liver and increased activity of ferroportin 1. This results in enhanced iron transfer from the small intestine and enhanced release of iron from phagocytes breaking down senescent red cells. The consequence is an increase in systemic iron load which is manifested by elevated plasma iron and ferritin concentrations and increased iron in liver parenchymal cells.

60. In type 4A (the more frequent form of ferroportin disease) iron release from macrophages, by the mutated protein, is decreased and iron accumulates in these cells. In type 4B the mutated protein is resistant to the action of hepcidin. Consequently, iron release from intestinal epithelial cells and macrophages is increased (as in types 1, 2 and 3) which leads to iron accumulation in hepatic parenchymal cells (Brissot *et al*, 2008).

The effect of infection and inflammation on iron metabolism

61. Acute and chronic inflammation affects the systemic distribution and turnover of iron: deposition of iron in tissue ferritin is increased, and availability of iron for distribution to functional sites, as well as gastrointestinal iron absorption, is reduced; concentrations of circulating iron are decreased and those of ferritin increased. This paradoxical situation, of red cell and systemic functional iron deficiency accompanied by an increased systemic and macrophage iron deposits, can become sustained with chronic inflammatory conditions and is known as the anaemia of chronic disease,

62. Infection and inflammation are followed by an acute phase response which involves the hepatic synthesis and release of a series of proteins known as acute phase reactants. Hepcidin, a key regulator of iron absorption, is an acute phase protein and its production is increased as part of the acute phase reaction (Nemeth *et al*, 2003); therefore increased amounts of hepcidin may contribute to the development of the anaemia of inflammation by reducing iron absorption and preventing the release of iron from macrophages. Ferritin also is an acute phase reactant.

63. The rapid drop in serum iron concentration following the induction of inflammation is due to an increase in apoferritin synthesis which sequesters iron and inhibits its release in to the plasma (Konijn & Hershko, 1977). Interleukin-I (IL-I), which is released from macrophages and monocytes, is the primary mediator of the acute-phase response (Dinarello, 1984). Studies of cultured human hepatoma cells have shown that IL-1ß directly enhances the rate of apoferritin synthesis by translational control of its mRNA (Rogers *et al*, 1990).

64. In the anaemia of chronic disease, serum ferritin concentrations are higher than those of patients with similar levels of tissue iron deposits but without infection and inflammation. This condition is observed frequently in clinical practice and chronic disease and is likely to be a significant confounder in population studies, particularly of older people. Such transient disturbances of iron metabolism in response to intercurrent infections need to be considered when interpreting the standard markers of iron metabolism (see section 4). In developing countries poverty, malnutrition, and infection are associated with the acute phase response and a correspondingly high prevalence of the anaemia of chronic disease.

3. Physiological Requirements

Current recommendations for iron intake in the UK

65. Dietary reference values (DRVs) for food energy and nutrients in the UK were revised by COMA in 1991 (DH, 1991). DRVs provide benchmark levels of nutrient requirements which can be used to compare mean values for population intakes. Although information is usually inadequate to calculate precisely and accurately the range of requirements for a nutrient in a group of individuals, it has been assumed to be normally distributed. This gives a notional mean requirement or Estimated Average Requirement (EAR) with the Reference Nutrient Intake (RNI) defined as two notional standard deviations above the EAR; intakes above the RNI will almost certainly be adequate to meet the needs of 97.5% of the population. The Lower Reference Nutrient Intake (LRNI), which is two notional standard deviations below the EAR, represents the lowest intakes which will meet the needs of approximately 2.5% of individuals in the group. Intakes below this level are almost certainly inadequate for most individuals.

66. Although the DRVs are often regarded as gold standards, they are not soundly established since there were insufficient data for most nutrients (DH, 1991). There are also inherent errors in the data, such as inaccuracies in assessment of food intakes, day-to-day variation in nutrient intakes, limitations in food composition data, as well as uncertainties about the appropriate biological marker to assess an individual's 'status' for a particular nutrient. These considerations are relevant to iron and it is important to recognise that, as a result, reference values are usually translated conservatively from the data; as a consequence, they may be more than populations or individuals actually need.

67. Since the COMA DRV report (DH, 1991), iron requirements for some or all population groups, have been considered by a number of expert committees including in the US, (National Academy of Sciences, 2001), the EU (SCF, 1993) and by the FAO/WHO (2002).

68. The universal approach taken to assess iron requirements has been to use a factorial method based on estimates of obligatory losses, menstrual losses and accretion of iron in synthesised tissues. Requirements for dietary iron are then estimated using an average figure for the absorption of iron from a typical diet. In the case of iron, this can be problematic, as these estimates are based on short-term studies that are usually carried out in iron replete individuals. Iron absorption will be down regulated in these individuals and might therefore not accurately reflect absorption of iron from the study diets (see section 5).

69. Other uncertainties such as the paucity of data for some population groups, difficulties in comparing long and short term studies, and problems measuring menstrual blood loss as well as variability in individuals limit the certainty with which requirements can be defined.

70. The DRVs for iron recommended by COMA (DH, 1991) are provided in Table 3.1. In their considerations, COMA assumed the following daily losses of endogenous iron: desquamated gastrointestinal cells (0.14 mg), haemoglobin (0.38 mg), bile (0.24 mg) and urine (0.1 mg) (Green *et al*, 1968); negligible amounts lost through skin and sweat (Brune *et al*, 1986); and blood loss (1 g of haemoglobin contains 3.47 mg of iron). Basal iron losses among normal healthy individuals were assumed to have a coefficient of variation of 15%; for infants, children, and adolescents, iron required for expanding red cell mass and growing body tissues was added to basal losses; in women of child bearing age, menstrual losses (average of 20 mg/28 day cycle[2]) were added to basal losses. It was assumed that only 15% of iron in the diet is absorbed, which is considered typical for most population groups in industrialised countries (FAO, 1988); for infants up to the age of three months, iron absorption was assumed to be 10% from breast milk substitutes (Flanagan, 1989). The limitations of these assumptions were previously described in paragraphs 66-69 and are further considered in section 4 (measuring iron status) and section 5 (iron in the diet).

Table 3.1: Dietary Reference Values for Iron mg/d (µmol/d[1]) (DH, 1991)

AGE	LRNI[2]	EAR[3]	RNI[4]
0-3 months	0.9 (15)	1.3 (20)	1.7 (30)
4-6 months	2.3 (40)	3.3 (60)	4.3 (80)
7-9 months	4.2 (75)	6.0 (110)	7.8 (140)
10-12 months	4.2 (75)	6.0 (110)	7.8 (140)
1-3 years	3.7 (65)	5.3 (95)	6.9 (120)
4-6 years	3.3 (60)	4.7 (80)	6.1 (110)
7-10 years	4.7 (80)	6.7 (120)	8.7 (160)
11-14 years (males)	6.1 (110)	8.7 (160)	11.3 (200)
11-14 years (females)	8.0 (140)[5]	11.4 (200)[5]	14.8 (260)[5]
15-18 years (males)	6.1 (110)	8.7 (160)	11.3 (200)
15-18 years(females)	8.0 (140)[5]	11.4 (200)[5]	14.8 (260)[5]
19-50 years (males)	4.7 (80)	6.7 (120)	8.7 (160)
19-50 years (females)	8.0 (140)[5]	11.4 (200)[5]	14.8 (260)[5]
50+ years	4.7 (80)	6.7 (120)	8.7 (160)

[1] 1µmol = 55.9µg
[2] Lower reference nutrient intake
[3] Estimated average requirement
[4] Reference nutrient intake
[5] COMA considered the distribution of iron requirements in women of child-bearing age to be skewed and the DRVs exclude those with high menstrual losses resulting in iron requirement above the EAR which is set at the 75th centile.

71. Other international reference values are provided in Table 3.2. Although the values are derived from similar sets of data, the differences in the references between committees are primarily due to differences in assumptions regarding the efficiency of iron absorption and utilisation in different population groups.

[2] Based on study in Sweden (Hallberg et al, 1966).

Table 3.2: International dietary reference values for iron (mg/d)

UK (Department of Health, 1991)		USA & Canada (National Academy of Sciences, 2001)		FAO/WHO (2002)			EU EC Scientific (Committee on Food 1993)	
Age	Recommended Nutrient Intake (based on 15% absorption)	**Age**	Recommended Dietary Allowance (based on 18% absorption)	**Age**	Recommended Nutrient Intake (based on 15% absorption)	Recommended Nutrient Intake (based on 10% absorption	**Age**	Population Reference Intake (based on 15% absorption)
0-3 m	1.7	_	_	_	_	_	_	_
4-6 m	4.3	0-6 m[1]	0.27	_	_	_	_	_
7-9 m	7.8	_	_	_	_	_	_	_
10-12 m	7.8	7-12 m[2]	11.0	6-12 m[5]	6.2	9.3	6-12 m[5]	6.2
1-3 y	6.9	1-3 y	7.0	1-3 y	3.9	5.8	1-3 y	3.9
4-6 y	6.1	4-8 y	10.0	4-6 y	4.2	6.3	4-6 y	4.2
7-10 y	8.7	_	_	7-10 y	5.9	8.9	7-10 y	5.9
MALES								
11-14 y	11.3	9-13 y	8.0	11-14 y	9.7	14.6	11-14 y	9.7
15-18 y	11.3	14-18 y	11.0	15-17 y	12.5	18.8	15-17 y	12.5
19-50 y	8.7	19-50 y	8.0	18+ y	9.1	13.7	18+ y	9.1
50 + y	8.7	50+ y	8.0	_	_	_	_	_
FEMALES								
11-14 y	14.8	9-13 y[3]	8.0	11-14 y[6]	9.3	14.0	11-14 y[6]	9.3
15-18 y	14.8	14-18 y[3]	15.0	11 -14 y	21.8	32.7	11-14 y	21.8
19-50 y	14.8	19-50 y	18.0	15-17 y	20.7	31.0	15-17 y	20.7
50+ y	8.7	50+ y	8.0	18+ y	19.6	29.4	18+ y	19.6
_	_	Pregnancy[4]	27.0	postmenopausal	7.5	11.3	postmenopausal	7.5
_	_	Lactation (14-18y)	10.0	lactating	10.0	15.0	lactating	10.0
_	_	Lactation (19-50 y)	9.0	_	_	_	_	_

[1] No functional criteria of iron status have been demonstrated that reflect response to dietary intake in young infants. Thus, recommended intakes of iron are based on an Adequate Intake (AI) that reflects the observed mean iron intake of infants principally fed human milk.
[2] Based on 10% absorption.
[3] Based on the assumption that girls younger than 14 years do not menstruate and that all girls 14 years and older do menstruate. For girls under age 14 who have started to menstruate, it would be appropriate to consider a median menstrual loss of 0.45mg/day of iron. Therefore, the requirement is increased by approximately 2.5mg/day of iron.
[4] The bioavailability in the first trimester is as estimated for nonpregnant females, in the second and third trimesters, it is increased to 25%.
[5] Bioavailability during this period varies greatly.
[6] Non menstruating

POPULATION GROUPS

The Neonate and Infant

72. At 20 and 40 weeks gestation, the fetus contains about 58 μg and 94 μg of iron respectively per gram of lean tissue (Dallman *et al*, 1988). During the last third of pregnancy the fetus accumulates about 2 mg of iron daily and the mature term neonate contains 150-250 mg of iron. Almost 80% of this iron is in haemoglobin (1 g of haemoglobin contains 3.4 mg of iron). Much of the remainder is in the reticuloendothelial and hepatic tissue iron depots.

73. To compensate for the low availability of oxygen *in utero*, the fetus has two main adaptations: it has a distinct form of haemoglobin, fetal haemoglobin, that has a greater affinity than adult haemoglobin for oxygen at low concentrations; and in addition, haemoglobin concentration in the circulation is high. After delivery the ambient concentration of oxygen is higher and the infant adapts by changing the type of haemoglobin and reducing its concentration by about 30%. At 2 months of age the haemoglobin concentration stabilises at 90-110 g/L. The iron released from degraded haemoglobin is retained in the reticuloendothelial system and redistributed peripherally for tissue synthesis. This is a major source of iron during the first 3-6 months of life; for example, in a term infant, a fall in haemoglobin of 60 g/L would release up to 60 mg of iron. This is then available to support lean tissue synthesis, which requires about 35 mg of iron per kg. The concomitant changes in iron deposition in ferritin is reflected by an initial increase in serum ferritin concentrations, which may approximate 400 μg/L at one month of age, followed by a decline, to about 30 μg/L at 6 months of age, as iron is used in synthesis of new tissue (Siimes *et al*, 1974). These postnatal changes in haemoglobin synthesis and iron stores occur irrespective of gestational age.

74. After birth, a delay in clamping the umbilical cord, until it has stopped pulsing, is associated with a 32% higher blood volume (Nelle *et al*, 1995) and a correspondingly increased transfer of iron (30-50mg) to the neonate (Pisacane, 1996). A randomised controlled trial of infants (n=358) in Mexico (Chaparro *et al*, 2006) reported that infants with delayed clamping (2 minutes after delivery) had significantly higher ($p<0.0002$) iron stores at 6m, reflected by serum ferritin concentrations, than infants with early clamping (around 10 seconds after delivery) even though there were no differences in haemoglobin concentration between the 2 groups.

75. A Cochrane review (McDonald and Middleton, 2008) reported that compared to early clamping, delayed cord clamping was associated with significantly higher newborn haemoglobin concentrations (mean difference, 21.7g/L; 95% CI: 2.8-40.6; 3 trials). Although this effect was not evident at 6 months (2 trials), serum ferritin concentrations were significantly higher at 3 months (mean difference, 17.9 μg/L; 95% CI: 16.59-19.21; 1 trial) and 6 months (mean difference, 11.8 μg/L; 95% CI: 4.07-19.53; 1 trial) suggesting higher systemic iron depots. However, significantly fewer infants in the early cord clamping group required phototherapy for jaundice compared with those in the late cord clamping group (RR: 0.59; 95% CI: 0.38-0.92; 5 trials).

Full term infants aged 0-6 months

76. Healthy term infants with a normal birth weight are born with sufficient iron reserves to cover their iron needs for about the first 4-6 months of life (Aggett *et al*, 2002; Griffin and Abrams, 2001) and this is their main source of iron during this period. Consequently requirements for exogenous iron during this period are low, but after 4-6 months, the demands of growth and haemoglobin synthesis result in a requirement for dietary iron.

77. Since the fetal accretion of iron is greatest in the last trimester of pregnancy, preterm infants are born with a lower total body content of iron and consequently have higher needs for iron to support their growth after birth (Oski, 1985). The iron requirements for preterm infants need special consideration and are not within the remit of this report.

78. Iron concentrations in human breast milk are low (0.2-0.4 mg/L) (Domellof *et al*, 2002a) and decline during lactation, decreasing from 0.97 mg/L in early transitional milk (Vuori, 1979) to 0.3 mg/L at 5 months (Siimes *et al*, 1979). Iron deficiency in the mother has no or little effect on the iron content of breast milk and iron supplementation during lactation does not increase the iron content of breast milk (Zavaleta *et al*, 1995).

79. Stable isotope studies have reported fractional iron absorption (i.e. incorporation of iron into erythrocytes) from breast milk of approximately 16%-25% at 6 months of age (Davidsson *et al*, 1994; Abrams *et al*, 1997; Domellof *et al*, 2002a). This means that an infant receiving 800 ml/day of breast milk with an iron content of 0.3 mg/L and a bioavailability of 20% will absorb about 0.05 mg/day of iron. This is compatible with meeting systemic needs for iron from reserves.

80. There are conflicting data on the risk of term infants who have been exclusively breast fed for six months developing iron deficiency. A trial in Honduras (n=139) reported that mean haemoglobin and plasma ferritin concentrations were significantly lower at 6 months in infants that had been exclusively breast fed compared to those who had been introduced to complementary food at 4 months (Dewey *et al*, 1998). However, birth weight was the factor most strongly associated with haemoglobin and ferritin concentrations; infants with birth weights <2500 g were at greater risk of low haemoglobin (<103 g/L) and low plasma ferritin (<12 µg/L) concentrations compared to infants with birth weights >3000 g. A study in Italy of infants with birth weight >2500 g (n=30) reported that mean haemoglobin concentrations were significantly higher in infants aged 12 months who had been exclusively breast fed for 7 months or longer compared to infants who received other foods at an earlier age. There was no difference in mean serum ferritin concentration between the two groups (Pisacane *et al*, 1995).

Infants aged 6 months to 3 years

81. Iron requirements of infants are highest during this period of rapid growth. After 6 months of age dietary requirements for iron are increased as iron stores become depleted and the amount of iron provided from breast milk is no longer sufficient to meet the increasing demands for growth and blood volume expansion.

82. Domellof *et al* (2002b) found no difference in iron absorption from breast milk between iron-supplemented and unsupplemented infants at 6 months of age (n=25);

at 9 months of age, however, absorption from breast milk was considerably higher in the infants not receiving iron supplements. No correlation was found between iron absorption and plasma ferritin concentration at both 6 and 9 months.

83. There are difficulties in setting reference standards to guide iron nutriture during the rapid transition of early childhood. This is demonstrated in the study by Domellof *et al* (2002b) (see previous paragraph) and further illustrated by a study of infants in the first 2 years of life (n=59) which reported wide variation in haemoglobin concentrations among children with similar iron intakes (Beal & Meyers, 1970). Consequently reference values are inclined to be conservative and cautious, assuming, for example, an estimated percentage absorption of iron from breast milk of 10%.

Children aged over 3 years

84. For children aged over 3 years iron is required to replace basal losses, for expanding red cell mass, and for growth which is an important determinant of body iron accretion.

Adolescents

85. Increased iron requirements for this age group are driven by the pubertal growth spurt which results in increased blood volume, haemoglobin, and functional use of iron by tissue as lean body mass expands. Compared with boys, these changes are less for girls due to lower growth velocity and differences in body composition; whereas lean tissue as a percentage of body mass percentage increases during puberty in boys, there is actually a small decrease in girls (Mølgaard *et al*, 1998). Serum ferritin concentrations may fall to levels at or below the usual reference range during this period of increased growth however this phenomenon does not necessarily indicate iron deficiency *per se* but suggests an increased *risk* of deficiency in adolescents.

86. For adolescent girls, iron requirements also increase to cover additional losses due to menstruation. Although menstrual blood losses are initially small, the menstrual blood loss of 15-year old adolescent girls is only marginally lower than in older women and it is generally assumed that adolescent menstrual losses are at the same level as those for adults (Hallberg and Rossander-Hulten, 1991).

Women in reproductive years

87. Menstruation, pregnancy, lactation, and growth during adolescence, all influence the systemic metabolism of iron and dietary requirements.

88. There are concerns that women in their reproductive years are at increased risk of iron deficiency due to changes in dietary habits and lifestyle which have led to lower energy expenditure and lower dietary energy intakes (Eaton and Konner, 1985; Hallberg and Rossander-Hulten, 1991). The iron nutriture of women of childbearing age in the UK is considered in section 9.

Menstruation

89. Menstrual blood loss is an important determinant of iron stores in women of reproductive age. A number of studies have observed an association between serum ferritin concentration and length of the menstrual period (Galan *et al*, 1985;

Soustre *et al*, 1986; Milman *et al*, 1993). Harvey *et al* (2005) reported a strong inverse correlation between menstrual blood loss and serum ferritin concentration with higher menstrual iron losses resulting in lower iron stores ($p<0.001$).

90. There is little intra-individual variation between menstrual cycles (Hallberg & Nilsson, 1964) but considerable inter-individual variation which follows a right skewed distribution in a population (see paragraph 91) (Hallberg *et al*, 1966). Calculation of DRVs for women in their reproductive years takes account of this distribution. Menstrual losses are affected by method of contraception: they are significantly increased with intrauterine devices (Milsom *et a*l, 1995) and reduced with oral contraceptives (Larsson *et al*, 1992).

91. Menstrual blood loss, and the associated iron loss, is difficult to measure accurately. Self-reported menstrual blood loss is inaccurate (Hallberg *et al,* 1966) and conclusions from qualitative or semi-quantitative studies are not considered reliable. A study in Sweden (Hallberg *et al*, 1966) reported that menstrual blood losses in women had a skewed distribution: 95% per cent of women lost 118 ml or less of blood per cycle; the median and mean menstrual blood loss was 30 ml and 44 ml respectively, equating to a median iron loss of 0.49 mg/d and a mean iron loss of 0.7 mg/d. These data were obtained before the widespread use of oral contraceptives.

92. A study in the UK (Harvey *et al*, 2005), which determined menstrual iron losses (n=90) by direct measurement of menstrual blood loss during one menstrual cycle, reported that median blood loss was18 ml which corresponds to a median iron loss of 0.26 mg/day; mean iron loss was 0.43 mg/d and 70% of the women lost less than 0.5 mg/d of iron through menstruation. In this study, the median blood loss of oral contraceptive users was significantly lower ($p<0.001$) than for those using other forms of contraceptives (excluding IUD users as there were too few to draw any conclusions).

93. The DRVs for women of reproductive age may have been set too high as they do not take account of adaptative responses to compensate for blood losses through menstruation by increasing the amount of iron absorbed from the diet; the extent of this compensation is not clear.

94. The long-term implications of menstrual iron losses over 20-30 years in the development of iron deficiency anaemia in women of reproductive age and the volume of menstrual blood loss which should be regarded as abnormal and needing clinical management to treat iron deficiency anaemia are not well characterised.

Pregnancy and Lactation

95. Physiological adaptations during pregnancy and lactation ensure an adequate supply of iron to the fetus and developing infant, even in the presence of iron deficiency. However, severe iron deficiency can affect reproductive efficiency (see section 6).

The effect of pregnancy on iron metabolism

96. Adaptations during pregnancy ensure the supply of nutrients to the fetus and other products of conception and sustain the additional metabolic burden of pregnancy. Maternal plasma volume and red cell mass increase early in pregnancy in advance of the systemic metabolic changes and significant growth that occurs in the fetus during the latter half of gestation. Iron supply to the conceptus is ensured by changes in the

structural and compositional characteristics of transferrin which facilitate preferential delivery of iron to placental rather than systemic transferrin receptors.

97. Plasma volume, which increases steadily until 32-34 weeks of gestation, is related to the size and health of the fetus. In a non-pregnant woman (55-60 kg), the plasma volume is approximately 2.6 L. This increases by 1.3L in a singleton pregnancy and by about 1.96 L and 2.4 L with twins and triplets respectively. The increase in plasma volume is not related to the preceding non-pregnant plasma volume. Consequently, since volume increases in small women are as large as those in bigger women, the dilutional effects on standard blood parameters of iron metabolism and haemoglobin are greater in small than in large women (Letsky, 1991). This is an important consideration in the interpretation of the customary parameters of iron status in pregnant women. Therefore, despite an increase in red cell mass and haemoglobin during pregnancy, the dilutional effect of the increased plasma volume produces lower values of iron markers which are not necessarily indicative of anaemia or of iron deficiency.

98. Red cell mass increases linearly from the end of the first trimester and may be related to the size of the fetus, but this is not established. The increase in red cell mass is associated with a 2-4 fold increase in erythropoietin which is most evident in the first 16 weeks of gestation. In non-pregnant women there is an inverse relationship between erythropoietin levels and haemoglobin, which is lost during the first and second trimesters of pregnancy but becomes evident again in the third trimester only in women with haemoglobin less than 90 g/L. The effects of iron supplementation, maternal age, and fetal size on red cell mass are uncertain. The fetus is unaffected by maternal erythropoietin and there is no evidence that fetal erythropoietin influences the mother (Schneider & Malek, 1995; Reisenberger *et al*, 1997).

99. The increase in plasma volume, concomitant with the rise in red cell mass, causes a reduction in peripheral resistance to blood flow which offsets any increase in blood viscosity caused by the increased red cell volume.

100. Since the relative balance between plasma volume and red cell mass changes throughout pregnancy, different cut-off points have been established for the diagnosis of anaemia during this period. In healthy, iron-supplemented women, haemoglobin concentrations decrease until the 25-30th week of gestation and then increase towards term.

101. The WHO (2001) recommends a cut-off of 110 g/L for anaemia, throughout pregnancy. In the UK, the National Institute for Clinical Excellence (NICE) recommends that iron supplementation should be considered for women with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks (NICE, 2008). In the USA, the Centers for Disease Control and Prevention (CDC) recommends a series of cut-offs for the '*physiological anaemia of pregnancy'* set at the 5th centile of haemoglobin, for 4-weekly intervals, from 12 weeks gestation onwards (CDC, 1989) while the Institute of Medicine advises screening of pregnant women in their first and second trimesters, and recommends that iron supplements are not supplied to women with haemoglobin $\geq$110 g/L or ferritin concentration >20 μg/L (IOM, 1993).

102. In a study of pregnant women (n=501) in the UK (Foulkes and Goldie, 1982), 12% (n=59) developed haemoglobin concentrations below 105 g/L at 36 weeks; out of these, 12 (20%) were taking iron supplements (100 mg/d) and 47 (80%) were not taking iron supplements. Eighty-seven per cent of the women not taking iron supplements had serum ferritin concentrations below 50 μg/L at 12-16 weeks gestation. This suggests the use of selective iron supplementation of pregnant women with serum ferritin concentration < 50 μg/L in the first trimester.

103. The relationship between maternal iron status and pregnancy outcomes is considered in section 6.

Intestinal absorption of iron during pregnancy

104. Intestinal uptake and transfer of iron increases during pregnancy (Whittaker *et al,* 2001). Svanburg *et al* (1975) reported that iron absorption from a standard meal was 3.1% in non-pregnant women, 0.8% in early pregnancy, 4.5% at 24 weeks gestation and 13.5% at 36 weeks gestation. A longitudinal study in 12 healthy pregnant women (Barrett *et al*, 1994), found that the mean absorption of iron from a standardised meal, extrinsically labelled with a stable isotope of iron, increased from 7% at 12 weeks to 36% and 66% percent at 24 and 36 weeks gestation respectively; at 16-24 weeks postpartum, absorption decreased to 11%.

Lactation

105. Assuming an average iron content of 0.4 mg/L and milk production of 800 ml/d, the average daily iron loss in breast milk is 0.32 mg, which represents about half the average iron loss with menstruation (DH, 1991). Thus, a breastfeeding woman with lactational amenorrhoea has a more positive iron balance than during pregnancy or menstruation. Long periods of lactation may therefore have a positive effect on the iron stores of fertile women, especially if they have many pregnancies.

106. Reference intakes for iron for lactating women differ between countries. In the UK, the recommendation for iron is the same as that of non-pregnant women (DH, 1991), while the FAO/WHO recommendation (2002) is about half more that of non-pregnant, menstruating women. The EU (1993), US and Canadian recommendations (2001) are similar to those of FAO/WHO.

Older people

107. Physiological requirements for iron are not increased in older people and iron depots usually increase with age (Lynch, 1982); this increase is greater in women then men because women have lower iron depots at younger ages. Iron requirements for older people are therefore the same as those of adults aged 19-50y and requirements for women after the menopause are the same as those of men (DH, 1991). The most common cause of iron deficiency in older people is gastrointestinal blood loss which can be caused by a number of factors including chronic disease (such as colorectal cancer), anti-inflammatory drugs (e.g. aspirin), diseases of the genitourinary system, or frequent blood drawings (Lipschitz, 1991)

Summary and conclusions

108. The dietary reference values for iron are based on limited data and few new data have emerged since COMA considered DRVs for iron in 1991. There are insufficient new data to allow recalculation of the DRVs for iron.

109. The paucity of available data makes it difficult to estimate accurately requirements for different population groups, particularly for infants, children, and women in their reproductive years.

110. The DRVs set for iron may be too high as assumptions about the availability of dietary iron for mucosal uptake and about absorptive adaptation are cautious.

111. Maternal adaptations during pregnancy facilitate the effective delivery of nutrients, including iron, to the conceptus.

4. MEASURING IRON STATUS: MARKERS OF DEPLETION, DEFICIENCY, SUFFICIENCY, AND EXCESS

Iron status

112. The term "*iron status*" is used to describe whether an individual has too little, enough, or too much iron in their body for their needs as well as to indicate the possible *risk* of deficiency or excess. However, it is of limited descriptional value unless it is more specifically qualified.

113. The spectrum of iron status (see Table 4.1) extends between the extremes of iron excess and iron deficiency, at which homeostasis has failed and functional and structural defects develop. In between these extremes, when the body needs iron, it is first mobilised from ferritin depots; when these fall to a certain level, hepatic production of hepcidin decreases, which facilitates increased intestinal uptake and transfer of iron from the diet. Simultaneously, peripheral needs for iron are manifest by increased expression of transferrin receptors and by changes in the amount of iron being transported by transferrin. If the body does not need iron, tissue ferritin depots increase, hepcidin concentrations increase, and intestinal uptake and transfer of iron is downregulated. Usually, in healthy individuals, when exposure to iron is solely from the diet, this adaptation is enough to prevent excessive systemic accumulation of iron. However, if iron is provided parenterally (e.g. in repeated blood transfusions) or if intestinal regulation of iron absorption is defective (as in the haemochromatoses), the body accumulates excess iron which is sequestered in ferritin, and there is an increased risk of systemic iron toxicity.

114. Assessment of an individual's iron status depends, therefore, on being able to determine where they lie on the above spectrum. This assessment would depend on measurement, interpretation and synthesis of various markers of iron metabolism. Adequate iron status (iron sufficiency) implies presence of normal erythropoiesis and iron dependent functions that are not limited by iron supply, as well as a small contingency reserve of "storage iron" to supply other physiological functions. Determination of "adequacy" or sufficiency is dependent on measurement of more than one marker; it is important to recognise that there is no single reliable marker of iron status, except at the extremes of deficiency and excess.

Table 4.1: Spectrum of iron status

Iron excess	Cellular and tissue architectural and functional damage	Damage to liver and other tissues
	Degradation of ferritin	Increased tissue haemosiderin
Iron adequacy	Deposition in depots	Increased tissue and serum ferritin
	Reduced intestinal uptake and transfer of iron	Increased hepcidin
Iron deficiency	Mobilisation of depots	Reduced hepcidin
		Reduced iron transfer between tissues reduced
		Reduced saturation of serum transferrin
		Increased transferrin receptors
		Defective haemoglobin synthesis
		Reduced haemoglobin
		Impaired muscle metabolism
	Increased intestinal uptake and transfer of iron	
	Functional defects in iron dependent activities	

Establishment of reference values for markers used to assess iron status

115. Iron status is a qualitative concept as there are no good dose-response data to determine thresholds for adverse events associated with iron deficiency or excess.

116. The selection of reference markers has been limited by their accessibility and by the reliability of their measurement. Each of the markers have limitations and are subject to other influences apart from those directly involved in the supply, acquisition, distribution, deposition, and use of iron. There is no good single marker of iron deficiency, adequacy, or excess. Any assessment of iron status depends on an integrated interpretation of a battery of markers which represent functional, storage, distribution, regulatory, or adaptive mediators.

117. The reference ranges are themselves not diagnostic but essentially describe sufficiency or adequacy. It does not necessarily follow that values outside the reference ranges indicate deficiency or excess; however the further a value lies from the reference limits, the more likely it is to represent a systemic deficiency (and a need for more exogenous iron) or excess of iron.

118. Two approaches have been used to establish thresholds and reference values for these markers. The first is to determine values in a sample of healthy subjects who are considered to be representative of the population from which they are taken and who are unlikely to be iron deficient or have a high iron body burden; cut-offs are then based on 90 or 95% confidence intervals (normative population method). This method requires, or assumes, the exclusion or absence of individuals with iron deficiency or iron overload from the selected population sample and an assumption that the rest of the sample population is otherwise “healthy” and unaffected by any conditions which may affect the markers being measured.

119. The second approach is to compare concentrations of any iron marker from individuals identified with iron deficiency anaemia (based on the absence of stainable iron in the bone marrow and/or a positive response to iron supplementation) with

those in non-iron deficient individuals (non-responders to iron supplementation). Cut-offs can then be established from the distribution of values for the two groups. Lower levels determined in this way are more likely to reflect values which are representative of functional outcomes of iron deficiency. However, with the exception of serum ferritin, this approach has not been widely used to assess markers of iron deficiency.

120. The cut-offs selected for markers of iron status can vary between laboratories and are not based on the appearance of functional deficits. The limits simply indicate values which signal the possibility of iron depletion, deficiency, or excess.

121. A low value for a *single* marker has a low diagnostic efficiency for iron deficiency. Approaches based on combinations of markers have been explored to improve the specificity and sensitivity of diagnostic approaches (see paragraphs 142-144). Markers need to be evaluated according to their functional relevance, potential confounders, and relevance of the indicative risk.

Markers of iron status

122. Markers that have been used to assess iron status, their representative reference ranges, use, and limitations are summarised in Table 4.2. They are categorised according to whether they represent: a functional use of iron in the synthesis of haemoglobin; the distribution or transport and supply of iron to tissues; and iron stores in tissues.

Table 4.2: Markers used for assessment of body iron status (adapted from BNF, 1995)

Measurement	Representative reference range (adults)	Confounding factors	Diagnostic use
Functional iron			
Haemoglobin concentration Males Females	 130-180 g/L 120-160 g/L	Other causes for anaemia besides iron deficiency; a reciprocal relationship with iron stores should be expected in all anaemias except in iron deficiency anaemia	Assess severity of IDA; response to a therapeutic trial of iron confirms IDA. Not applicable to assessment of iron overload
Red cell indices MCV* MCH	 84-99 fl 27-32 pg	May be reduced in other disorders of haemoglobin synthesis (e.g. thalassaemia, sideroblastic anaemias) in addition to iron deficiency	
Tissue iron supply			
Serum iron Saturation of transferrin	10-30 μmol/L 16-50%	Normal short-term fluctuations mean that a single value may not reflect iron supply over a longer period. Both measures reduced in chronic disease.	Raised saturation of transferrin used to assess risk of tissue iron loading (e.g. in haemochromatosis or iron-loading anaemias).
Serum transferrin receptor	2.8-8.5 mg/L**	Directly related to extent of erythroid activity as well as being inversely related to iron supply to cells.	Decreased saturation of transferrin, reduced red cell ferritin, increased zinc protoporphyrin, and increased serum transferrin receptors indicate impaired iron supply to the erythroid marrow.
Red cell zinc protoporphyrin*	<70 μmol/mol Hb (<80 g/L red cells)	Stable measures: reduced iron supply at time of red cell formation leads to increases in free protoporphyrin and hypochromic red cells, and reduced red cell ferritin. However, values may not reflect current iron supply	
Red cell ferritin (basic)	3-40 ag/cell		Serum transferrin receptors may have particular value in identifying early iron deficiency and, in conjunction with serum ferritin, distinguishing this from anaemia of chronic disorders
% hypochromic red cells	<6%	May be increased by other causes of impaired iron incorporation into haem (e.g. lead poisoning, aluminium toxicity in chronic renal failure, sideroblastic anaemias)	
Iron stores			
Serum ferritin males Females	15-300 μg/L 15-200 μg/L	Increased: as an acute phase protein and by release of tissue ferritins after organ damage.	All measures are positively correlated with iron stores except TIBC which is negatively correlated. Serum ferritin is of value throughout the range of iron stores. Quantitative phlebotomy, liver iron concentration, chelatable iron and MRI are of value only in iron overload. Bone marrow iron may be graded as absent, normal or increased and is most commonly used to differentiate ACD from IDA.
Tissue biopsy iron - Liver (chemical assay)	3-33 μmol/g dry wt	Potential for sampling error on needle biopsy, especially when this is <0.5 mg, or liver is nodular. But remains the "gold standard" in iron overload.	
Bone marrow (Perls' stain)			
Quantitative phlebotomy	< 2g iron		
Serum TIBC (may be measured directly or calculated from transferrin concentration)	50-70 μmol/L*		In IDA, a raised TIBC is characteristic.
Urine chelatable iron (after 0.5 g IM desferrioxamine)	< 2 mg/24h		
Non-invasive imaging MRI	–	Not yet sufficiently sensitive and reproducible for quantitation of normal levels of storage iron. Useful for detecting iron overload.	
SQUID (Magnetic susceptibility)		Sensitive, accurate and reproducible but only a few machines in the world.	

ACD=Anaemia of chronic disease; Hb, haemoglobin; IDA=iron deficiency anaemia; MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MRI=Magnetic resonance imaging TIBC=Total iron binding capacity;
*No internationally accepted cut-off values for MCV, TIBC, or ZPP have been developed because of analytical differences between laboratories and because these indicators can be influenced by variations in the conditions under which the blood samples were collected (e.g. fasting/non-fasting, time of day) and by the methods used for transportation, storage and processing.
** There is a major problem with the different units and reference ranges for the various assays in use (Akesson *et al*, 1999;Worwood, 2002).

Characterising and detecting iron depletion and deficiency

123. A direct way to assess iron stores is to measure iron concentration in bone marrow using the Prussian blue stain[3]: absence of stainable iron indicates depletion of iron stores. This is widely considered as the gold standard for evaluating iron depletion and deficiency however its use in population surveys is limited by its impracticality and cost. The most commonly used markers for population surveys are considered below.

Functional iron

Haemoglobin

124. Haemoglobin and haematocrit have been widely used to assess anaemia, which is a functional consequence of iron deficiency. However, these measurements lack sensitivity[4] because anaemia associated with nutritional iron deficiency is usually mild resulting in extensive overlap between healthy and iron deficient populations. The specificity[5] of these measures are also poor since there are many other causes of anaemia (DeMaeyer *et al*, 1985). The shape and size of the red cells and other indicators of iron deprivation are necessary to determine whether low haemoglobin concentrations are due to iron deficiency in a population. Even then, other causes of anaemia, including other nutritional deficiencies, may co-exist.

125. Internationally accepted values have been developed for diagnostic and screening purposes which correspond to the concentration of haemoglobin below which the functional consequences of anaemia are encountered but the levels themselves are not necessarily those at which specific functional defects arise.

126. The WHO criteria of anaemia are widely used (see Table 4.3) although there is evidence of significant racial differences in "normal" haemoglobin values. In the USA, data from national surveys have generally shown that individuals of African extraction with adequate iron stores have haemoglobin concentrations which are 4 to 10 g/L lower than those of Caucasian origin (Perry *et al*, 1992). Haemoglobin concentrations are also influenced by smoking and altitude (>1000 m) (WHO, 2001) and individual haemoglobin concentrations show strong tracking, implying genetic influences.

Table 4.3: WHO (2001) - Haemoglobin thresholds used to define anaemia at sea level

Age	Haemoglobin concentration (g/L)
Children 0.5-4.99 years	110
Children 5-11.99 years	115
Children 12-14.99 years	120
Non-pregnant women (>15 years)	120
Pregnant women	130
Men (>15 years)	130

[3] Prussian blue stain is used to detect the presence of iron which has not been incorporated into haemoglobin.
[4] The sensitivity represents the proportion of a population with a certain characteristic (e.g. disease, health status) that are correctly identified by the measurement. It refers to the proportion of true positives that are correctly identified by the measurement.
[5] The specificity of a measurement represents the proportion of a population correctly identified without a certain characteristic. Specificity refers to the proportion of true negatives that are correctly identified by the measurement.

Tissue iron supply

Zinc protoporphyrin

127. Measurement of zinc protoporphyrin (ZPP) has largely replaced the free erythrocyte protoporphyrin assay which was previously used in population surveys, for example, in the National Health and Nutritional Examination Survey (NHANES) II and NHANES III in the USA. ZPP is the form in which most protoporphyrin IX exists in the red cell in iron deficiency as zinc is inserted into the protoporphyrin molecule instead of iron. The cut-offs recommended by the WHO (2001) for ZPP are >61 mmol/mol haem (<5y) and >70 mmol/mol haem (5y+). Although ZPP is a sensitive indicator of iron-deficient erythropoiesis, concentrations are raised in both iron deficiency and anaemia due to infection and inflammation.

Serum Iron

128. Serum iron has very limited value in assessing iron deficiency or chronic excess in population studies because concentrations within an individual are affected by other influences such as diurnal and post prandial variations and concentrations are rapidly reduced following infection or inflammation.

Transferrin saturation

129. Measurement of transferrin saturation (serum iron/total iron binding capacity x 100%) is a more sensitive and specific indicator of iron deficiency than serum iron alone (Bainton & Finch, 1964). However the limitations of using transferrin saturation are similar to those of serum iron.

130. Although total iron binding capacity (or transferrin concentration) specifically increases in iron deficiency, measurements of total iron binding capacity are rarely used alone as reliable reference ranges are not available because of analytical differences between laboratories and because TIBC can be influenced by the conditions in which the blood samples were collected (e.g., fasting/non-fasting, time of day), transported, stored, and processed.

131. Transferrin saturation below 16% indicates the point at which iron-deficient erythropoiesis develops (Bainton & Finch, 1964) and allows assessment of iron deprivation based on a reduction of the transport pool. Koerper & Dallman (1977) defined the lower limit of the 95% range for normal infants and children (aged 0.5-18y) as 7%. Cut-off values have also been derived from NHANES II and NHANES III data (1-2y <10%; 3-5y, 12%; 6-15y, 14%; over 16y, 15%).

Serum transferrin receptor

132. Cells increase their production of transferrin receptors when they need iron. Circulating receptors are derived from cellular transferrin receptors which are mostly released from developing red cells. Iron deficiency causes increased synthesis and the assay for serum transferrin receptors provides an indicator of the adaptations of cells, particularly red cell precursors, to acquire iron. However, there are a variety of assay reagents, units of measurement and reference ranges and no agreed methodological approach for measuring serum transferrin receptors or establishing reference ranges (Worwood *et al*, 2000).

133. Concentrations of serum transferrin receptors are higher in infants than adults (Choi *et al*, 1999; Olivares *et al,* 2000; Suominen *et al,* 2001) and decrease steadily throughout childhood to adult values. Values are higher at high altitudes (Allen *et al*, 1998).

134. The serum transferrin receptor assay provides a sensitive indicator of iron deficient erythropoiesis and is relatively unaffected by inflammatory phase reactions. Serum transferrin receptors, however, represent tissue requirements for iron rather than deficiency *per se*. They are therefore raised in the absence of iron deficiency when iron needs are increased for other reasons such as growth, erythropoiesis to compensate red cell breakdown in other conditions, and some malignancies. Consequently, in clinical practice, measurement of serum transferrin receptors has not enhanced the sensitivity and specificity for detecting iron deficiency in patients with the anaemia of chronic disease (Worwood, 2005).

Total body iron depots

Serum ferritin

135. Serum ferritin concentration reflects systemic ferritin iron depots: in normal subjects without infection (see paragraph 62) 1 μg/L of serum ferritin represents 8 mg of stored iron (Walters *et al*, 1973). This approximation does not take account of body weight. In healthy subjects total body iron stores may be calculated from measurements of serum ferritin and serum transferrin receptors. These values may be positive (stores present) and negative (reflecting a loss of haemoglobin iron) (Cook *et al*, 2003) and are related to body weight.

136. Cut-offs for serum ferritin concentration have been derived by examining the highest values found in patients with iron deficiency anaemia, defined as microcytic anaemia with either an absence of storage iron in the bone or a subsequent response to therapeutic iron (Worwood, 1979). Hallberg *et al* (1993) measured serum ferritin concentrations in healthy women with no stainable iron in the bone marrow. Both these approaches suggested a limit of 15 μg/L and this cut-off has been widely used in population surveys in Scandinavia and other parts of Europe to identify iron depletion (Hallberg, 1995); in the USA a slightly lower limit of 12 μg/L has been used (Looker *et al*, 1997). These different thresholds reflect methodological variations and their application (e.g. for clinical purposes or for population surveys). Lower values have generally been recommended for children. The WHO criteria used to define depleted storage iron are 12 μg/L for children under 5 years and 15 μg/L for males and females over 5 years (WHO, 2001).

137. It is important to recognise that low serum ferritin concentrations represent low iron depots in tissues; they do not represent a functional deficiency of iron. Cook *et al* (1976) showed there was little difference in the prevalence of iron deficiency anaemia[6] in women with a single low result for serum ferritin, erythrocyte protoporphyrin or transferrin saturation than in subjects with normal results for all three tests.

[6] Iron deficiency anaemia defined as transferrin saturation <15%, red cell protoporphyrin > 100 g/L packed red blood cells, serum ferritin <12 μg/L

138. Since ferritin behaves as an acute phase reactant, serum concentrations are increased in response to acute and chronic inflammation and even a mild infection can raise concentrations (Hulthen *et al*, 1998). This means that serum ferritin concentrations can fall in the normal range even when iron stores are low. This limits the sensitivity of serum ferritin as an indicator of iron deficiency or excess, particularly in places where infectious diseases are common.

139. Markers that can be used concomitantly for detection of infection and/or inflammation include: C-reactive protein (CRP), which provides an indication of acute disease and α-1-acid glycoprotein (AGP), which provides a marker of chronic infection (although this assay is not widely used in the UK). The acute phase protein most commonly used for this purpose is CRP. Although CRP concentration rises quickly in response to inflammation, it also falls quickly. It has been suggested that AGP concentration, which is slower to respond to inflammation, may be a better indicator than CRP because it remains at a higher concentration for a longer time (WHO/CDC, 2004). Although high sensitivity CRP assays may improve the value of the CRP assay, such measurements will not detect minor infections that may increase ferritin levels (Hulthen *et al*, 2001) and ideally, a health questionnaire needs to be completed for each subject to identify possible infection.

140. It has also been proposed that a higher threshold of <30 µg/L should be recommended in the presence of infection, but this applies only for children aged under 5 years (WHO, 2001); there has been no consensus regarding a threshold for adults.

Serum transferrin receptor/serum ferritin ratio

141. The ratio of serum transferrin receptors/serum ferritin concentration may provide a quantitative measure of iron stores in normal subjects. Cook *et al* (2003) demonstrated the use of this ratio[7] in studies of iron supplementation and fortification. However, the serum transferrin receptors/serum ferritin ratio applies to healthy subjects and its validity has not been established in populations with a high degree of infection. It is unlikely that direct confirmation of the validity of the relationship will be possible for neonates, children and pregnant women. Secondly, until the assay is standardised, it will not be possible to compare results between studies.

Multiple indices: using a combination of parameters to assess iron depletion and deficiency

142. All the individual indices used to measure biochemical iron status have limitations in terms of their sensitivity and specificity and no single indicator can assess the entire range of deficiency. A combination of haemoglobin and other indicators have been used to better characterise iron depletion and deficiency, and to distinguish between anaemia associated with iron deficiency from that associated with other causes.

143. A multiple analyte approach was developed for the analysis and interpretation of NHANES II data (Expert Scientific Working Group, 1985). Two models, the ferritin model and the mean red cell volume (MCV) model, were used to estimate the prevalence of abnormal values. The ferritin model used serum ferritin, transferrin saturation, and erythrocyte protoporphyrin as indicators whereas the MCV model used MCV, transferrin saturation, and erythrocyte protoporphyrin. Participants with 2

[7] The logarithm of concentrations [µg/L] of serum transferrin receptors/serum ferritin

or more abnormal values were considered to be iron-deficient. Relatively large differences between the two models, in prevalence estimates for impaired iron status, were observed in males aged 11-14 years and females aged 15-19 years. This would be expected because low serum ferritin concentrations detect early stages of reduced iron stores and by implication iron depletion, whereas mean red cell volume is reduced when haemoglobin synthesis is impaired in iron deficiency.

144. A WHO/CDC consultation which reviewed the various indicators used to assess the iron status of populations recommended that measurement of haemoglobin concentration could provide useful information about the severity of iron deficiency when used with other measurements of iron status (WHO/CDC, 2004). Serum ferritin concentration was considered the best single indicator of iron status except in populations with widespread infection; in such populations, measurement of transferrin receptor concentration was also recommended. Transferrin receptor concentration is not increased with inflammation which makes it possible to distinguish between iron deficiency and inflammation when both transferrin receptor and serum ferritin concentrations are measured. Serum ferritin concentration, together with haemoglobin concentration, was considered to be the most useful indicator to assess the response to an intervention to treat iron deficiency.

Assessment of iron status in infants and young children

145. For children aged 6 months to 5 years, current WHO guidelines (2001) give a cut-off value for anaemia as haemoglobin concentration of 110 g/L, while depleted iron stores are defined as a ferritin value below 12 μg/L.

146. The ESPGHAN[8] Committee on Nutrition has criticised these values, suggesting that they do not represent current knowledge of developmental aspects of erythropoiesis and iron metabolism; consequently, they are too high for the first two years of life and overestimate the prevalence of anaemia and iron deficiency (Aggett *et al*, 2002). The Committee identified the need for evidence to demonstrate the concentrations of haemoglobin and ferritin associated with functional defects that are responsive to iron therapy and which can therefore be regarded as indicators of deficiency.

147. Many studies have suggested lower haemoglobin concentrations based on the distribution of the parameters in groups of infants that are likely to be iron replete. These cut-offs are, however, not based on functional criteria such as stainable iron in bone marrow aspirates (Aggett *et al*, 2002) or on any systematic study of the response to iron supplementation.

148. A study in Sweden and Honduras identified iron replete infants using 3 markers of iron adequacy and lower cut off levels (- 2SD) were identified by the normative population method (Domellof *et al*, 2002). For haemoglobin, the values identified were 105 g/L at 4-6 months and 100 g/L at 9 months. For ferritin, the suggested values were 20 μg/L at 4 months, 9 μg/L at 6 months, and 5 μg/L at 9 months (Table 4.4). In this study haemoglobin response to iron supplementation was also used to identify infants with iron deficiency, but gave a higher haemoglobin cut-off (113 g/L) than using the normative population method. In the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC), a large randomly selected representative (n=1175) population sample in the UK, the 5th percentile for haemoglobin was 97 g/L

[8] European Society for Paediatric Gastroenterology, Hepatology and Nutrition

at 8 months and 100 g/L at 12 and 18 months (Emond *et al*, 1996; Sherriff *et al*, 1999); ferritin concentrations were 16 μg/L at 8 and 12 months and 12 μg/L at 18 months.

Table 4.4: Suggested cut-off values for iron deficiency at 4, 6 and 9 months based on iron-replete, breast-fed infants (Domellof *et al*, 2002)

Iron marker	4 months	6 months	9 months
Haemoglbin g/L	<105	<105	<100
Mean cell volume fL	<73	<71	<71
Zinc protoporphyrin μmol/mol haem	>75	>75	>90
Ferritin μg/l	<20	<9	<5
Transferrin receptor mg/l*	>11	>11	>11

*Ramco assay (cannot be compared directly with other assays but should relate to the assay of Flowers *et al*, 1989). For infants (8-15 m) the upper reference value (95% CI) for sTfR is 13.5 mg/l (Olivares *et al*, 2000)

Iron overload

149. In Genetic Haemochromatosis (type 1), measurement of transferrin saturation provides an early indication of the risk of iron accumulation and measurement of serum ferritin concentration provides an indication of developing iron overload. Subjects may have slightly higher blood haemoglobin levels and mean cell volume or mean cell haemoglobin (Barton *et al*, 2000) but these are not diagnostically useful. Serum transferrin receptors also do not have diagnostic value; although these are lower in subjects with iron overload, there is considerable overlap with concentrations for subjects with normal iron depots (Baynes *et al*, 1994; Ledue & Craig, 1995; Looker *et al*, 1999).

150. Increased hepatic iron deposition and concentration is not fully reflected by serum ferritin concentrations during the early stages of excessive iron accumulation, for example in patients with haemochromatosis (homozygous for C282Y) and for heterozygotes (Edwards *et al*, 2000). This is because serum ferritin is usually derived from phagocytic cells of the reticulo-endothelial system whereas excess iron is first deposited in the ferritin pool of hepatic parenchymal cells (Worwood, 1990).

151. Suggested upper reference thresholds for serum ferritin concentrations are uncertain and have also varied widely. In the UK, values of >300 μg/L for men and postmenopausal women (200 μg/L for premenopausal women) have been suggested (Dooley & Worwood, 2000); the same thresholds were used in the Haemochromatosis and Iron Overload Screening (HEIRS) study of 100,000 adults in the USA (Adams *et al*, 2005). For men, cut-off values ranging from 200 μg/L (Mainous *et a*l, 2002) to 400 μg/L (Looker & Johnson, 1998) have been applied. For premenopausal women, a concentration of 200 μg/L has commonly been selected, although 100 μg/L has also been used (Asberg *et al,* 2001).

152. In the USA approximately 6% of adults in NHANES III (1988-94), had a transferrin saturation > 45% (Looker & Johnson, 1998) however this cut off is associated with low specificity for haemochromatosis. A threshold as high as 62% has been applied (Edwards, 1988) and intermediate thresholds of > 55% (men) and 50% (women) have also been used (Dooley & Worwood, 2000). In the HEIRS study (Adams *et al*, 2005) and a systematic review for the US Preventative Services Task Force (Whitlock *et al*, 2006), a transferrin saturation threshold of 50% was selected for men and 45% for women. Unsaturated iron-binding capacity is as sensitive and specific

as transferrin saturation but reference ranges vary widely for different assay systems (Adams *et al*, 2000; Jackson *et al*, 2001; Murtagh *et al*, 2002).

153. Results from studies which have examined the association of HFE genotype and iron status have generally found slightly but significantly higher values for serum iron and transferrin saturation in heterozygotes for either C282Y or H63D compared with those lacking these variants but no significant difference between heterozygotes and "wild type" individuals for serum ferritin concentration (Burt *et al,* 1998; Datz *et al*, 1998; Beutler *et al,* 2000; Jackson *et al*, 2001; van Aken *et al*, 2002; Chambers *et al*, 2003; Raddatz *et al*, 2003; Sanchez *et al*, 2003)).

Summary and conclusions

154. Iron status represents a risk assessment of adequacy, deficiency, and excess. There are a number of haematological and biochemical indicators which are used to assess iron status; these are based on iron depots, distribution of iron between tissues and iron utilisation.

155. Iron status is essentially a qualitative concept. It can not be precisely quantified because of difficulties in determining accurate thresholds for adaptive responses and for adverse events associated with iron deficiency or excess. The thresholds selected for use are not based on functional outcomes.

156. Reference ranges for various indices are affected by analytical differences between laboratories and other conditions such as diurnal and post prandial variations.

157. No single marker of iron metabolism is considered ideal for the assessment of iron deficiency, adequacy, or excess as all the individual indices have limitations in terms of their sensitivity and specificity. However, in agreement with pragmatic decisions made by others such as the WHO/CDC (2004) and COMA (DH, 1994), haemoglobin (functional iron) & ferritin (iron stores) are considered to be the most useful indicators of iron status.

158. The WHO criteria for identification of anaemia are haemoglobin concentrations of: 110g/L, children under 5y; 115 g/L, children 5-11.99y; 120 g/L, children 12-14.99y and non-pregnant females over 15y; 130 g/L males over 15y. The WHO criteria used to define depleted storage iron are serum ferritin concentrations of: 12 µg/L, children under 5y; 15 µg/L, males and females over 5y.

5. IRON IN THE DIET

Dietary iron

159. Dietary iron exists in two main forms: haem iron and non-haem iron. Haem iron is found almost entirely in food of animal origin but small amounts (<0.1% of total iron) are found in plants (Tang *et al*, 1990). Non-haem iron is found in animal and plant tissues as Fe^{2+} bound to insoluble proteins, phytates, oxalates, phosphates, carbonates, and as ferritin. The richest sources of non-haem iron are cereals, vegetables, nuts, eggs, fish and meat. The contribution of these foods to dietary iron intakes in the UK is described in section 9.

160. Iron is also added to a number of foods during its manufacture (iron fortificants) and is available in supplemental form (capsules, tablets or other over-the-counter preparations containing iron, either alone or in combination with other micronutrients).

161. In some developing countries, contamination iron from mechanical or chemical reactions occurring during food production and preparation may be an important source of iron (Adish *et al*, 1999; Kroger-Ohlsen *et al*, 2002). The iron content of food is increased by being cooked in cast-iron cookware, particularly when acidic foods are cooked for extended periods of time (Brittin & Noassaman, 1986; Fairweather-Tait *et al,* 1995). Contamination iron is not considered to be a significant source of iron in the UK as the use of cast-iron cookware is not widespread in the UK.

162. There is no direct method for measuring the haem iron content of meats and foods; values are derived by measuring inorganic iron and total iron and assuming the difference represents the amount of haem iron. The haem iron content of meat is estimated to be 40% of total iron (Monsen *et al,* 1976), however the percentage of total iron present as haem in different non-milk animal food products is very variable; later estimates suggest that haem iron content of different meats ranges between 22 and 80% (Carpenter & Clark, 1995).

163. Processes that are most likely to affect iron content and/or absorption include milling, soaking, cooking, germination, fermentation and heat.

Bioavailability of dietary iron

164. Absorption refers to the passage of a nutrient, or other dietary constituent, from the intestinal lumen into the body. It usually comprises uptake of nutrient into the enterocytes of the gut mucosa and its subsequent transfer across the cell and into the body Bioavailability describes the systemic utilisation of a dietary nutrient and refers to the proportion of a nutrient that can be taken up and transferred by the intestinal mucosa and and which is subsequently used systemically.

165. Haem and non-haem iron are transported into the intestinal mucosal cells by independent mechanisms (see section 2). The intestinal uptake and transfer of iron is dependent on the body's need for iron (see paragraph 23). The availability of dietary iron for uptake by the gut mucosa is affected by the chemical form of iron (haem iron or inorganic non-haem iron) and the character of the complexes of inorganic iron with other dietary constituents.

166. Dietary components that affect iron absorption have been identified and partly characterised from single meal studies using isotope labels (see paragraph 172). Single meal studies have shown that haem iron is more efficiently absorbed from the diet (20-30%) than non-haem iron (5-15%) (Martinez-Torres & Layrisse, 1971; FAO, 1988). Non-haem iron absorption in single meal studies is very variable and is influenced by the balance of dietary factors enhancing and inhibiting absorption (see paragraphs 176-195). With the exception of calcium, which has an inhibitory effect on both haem and non-haem iron (Hallberg *et al*, 1991), absorption of haem iron is not affected by other components of the diet.

167. Homeostatic control of non-haem iron absorption is more pronounced than that of haem iron. Lynch *et al* (1989) reported that absorption of non-haem iron from a standard meal containing 4.8 mg/L of iron, was shown to be nearly tenfold higher with decreasing serum ferritin concentrations (from 100μg/L to 10 μg/L) compared to 2-3 fold increase for absorption of haem iron (Lynch *et al*, 1989). Hallberg *et al* (1997) reported that fractional absorption of haem and non-haem iron was similar (about 40%) at serum ferritin concentrations of 10 μg/L; at higher serum ferritin concentrations absorption of both haem and non-haem iron was decreased but the decrease in absorption was greater for non-haem; at serum ferritin concentrations of 15, 20, and 30 μg/L, haem iron absorption was 40%, 80%, and 140% higher than that of non-haem iron.

168. Since the systemic need for iron is the major determinant of iron uptake and transfer, bioavailability is not strictly a characteristic of a food or diet *per se.* However as systemic needs for iron increase, the type of diet and its influence on the availability of iron may become increasingly relevant and could therefore be of particular importance in growing children or in populations with iron deficiency secondary to intestinal parasites and infection when the iron content of the diet is very low. The presence of iron deficiency in a population where there is no evidence of any diseases that affect iron metabolism suggests that dietary iron supply may be inadequate in terms of absolute amounts of absorbable iron relative to iron requirements. This demonstrates the need for greater understanding of the dietary factors influencing the amount of intraluminal iron available for uptake by the intestinal mucosa.

169. In general, iron compounds that are water-soluble have the highest bioavailability, followed by those that are soluble in dilute acid (equivalent to gastric conditions). Compounds that are insoluble in water or dilute acid have a lower bioavailability because the iron precipitates and cannot be taken up by the enterocyte (Hurrell, 1997).

Measuring bioavailability and absorption of dietary iron

170. The influence of dietary factors on iron bioavailability and absorption has been determined from single isotopically labelled meals which are consumed by subjects following an overnight fast.

171. The true bioavailability of iron in the diet can only be assessed when the technique used is not limited by the intestinal setting for iron absorption, i.e., individuals with a high setting for iron uptake and transfer (iron deficient individuals) or without regulation of iron absorption (e.g., indivdiuals with haemochromatosis). If iron bioavailability is assessed in individuals in which intestinal uptake and transfer of iron

is down regulated, i.e. normal healthy indivduals whose only requirements for iron are to replace systemic losses, the values will probably underestimate the bioavilability of iron from food. Such studies are useful for comparing and ranking iron bioavailability from various foods but their absolute values cannot be translated to their effect on meeting systemic needs for iron. Additionally single meal studies do not allow for intestinal adaptation.

172. The only method of determining iron bioavailability, i.e., the amount that is available for sytstemic utilisation, is to measure the fraction of dietary iron that is absorbed and incorporated into haemoglobin as a functional measure of iron utilisation. Foods or meals are intrinsically or, more commonly, extrinsically tagged with radioisotopes of iron (^{59}Fe and ^{55}Fe) and the percentage of the isotope incorporated into haemoglobin is measured 14 days later (Cook *et al*, 1969). This is based on the assumption that most (80-100%) of the absorbed iron is incorporated into haemoglobin in newly formed erythrocytes (Cook *et al,* 1991). Enriched amounts of naturally occurring low abundance stable isotopic tracers (^{54}Fe, ^{57}Fe, ^{59}Fe) can also be used; as this method avoids the use of ionising radiation it is preferred for vulnerable groups such as pregnant women and children.

173. A technique that has been used to measure iron absorption is whole body counting in which foods or meals labelled with a radioisotope of iron are consumed and the fraction of radioactivity remaining in the body is measured after 14 days using a whole-body counter. This approach actually measures iron retention but as there is no usual means of iron excretion, iron retention can be assumed to closely match iron absorption. Iron absorption has also been calculated as the difference between oral iron intake and faecal excretion of an isotope. This method requires a study period of sufficient duration to ensure the complete faecal loss of iron trapped in the gut mucosa otherwise it only measures the luminal disappearance of iron.

174. The influence of systemic iron needs on iron absorption means that absorption values of individuals in studies can only really be compared if allowance is made for inter-individual variability. Three methods have been developed to enable a standardised approach:

- Use of a reference dose of inorganic iron which is compared to the source of iron under investigation (Layrisse *et al*, 1969); results for each individual are presented as ratios.
- Measurement of absorption from a reference dose of inorganic iron and correction of food absorption data to a mean reference value of 40% by multiplying by 40/R, where R is the reference dose absorption. The reference value of 40% represents the amount of iron which corresponds to absorption by subjects with borderline iron stores (Magnusson *et al*, 1981).
- Correction of dietary absorption data to a value corresponding to low levels of iron stores, i.e. serum ferritin concentration of 40µg/L (Cook *et* al, 1991) or 30µg/L (Reddy *et al*, 2000).

DIETARY FACTORS INFLUENCING IRON ABSORPTION

Human studies of iron absorption based on single meals

175. Although single meal absorption studies can control for all confounding variables and provide a robust method for ranking the relative absorption and evaluating dose

response, they cannot be used with confidence to predict the efficiency of iron absorption from foods because they do not take account of adaptive, absorptive, and homeostatic responses that might occur if the foods or diets were consumed over a more extensive time period.

Enhancers of iron absorption

176. The most widely researched enhancers of non-haem iron absorption are meat and ascorbic acid (vitamin C).

Meat

177. Meat and fish contain haem iron, which is relatively well absorbed (20-30%); they also enhance non-haem iron absorption from foods consumed at the same time (Cook and Monsen, 1976; Hallberg & Rossander, 1984; Layrisse *et al*, 1968; Martinez-Torrez *et al*, 1971) in a dose-dependent manner (Baech *et al*, 2003; Cook and Monsen, 1975; Layrisse *et al*, 1984). Hallberg and Rossander (1984) found a 2.5 fold increase ($p<0.01$) in non-haem iron absorption after addition of meat (75g) to a meal of maize, rice, and black beans.

178. It has been suggested that meat enhances absorption of non-haem iron only when ingested with notably inhibitory foods (Bjorn-Rasmussen and Hallberg, 1979). Hurrell (1988) reported that addition of beef (92g) to maize porridge significantly increased non-haem iron absorption threefold but had no effect on a bread meal made from wheat flour. Maize has a greater inhibitory effect on non-haem iron absorption than wheat and it has been estimated that the percentage of non-haem iron absorption from wheat meals is six times that from maize meals (International Nutritional Anaemia Consultative Group, 1982).

179. The mechanism for the enhancing effect of meat on non-haem iron absorption is unknown.

Ascorbic acid

180. The primary mechanism for the enhancing effect of ascorbic acid on iron absorption is thought to be by formation of a more soluble complex than iron alone and by its ability to reduce Fe (3+) to Fe (2+), the form in which iron is taken up into the mucosal cells (Plug *et al,* 1984).

181. Cook and Monsen (1977) reported that non-haem iron absorption from a semisynthetic meal increased in a dose-dependent manner over a range of 25-1000 mg of added ascorbic acid. A review of 24 studies (Bendich & Cohen, 1990) reported a clear dose-response effect of ascorbic acid on non-haem iron absorption which appeared to level off at doses above 500 mg, however the authors noted that the sample size at high doses of ascorbic acid was too small to reach firm conclusions.

182. The influence of ascorbic acid on increasing non-haem iron absorption is reported to be most effective with meals containing high levels of phytates and polyphenols which are inhibitors of iron absorption (Hallberg *et al,* 1989; Siegenberg *et al*, 1991) (see paragraphs 185-192).

183. The effects of ascorbic acid and meat in facilitating non-haem iron absorption are not additive. Cook & Monsen (1977) reported that addition of ascorbic acid (100g) to a

meal containing beef increased absorption 1.67-fold compared to a 4-fold increase observed when the same amount was added to a meal without beef.

184. Erythorbic acid, a stereoisomer of ascorbic acid used as an antioxidant in processed foods, is a stronger enhancer of non-haem iron absorption than ascorbic acid (Fidler *et al,* 2004). Other organic acids occurring naturally in fruit and vegetables, including malic, citric, and tartaric acid, have also been shown to increase non-haem iron absorption (Gillooly *et al,* 1983; Ballot *et al*, 1987). Lactic acid, which is produced during the brewing process, is one of the factors responsible for the high absorption of iron from maize and sorghum beers in southern Africa (Derman *et al*, 1980).

Inhibitors of iron absorption

Phytic acid

185. Phytic acid (myoinositol hexaphosphate and some lesser phosphorylated forms of myoinositol), found in whole grain cereals, legumes, nuts and seeds, has been shown to be a dose-dependent inhibitor of non-haem iron absorption (Hallberg *et al* 1989). Addition of 2, 25, and 250 mg doses of phytate phosphorus (phytate P) to phytate-free bread rolls significantly reduced iron absorption by 18%, 64%, and 82% respectively (Hallberg *et al*, 1989).

186. Many Western-style meals contain 10-100 mg phytate P and vegetarian meals can contain 250 mg of phytate P. An 80 g wheat roll made with 70% extraction flour contains 30mg phytate P and a roll made with 80% extraction flour contains 60 mg phytate P.

187. The inhibitory effects of phytates on iron absorption can be ameliorated by ascorbic acid (Hallberg *et al* 1989; Siegenberg *et al*, 1991). Hallberg *et al* (1989) estimated that approximately 80 mg of ascorbic acid (more than is usually contained in a 100g portion of fruit) would be needed to counteract the inhibitory effect of 25 mg of phytate P and that very large amounts (several hundred mg) would be required to overcome the inhibitory effects of high phytate diets (250 mg phytate P). Siegenberg *et al* (1991) reported that 30 mg of ascorbic acid was required to overcome the inhibitory effects of 58 mg of phytate P contained in a bread roll made of maize bran.

188. Meat has also been shown to ameliorate the inhibitory effect of phytate (Hallberg *et al*, 1989; Baech *et al,* 2003). Addition of 75 g of beef to a meal containing inhibitors of iron absorption increased absorption of non-haem iron 2.5 fold (Hallberg *et al*, 1989). Baech *et al* (2003) reported no effect of 25 g of pork added to a meal containing 62 mg of phytate P, however addition of 50 g and 75 g of pork significantly increased absorption 1.4 fold and 1.6 fold respectively.

Soy protein

189. Soy protein products have been reported to inhibit non-haem iron absorption (Cook *et al*, 1981). Soy protein products contain high levels of phytate which is an important inhibitor of iron absorption (see paragraphs 185-187). However, phytate-free soy proteins have also been shown to inhibit iron absorption. Hurrell et al (1992) examined the effect of soy protein isolates on non-haem iron absorption from a liquid meal to which either egg white (control) or soy protein isolate was added. Reduction of the phytate content of the soy isolate from its native amount (4.9-8.4 mg/g isolate) to 0.2 mg/g resulted in a significant 2.3-fold increase in iron absorption. After complete removal of the phytate from the soy protein isolate, iron absorption was

approximately 55% of the absorption from the egg white control meal, suggesting that soy protein itself is inhibitory.

190. Lynch *et al* (1994) demonstrated that a protein related moiety in soy protein isolate, had an inhibitory effect on non-haem iron absorption independently of phytate. After dephytinisation of the two main protein fractions in soy beans, conglycinin and glycinin, the glycinin fraction was only inhibitory in the presence of phytate; however, the phytate free conglycinin fraction had an inhibitory effect similar to that of phytate suggesting that conglycinin fraction in soy protein inhibits iron absorption.

Polyphenols

191. Polyphenols, found particularly in tea (Disler *et al,* 1975) and coffee (Morck *et al*, 1983), have a dose-dependent effect on non-haem iron absorption**,** with coffee having approximately half the inhibitory effect of tea. Other beverages such as red wine (Bezwoda *et al*, 1985), cocoa (Gillooly *et al*, 1984) and herb teas (Hurrell *et al*, 1999) have also been shown to inhibit non-haem iron absorption. Black tea polyphenols are more powerful inhibitors than those from herb teas, cocoa, or wine, possibly due to higher levels of galloyl esters (Hurrell *et al* 1999). Hurrell *et al* (1999) reported that 20-50 mg total polyphenols reduced non-haem iron absorption from a bread meal by 50-70%, while 100-400 mg total polyphenols (equivalent to one cup of tea or instant coffee) reduced non-haem iron absorption by 60-90%.

192. Although polyphenols bind strongly to protein, the addition of milk to black tea or coffee does not reduce their inhibitory effect (Hurrell *et al* 1999). Ascorbic acid has been shown to counteract the inhibitory effects of the polyphenol tannin on non-haem iron absorption: 50 mg of ascorbic acid was required to overcome the effects of >100 mg tannic acid (Siegenberg *et al,* 1991).

Calcium

193. Both supplemental and dietary calcium has been reported to reduce absorption of non-haem iron (Monsen & Cook, 1976; Deehr *et al*, 1990; Hallberg *et al,* 1991; Gleerup *et al,* 1995; Minihane & Fairweather-Tait, 1998) and haem iron (Hallberg *et al,* 1993). The mechanism for the inhibitory effect of calcium on iron absorption is unknown. It has been suggested that calcium and iron may compete for binding with one or more substances that are important in the absorption pathway (Hallberg *et al*, 1991).

194. Hallberg *et al* (1991) reported that addition of calcium (40-600 mg) to wheat rolls significantly reduced iron absorption; the extent of inhibition was clearly dose related up to levels of 300 mg. The inhibiting effect was greater when calcium was added to the dough as it reduced phytate degradation during fermentation and baking. Addition of 40 mg of calcium to dough reduced iron absorption by 40% and 300-600mg of calcium reduced absorption by approximately 75-80%. When calcium was added after baking, 40 mg of calcium did not inhibit iron absorption; however addition of increasing amounts of calcium, up to 300 mg, inhibited iron absorption by approximately 60%. The decrease in iron absorption between 300-600 mg was not statistically significant. Consumption of milk or cheese (equivalent to 165mg calcium) with the rolls, reduced iron absorption by 57% and 46% respectively.

195. The inhibiting effect of calcium appears to be more pronounced in simple meals (such as bread rolls) compared to more complex meals. Galan *et al* (1991) found no

difference in iron absorption (haem or non-haem) from a typical French meal (comprising meat, vegetables, potatoes, cheese, bread, fruit) when it was consumed with or without 150 ml of milk or yoghurt. The calcium content of the basal meal was 320 mg, so maximal inhibition of iron absorption may already have occurred prior to the addition of the milk or yoghurt. However, iron absorption from a meal low in calcium (hamburger, string beans, potatoes) was not reduced when it was consumed with milk (250 ml) (Hallberg & Rossander, 1982).

Studies of iron absorption based on the whole diet

196. A number of studies have suggested that single meal studies overestimate or underestimate the magnitude of the effect of dietary modulators on non-haem iron absorption. This could be because absorptive efficiency is maximised after an overnight fast, effects of key modulators may be diluted substantially when they are consumed with other foods as part of the whole diet, and the intestinal setting for uptake and transfer of iron has time to adapt to the change of diet over longer periods.

197. Tidehag *et al* (1996) compared non-haem iron absorption from a morning meal with that from all three meals of the day for two consecutive days (n=10; 34-65y). Meals were extrinsically labelled and iron absorption was determined from whole-body counting. On a low-fibre diet, absorption was approximately 78% greater from the morning meals than from all meals during the two-day period. With a high-fibre diet, absorption from the morning meals was 48% more than the average from all meals but this difference was not significant.

198. Cook *et al* (1991) compared the bioavailability of non-haem iron over a two-week period with that from single meals. Extrinsically tagged bread rolls were consumed with each meal for 14 days and bioavailability was measured by erythrocyte incorporation of radioactivity. Participants (n=45; 21-40y) modified their diets to either maximally enhance or inhibit absorption of non-haem iron. Mean iron bioavailability from a diet modified to maximise iron absorption was 2.5 fold higher than from a diet of very low bioavailability. When the same diets were compared using single meals, iron absorption was 5 times higher from the meal modified to maximise non-haem iron absorption compared to the meal high in inhibitors of non-haem iron absorption.

199. Hunt and Roughead (2000) compared short-term measurements of iron absorption with longer-term measurements in men (n=31; 32-56y) fed diets containing either high amounts of enhancers of iron absorption or high amounts of inhibitors of iron absorption[9] for 12 weeks. All food consumed during this period were isotopically labelled. Haem and non-haem iron absorption were measured at 0 and 10 weeks by whole-body counting. With time, there was a significant decrease in non-haem iron absorption on the diet high in enhancers of iron absorption and a significant increase in non-haem iron absorption on the diet high in inhibitors of iron absorption. After 10 weeks, a 5-fold difference in non-haem iron absorption between the two diets at the beginning of the study was significantly reduced to a 2-fold difference. There was no significant difference in haem iron absorption from the 2 diets or any adaptation in absorption with time.

[9] Meals contained different amounts of meat, ascorbic acid, whole grains, legumes, and tea.

200. Cook and Reddy (2001) evaluated the effect of ascorbic acid on non-haem iron bioavailability from a complete diet over three 5-day periods. Participants (n=12; 20-38y) consumed extrinsically tagged bread rolls with each meal during the dietary period and non-haem iron bioavailability was determined from erythrocyte incorporation of the radioisotope. Diet was self-selected for the first 5-day period and then altered to maximally increase or decrease ascorbic acid for the second and third dietary periods. Mean ascorbic acid intake was 90 mg/d with the self-selected diet, 247 mg/d with the maximal ascorbic acid diet, and 51 mg/d with the decreased ascorbic acid diet. No significant difference was found in iron bioavailability between the three dietary periods.

201. Reddy *et al* (2006) examined the effect of meat intake on non-haem iron bioavailability over three 5-day dietary periods. Participants (n=14) initially consumed a self-selected diet followed by diets to eliminate or maximally increase intakes of meat and seafood. A radiolabelled wheat roll was consumed with each of 3 meals during the different diets and iron bioavailability was measured by erythrocyte incorporation. Although meat intake was significantly different between the three diets (0 g, no meat; 136 g, self-selected; 222 g, high meat), there were no differences in non-haem iron bioavailability.

202. Variations in calcium intake (280, 684 and 1,281 mg/d), achieved by modifying food selection, did not affect total dietary non-haem iron bioavailability over three 5 day periods (n=14; 19-37y) (Reddy and Cook, 1997). The diet was labelled during each 5-day period by consumption of a radioisotopically tagged bread roll with each meal and iron absorption was measured by erythrocyte incorporation of radioactivity.

203. Grinder-Pedersen (2004) examined the effects of consuming 3 different sources of calcium, with 3 main meals over 4 days, on non-haem iron absorption in women (n=14; age 21-34y) with relatively low iron stores (serum ferritin$\leq$ 40μg/L). The basic diet, which was low in calcium (224 mg/d), was supplemented with either 1 glass of milk (826 mg Ca/d), calcium lactate (802mg Ca/d) or a milk mineral isolate (801 mg Ca/d). Meals were extrinsically labelled with a radioisotope of iron and absorption was determined from whole body retention of radioactivity. There were no significant differences in non-haem iron absorption between the different diets.

Models to predict iron bioavailability

204. Several models have been developed to estimate iron bioavailability from different meals and diets based on the iron status of the individual, the type of iron (haem or non-haem), and that take various account of the presence of enhancers or inhibitors of iron absorption (Monsen *et al*, 1978; Hallberg and Hulthen, 2000; Reddy *et al*, 2000).

205. Prediction equations to estimate iron bioavailability from meals have a number of limitations. They are based on absorption from single meals which may overestimate the effects of enhancers and inhibitors and do not take account of dietary complexity and variability or long-term adaptation to iron absorption.

206. Beard *et al* (2007) compared a number of prediction equations to the change in serum ferritin concentration of women (n=317) taking part in a 9 month feeding trial in the Philippines to assess the efficacy of iron fortified rice. Analysis of 6 equations showed highly significant differences in the predicted efficiency of iron absorption;

none of the equations agreed with dietary iron utilisation based on improvement in serum ferritin concentration.

207. Prediction equations to estimate iron absorption are based on a number of questionable assumptions about iron bioavailability. As a consequence, they are of little use in predicting efficiency of iron absorption.

THE INFLUENCE OF ENHANCERS AND INHIBITORS OF IRON ABSORPTION ON IRON STATUS

Epidemiological studies

208. Evidence on the relationship between dietary modulators of iron absorption and iron status is based mainly on observational data which have a number of limitations.

209. Although epidemiological studies take account of adaptive responses and the complexity of the whole diet, unless good quality dietary information is collected in conjunction with appropriate and sensitive measures of iron status, correlations between dietary constituents and iron status can be misleading. Multiple days of dietary records are required to obtain accurate estimates of habitual iron intake; 12 days provide a better representation of individual iron intake (Bingham, 1987). A questionnaire based on food frequency can also be used (Heath *et al*, 2005). Most studies have used dietary records of less than 12 days or questionnaires not specifically validated for iron intake. Additionally, there are only limited food composition data for some modifiers of iron absorption such as phytate and polyphenols.

210. A measurable effect of dietary enhancers and inhibitors may only be observed in individuals with low iron stores and, as a consequence, elevated absorptive capacity and not in populations where the majority of the population is iron replete (Garry *et al,* 2000). The relationship between iron intake and iron status is also complicated by a number of confounding factors which influence iron absorption, such as age, homeostatic metabolic responses, menstrual losses (in women), genetic influences (e.g., HFE and other polymorphisms).

211. Indices of iron status, such as serum ferritin and haemoglobin concentration, are also affected by other factors (Bates *et al*, 1997). Serum ferritin concentrations are raised when haemoglobin synthesis is inhibited, by the acute phase reaction in response to infection and inflammation, and in liver damage. Concentrations of haemoglobin can be a reflection of low vitamin B_{12} or folic acid intakes, haemoglobinopathies, and a variety of other diseases. Additionally, most studies have collected only 1 blood sample, which does not take account of day-to-day variability in iron status measurements.

Cross-sectional studies

212. Numerous cross-sectional studies have examined the association between total dietary iron intake and dietary modulators of iron absorption on biochemical markers of iron status (mainly serum ferritin and haemoglobin concentration). These studies suffer from a number of limitations including narrow range of exposures, small sample sizes, inadequate dietary assessment methods, and variability in the allowance made for other factors that affect iron status (e.g., most studies did not

measure markers of inflammation/infection which are also associated with increased serum ferritin concentration).

213. Most cross-sectional studies have reported better iron status with increased meat intake (Worthington-Roberts *et al*, 1988; Leggett *et al,* 1990; Fleming *et al*, 1998; Galan *et al*, 1998; Gibson *et al*, 1999; Heath *et al*, 2001; Milman *et al*, 2004;) and haem iron intake (Davis *et al*, 1992; Preziosi *et al*, 1994; Fleming *et al*, 1998, Galan *et al*, 1998) although some studies found no association (Fogelholm *et al*, 1993; Garry *et al*, 2000; Cowin *et al*, 2000).

214. Findings from cross-sectional studies assessing the effects of total iron intake, ascorbic acid, calcium, and polyphenols, have been inconsistent. Most did not find an association between markers of iron status and phytate consumption.

Prospective studies

215. A limited number of prospective studies have investigated the association between dietary factors and iron status. For details of the prospective studies considered in this report, including iron status of participants, dietary assessment methods, and allowance made for other factors affecting iron status, see Annex 2 (Table 5.1).

216. A prospective study in Costa Rica (Munoz *et al*, 1988) investigated the association between coffee consumption and iron deficiency anaemia (haemoglobin <110g/L, serum ferritin <10μg/L) among low-income women (n=48; 17-30y) during late pregnancy/early lactation. All women were receiving iron supplements (60 mg/d elemental Fe) by 6 months of pregnancy. There were no differences between coffee drinkers (>450 ml/d; ≥10g ground coffee/d) and non-drinkers (0 ml/d) in consumption of energy, protein, ascorbic acid, iron, red meat, and dark green vegetables. In multivariate analysis, haemoglobin concentrations were significantly lower in coffee drinkers at 8m gestation ($p<0.001$) and at 1 month post partum (p not provided); breast milk iron concentration was also significantly lower in the coffee drinkers group at 1 month post partum. Infants whose mothers consumed coffee had significantly lower birth weight ($p<0.001$) and haemoglobin concentration ($p<0.05$) at 1m of age; this association was independent of maternal iron status and birth weight. There were no differences in maternal and infant serum ferritin concentrations between the two groups.

217. A 10 year longitudinal study in the USA (n=125; 60-93y) investigated the influence of haem and non-haem iron intake on body iron stores (ferritin bound iron) (Garry *et al*, 2000) which were estimated from serum ferritin concentrations (Cook *et al,* 1986[10]). No significant association was found between dietary iron intake (haem or non-haem) and estimated iron stores; however, supplemental iron intake was significantly associated ($p=0.04$) with increased iron stores (estimated body ferritin was 64 mg greater for individuals consuming more than 18 mg/d of supplemental iron compared to those not consuming iron supplements).

218. Backstrand *et al* (2002) investigated the association of usual diet with haemoglobin and serum ferritin concentrations in a cohort of women (n=125; 16-44y) in Mexico. Higher plasma ferritin concentrations were significantly associated with increasing

[10] For participants with SF concentration >12μg/L, the following equation is used: iron stores (mg) = 400 x (*ln SF* – ln12), where 400 id the proportionality constant, ln is the natural logarithm and SF is serum ferritin. SF concentrations of all participants in the study were >12μg/L.

intakes of non-haem iron (p=0.003) and ascorbic acid (p=0.0395). Risk of low plasma ferritin concentration (<15 µg/L) was not associated with intake of non-haem iron (OR, 0.92; 95% CI, 0.83-1.01; p=0.09), haem iron (OR, 1.26; 95% CI, 0.77-2.05; p=0.36) or ascorbic acid (OR, 0.98; 95% CI, 0.94-1.00; p=0.13). No association was found between risk of low haemoglobin concentration (<130 g/L) and intakes of non-haem iron (OR, 0.95; 95% CI, 0.86-1.05; p=0.3) and haem iron (OR, 1.21; 95% CI, 0.74-1.98; p=0.45), however each additional mg of ascorbic acid was associated with a 7% reduction in risk of low haemoglobin concentration (OR, 0.93; 95% CI, 0.89-0.97; p=0.0009).

219. Liu *et al* (2003) examined dietary variables affecting serum ferritin concentrations of postmenopausal women in the USA followed for 10 years (n=620; 44-69y). Higher serum ferritin concentrations were significantly associated with increasing intakes of haem iron (mainly from red meat) (p trend = 0.01) and iron supplements (p trend=0.02). Dietary intakes of non-haem iron, calcium, vitamin C and coffee were not associated with serum ferritin concentrations.

220. A study in Sweden (Öhlund *et al*, 2008) evaluated factors affecting haemoglobin and serum ferritin concentrations in children (n=127) aged 6-12m who were followed up to age 4y. Haemoglobin concentration at 4y was significantly related to previous haemoglobin concentration (at 6, 12, 18m) and mothers' haemoglobin concentration (measured when child was 4y). No associations were found between haemoglobin concentration and mean daily intake of iron, meat, ascorbic acid, calcium, or dairy products. A significant association was found between serum ferritin concentration and meat intake in boys only (p=0.015).

Intervention studies

221. Further details of the studies considered including baseline serum ferritin and haemoglobin concentrations are provided in Annex 2 (Table 5.2).

Meat

222. Lyle *et al* (1992) compared the effects of supplemental iron intake with increased meat consumption on serum ferritin and haemoglobin concentrations of previously sedentary women in the USA (n=60) participating in a 12-week programme of moderate aerobic exercise (30 mins, 3 days/wk). Participants were assigned to one of five groups: 50 mg/d Fe supplement and a diet containing low food-iron; 10 mg Fe supplement and low food iron diet ; placebo; high food iron diet (mainly from meat) and meat supplements; and a control group with a free choice diet and no exercise. Mean dietary intakes of total iron for the groups (mg/d) were 57.8, 17.5, 8.8, 11.8, and 8.0 respectively. There were group differences in serum ferritin concentration at baseline.

223. After 12 weeks the haemoglobin concentration of the high food iron and meat supplement group was significantly higher than for all other groups (p<0.009). The serum ferritin concentrations of the high food iron/meat supplement group and the 50 mg/d Fe supplement group were significantly higher (p<0.02) than that of the control group; serum ferritin concentrations of the high food iron/meat supplement group were also significantly higher (p=0.04) than those of the placebo group.

224. Engelmann *et al* (1998) examined the effect of increased meat intake on haemoglobin and serum ferritin concentrations of partially breast fed infants in

Denmark (n=41; aged 8m). Infants were randomised to either a low meat group (10 g/d of meat) or a high meat group (27 g/d of meat) for 2 months. There were no significant differences in total iron intake between the two groups as infants in the low meat group consumed more commercial gruel which is fortified with iron. At the end of the intervention period there was a significant difference (p=0.008) in the change in haemoglobin concentration which decreased by 4.9 g/L (range -12.9-5.6 g/L) in the low meat group and by 0.6 g/L (-12.1-7.3 g/L) in the high meat group. There was no significant difference in change in serum ferritin concentration for both groups.

225. In the USA, Hunt and Roughead (1999) compared the effect of consuming a lacto-ovovegetarian (0 g/d meat) or non-vegetarian diet (184 g/d meat) for 8 weeks on serum ferritin concentrations of women (n=21; 20-42y) in a random crossover design. After 8 weeks, the type of diet was found to have no effect on serum ferritin concentrations.

226. Wells *et al* (2003) compared the effect of vegetarian and beef containing diets on serum ferritin and haemoglobin concentration of men (n=21; 59-78y) undergoing resistance training (3 days/wk) in the USA. All participants consumed a vegetarian diet supplemented with textured vegetable protein (0.6 g protein/kg body weight/day) for 2 weeks before being assigned to a beef containing or vegetarian diet for 12 weeks. In addition to the self selected vegetarian diet, the beef group received 0.6 g protein/kg body weight/day of beef products and the vegetarian group received the equivalent amount as texturized vegetable protein. After 12 weeks serum ferritin concentrations significantly decreased in both groups ($p<0.01$). There was a significant increase ($p<0.01$) in haemoglobin concentrations of the beef group compared to the vegetarian group, which remained stable.

227. Another study in the USA (Snetselaar *et al,* 2004) compared serum ferritin concentrations of adolescents (n=86; age not specified) randomised to an eating pattern emphasising either beef or poultry and fish for 3 months. Participants were asked to consume the study meat 5 times/wk and the comparison meat no more than twice/wk. After 3 months, the beef group increased their mean consumption of beef by 21 g/d (to 66 g/d) and decreased their mean poultry/fish intake by 13 g/d (to 18 g). The poultry/fish group increased their mean consumption of poultry/fish by 8g/d (to 50g) and decreased their mean beef intake by 4 g (to 36 g/d). There were significant differences between groups in the amount of beef and poultry/fish consumed. After 3 months there was a significant difference between groups in serum ferritin concentration which was unchanged for the beef group and decreased for the poultry/fish group.

228. In Denmark, Tetens *et al* (2007) compared the impact of a meat or vegetable based diet for 20 weeks on the serum ferritin and haemoglobin concentrations of women (n=57; 19-39y) with low iron status (defined as serum ferritin ≤30 µg/L; haemoglobin ≥120 g/L). Participants allocated to the meat based diet consumed their usual diet as well as 150 g/d of meat; participants in the vegetable based diet group consumed vegetable products with energy and iron content similar to that of the meat products and were instructed to consume no more than 250 g of meat/week. At the end of the intervention period, serum ferritin and haemoglobin concentrations were unchanged in the meat based diet group; there was a significant decline in serum ferritin concentrations ($p<0.001$) and haemoglobin concentrations ($p=0.003$) in the vegetable based diet group.

229. A 7-week controlled feeding study in the USA (Hunt *et al,* 1995) which compared the effect of a high meat (289 g/d) and low meat (38.5g/d) diet on haemoglobin and serum ferritin concentrations of postmenopausal women (n=14; 51-70y) reported a significant decrease in serum ferritin concentrations with the high meat diet; haemoglobin concentrations were unaffected by either diet.

Ascorbic acid

230. Cook *et al* (1984) reported no change in serum ferritin concentrations of adults (n=17; 20-30y) in the USA, when meals were supplemented with 2g of ascorbic acid/day (1g with each of 2 meals) for 16 weeks. The lack of effect was not due to adaptation to the ascorbic acid as enhancement of iron absorption by ascorbic acid from single test meals was observed at the beginning and end of the 16-week period. There was also no significant effect on serum ferritin concentrations of 4 iron deficient subjects (mean serum ferritin concentration < 6 µg/L) who continued to receive ascorbic acid for a further 20 months.

231. In a double blind placebo controlled trial in Ireland (Malone *et al*, 1986), healthy women (n=58; 17-21y) were supplemented with either 300 mg/d of ascorbic acid (100mg, 3 times/day with each meal) or placebo for 8 weeks. At the end of the supplementation period serum ferritin concentrations were unchanged in both groups.

232. In a study by Hunt *et al* in the USA (1990), premenopausal women (n=11; 22-36y) with low to moderate serum ferritin concentrations (8.5-55 µg/L) were depleted of iron stores by consuming a low iron diet and undergoing phlebotomy until serum ferritin concentrations were less than 8.5 µg/L. Participants then consumed a low iron bioavailability diet (minimal meat and ascorbic acid, containing 13.7mg of iron/2000kcal) which was supplemented with 1500 mg/d ascorbic acid (500 mg, 3 times/d with each meal) or placebo, for 5.5 weeks in a controlled metabolic environment. At the end of the supplementation period, there was a significant improvement ($p<0.05$) in haemoglobin concentration of the ascorbic acid supplemented group; serum ferritin concentrations were unchanged.

233. In another study by Hunt *et al* (1994), pre-menopausal women (n=25; 20-45y) with low serum ferritin concentrations (3.5-17.7 µg/L) consumed either a diet with low iron availability or a typical western diet for 10 weeks which was supplemented with ascorbic acid (500 mg, 3 times/d) or placebo for 5 out of 10 weeks using a double-blind crossover design. Participants were provided with all meals during the study. Ascorbic acid supplementation had no effect on serum ferritin concentration in either dietary group, however serum ferritin concentrations were slightly higher with ascorbic acid ($p<0.06$) when data from both diets were combined.

234. In a placebo controlled intervention trial in Mexico (Garcia *et al,* 2003) women (n=36; mean age 28y) with serum ferritin concentrations <12µg/L consumed either 50 mg ascorbic acid (as limeade containing 25 mg with each of two meals a day) or placebo, 6 days/wk, for 8 months. Stable isotope studies over 14 days had previously shown that this treatment more than doubled iron absorption of women with serum ferritin concentrations <12 µg/L (Diaz *et al*, 2003). After 8 months, no difference was found between the two groups in haemoglobin or serum ferritin concentrations.

Calcium

235. Sokoll and Dawson-Hughes (1992) investigated the effect of calcium supplements (1000 mg/d) on serum ferritin and haemoglobin concentrations of premenopausal women (n=109; 18-52y) in the USA. Two tablets containing 250 mg calcium were consumed with each of 2 meals/d for 12 weeks. Women in the control group were not given placebo tablets. At the end of the supplementation period, there were no significant differences in serum ferritin or haemoglobin concentrations for the treatment or control groups.

236. A randomised double-blind intervention trial (Ilich Ernst *et al*, 1998) in the USA examined the effect of calcium supplementation (1000 mg/d) over 4 years on serum ferritin concentration of girls (n=354) who were premenarcheal (8-13y) at baseline. Calcium or placebo tablets were taken after breakfast and before bedtime. No significant difference was found in serum ferritin concentration between the placebo and intervention group at 0, 1, 2, 3, and 4 years.

237. Kalkwarf and Harrast (1998) examined the effect of 6 months of calcium supplementation on serum ferritin concentrations in lactating and non-lactating women (n=187; mean age 31y) in the 6-12m postpartum period. Half the women in each feeding group were randomly assigned to either 1000 mg/d calcium carbonate or a placebo, in a double-blind design. The calcium supplements (two 500 mg tablets) or placebo were consumed with two separate meals for a period of 6 months. There were no significant differences between the 4 groups in baseline haemoglobin concentrations however baseline serum ferritin concentrations were significantly higher (p=0.044) in the lactating women (44.0 μg/L) compared to nonlactating women (34.5 μg/L). (Postpartum iron stores of lactating women are usually higher than those of nonlactating women because of the delayed return of menstruation.) At the end of the intervention, there was no significant effect of calcium supplementation on serum ferritin or haemoglobin concentrations in either group.

238. A study in the UK investigated effects of calcium supplementation (1200 mg/d) for 6 months, on serum ferritin and haemoglobin concentrations of adults (n=24; mean age 43y) with serum ferritin concentrations >12μg/L (Minihane and Fairweather-Tait, 1998). Participants consumed 400 mg of calcium with each of 3 meals daily. The control group did not receive any dietary intervention. After 6 months, there was no significant effect of calcium supplementation on serum ferritin or haemoglobin concentrations.

239. A review examining the effect of calcium supplementation on the iron status of women (Bendich, 2001) concluded that long term consumption of calcium supplements has no effect on various indicators of iron status, including serum ferritin concentration.

Phytate

240. Lind *et al* (2003) examined the effect of reducing the phytate content of infant cereals on serum ferritin and haemoglobin concentrations of infants (n=267; aged 6 m). In a double-blind design, infants (n=267) were randomly allocated to 3 cereal groups containing different amounts[11] of phytate for 6 months: a commercial milk-based cereal drink (MCD) and porridge (CC group), phytate reduced MCD and phytate-

[11] Mean daily intake of phytate for the different diets was: at 6-8m, 124 μmol/d on the CC diet, 48 μmol/d on the PR diet and 26 μmol/d on the IF diet 48 μmol/d; at 9-11m, 189 μmol/d on the CC diet, 36 μmol/d on the PR diet and 62 μmol/d on the IF diet 48 μmol/d.

reduced porridge (PR group); or milk based infant formula and porridge (IF group). After 6 months, serum ferritin concentrations were significantly lower ($p<0.05$) in all the groups compared to baseline but there were no differences in serum ferritin concentration between the three groups; haemoglobin concentration was significantly lower ($p<0.05$) in the CC and PR groups compared to baseline and significantly lower ($p=0.015$) in the IF group than the PR group.

241. Bach Kristensen *et al* (2005) examined the effects of long term (4 months) consumption of fibre rich wheat bread (300 g), prepared with and without phytase, on serum ferritin and haemoglobin concentrations of women (n=41; 19-37y). The molar ratio of phytic acid to iron was 8.5:1 in the wheat bread and 6.7:1 in the wheat + phytase bread. At the end of the intervention period serum ferritin concentrations were significantly decreased ($p<0.001$) in both groups but there was no difference between groups; haemoglobin concentrations were unchanged in both groups at the end of the intervention period.

Limitations of intervention studies

242. Overall, short-term or long-term intervention studies of modulators of dietary iron absorption have not shown corresponding increases in haemoglobin or serum ferritin concentrations.

243. Most of the intervention studies had small sample sizes (n=11-354) and may not have had sufficient power to detect an association.

244. The systemic need for iron is the most important determinant of iron absorption from the diet. Most studies were carried out in iron replete western populations who are unlikely to have a physiological response to additional iron in the diet. Particpants may already have been consuming diets containing promoters of non-haem iron absorption which would minimise any further effects.

245. Although beneficial effects of enhancers and inhibitors of iron absorption on markers of iron status might be expected in studies with iron deficienct individuals, the studies which included menstruating women, who tend to have low iron stores due to blood losses, and the study in Mexico of women with low serum ferritin concentration (mean=6 µg/L) also showed no beneficial effects of modulators of non-haem iron absorption on markers of iron status.

Fortification iron

246. Fortification of foods with iron (addition of iron to foods) has been the main approach used to improve the supply of iron in the diet. Iron has also been added to foods to replace iron lost during processing (restoration) and to ensure nutritional equivalence of products replacing common foods in the diet (e.g. meat substitutes).

247. The most common food vehicles used for iron fortification are staples such as wheat flour; in developing countries, condiments such as salt, sugar, fish sauce, soy sauce, and curry powder have also been used.

248. Iron is lost during the processing of wheat flour. In the UK, mandatory addition of iron to white and brown flour was introduced in 1953 to restore it to the level found in 80% extraction flour. The Bread and Flour Regulations (1998) require all flour other than wholemeal flour to contain not less than 1.65 mg iron/100 g flour, regardless of

intended use. The forms of iron used for fortification of flour include ferric ammonium citrate, ferrous sulphate and iron powder.

249. In the UK, many breakfast cereals are fortified on a voluntary basis with levels usually in the range of 70-120 mg/kg (EVM, 2003). Other foods fortified with iron on a voluntary basis include cereal bars, beverages, infant formulas, and some weaning foods. Information on the contribution of fortificant iron to iron intakes in the UK, can be found in section 9.

Bioavailability of iron fortification compounds

250. Four groups of iron compounds are used for fortification: freely water-soluble (ferrous sulphate); poorly water-soluble, but soluble in dilute acids such as gastric juice (ferrous fumarate); water-insoluble and poorly soluble in dilute acid (ferric pyrophosphate, elemental iron powders); and protected iron compounds (NaFeEDTA) (Hurrell, 1997).

251. Iron fortificants differ in their relative bioavailability and their potential to cause unfavourable sensory changes such as discoloration of the food vehicle and rancidity. The water-soluble compounds are the most bioavailable. Compounds which are water-insoluble but soluble in acid are also well absorbed as they dissolve in gastric juice. Elemental iron powders and iron phosphate compounds, which are water-insoluble and poorly absorbed in dilute acid, have the lowest and most variable bioavailability (Hurrell, 2002).

252. Elemental iron powders (electrolytic, hydrogen/carbon monoxide reduced, carbonyl) are most widely used for fortification of cereal products as they do not cause organoleptic changes, which increase their consumer acceptance and shelf life. Hydrogen reduced elemental iron is used to fortify flour in the UK and is also added to breakfast cereals and other fortified food products. However, lack of evidence about the effectiveness of iron fortification of flour on iron status in the UK led to a recommendation by COMA in 1981 that fortification of flour with iron should be discontinued (DH, 1981). Studies which have investigated the effect of iron fortification on markers of iron status are considered in paragraphs 254-255 below.

253. The WHO/FAO (2006) recommend that the order of priority for iron compounds used for fortification should be ferrous sulphate, ferrous fumarate, encapsulated ferrous sulphate/fumarate, electrolytic iron at twice the dose of ferrous sulphate/fumarate, ferric pyrophosphate at twice the dose of ferrous sulphate/fumarate; and NaFeEDTA for high phytate cereal flours.

Effect of iron fortification on iron status

Efficacy/effectiveness trials of iron fortification

254. The majority of trials which have examined the effect of consumption of iron fortified foods on markers of iron status were conducted in developing countries. Results from these trials suggest that increasing consumption of foods fortified with water-soluble iron compounds such as ferrous sulphate (Zimmermann *et al*, 2003 & 2005; Sun *et al*, 2007), or iron chelators such as NaFeEDTA (Huo *et al*, 2002; Zhao *et al* 2004; Chen *et al*, 2005), is associated with an improvement in markers of iron status.

255. A number of trials have examined the efficacy of elemental iron powders, which are most widely used to fortify flour in national fortification programmes, and have also compared their efficacy with other iron compounds. Three studies (Walter *et al*, 1993; Zimmermann *et al*, 2005; Sun *et al*, 2007) reported an improvement in iron status following consumption of foods fortified with elemental iron powder, while 4 trials reported no effect (Nestel *et al*, 2004; van Stuijvenberg *et al*, 2006; Andang'o *et al*, 2007; van Stuijvenberg *et al*, 2008).

Efficacy of country-wide iron fortification strategies

256. Although iron fortification of cereal flour (wheat and maize) and other food products is practised in several countries as a strategy to combat iron deficiency, there is limited evidence of a beneficial effect on iron status at a population level. No large scale fortification programmes have formally evaluated their impact on iron status.

257. In Denmark, flour was fortified with carbonyl iron (30 mg/kg flour) from 1954 until 1987, when mandatory fortification was discontinued. A study which compared serum ferritin concentrations of a cohort of men and women (n=238, aged 35-65y) in 1987/88 and 6 years later (1993/94), reported a significant increase in postmenopausal women and an increasing trend over time for men (p=0.07) (Osler *et al*, 1999). A population survey of Danish women in 1994 (n=1319; age 40-70y) and 1984 (n=880; age 30-60y) found no difference in the prevalence of iron deficiency (serum ferritin<16μg/L) and iron deficiency anaemia (serum ferritin <13μg/L & haemoglobin <121g/L) (Milman *et al*, 2000). The prevalence of iron deficiency (serum ferritin, 16μg/L) and iron deficiency anaemia (serum ferritin<13μg/L & haemoglobin<129g/L) in 1994 (n=1332; age 40-70y) compared with 1984 (n=1044; age 30-60y) was also unchanged for men (Milman, 2004).

258. In Venezuela, mandatory fortification of corn flour with ferrous fumarate (50 mg/kg flour) and voluntary fortification of wheat flour (20 mg ferrous fumarate/kg flour) was introduced in 1993. Comparison of surveys of schoolchildren (aged 7, 11, & 15y) before fortification in 1992 (n=282) and one year after fortification in 1994 (n=317) showed a significant reduction in the prevalence of iron deficiency (serum ferritin<12 μg/L) from 37% to 16% and the prevalence of anaemia (haemoglobin: 115g/L, children 7y ; 120g/L, females 11 & 15y; 125g/L, males 11y; 130g/L, males 15y) from 19% to 9%. There was no significant difference in the prevalence of iron deficiency anaemia (haemoglobin as above and serum ferritin<12 μg/L) between 1992 and 1994 (Layrisse *et al*, 1996). Later surveys, however, in 1997, 1998, and 1999 reported that the prevalence of anaemia increased to pre-fortification levels while serum ferritin concentrations were unchanged (Layrisse *et al*, 2002).

259. In Brazil, fortification of wheat flour with iron (42 mg/kg flour; type of iron not specified) became mandatory in 2004. A study which assessed the impact of iron fortification on haemoglobin concentrations of children under 6y reported no effect at 12 and 24 months post fortification (Assunção *et al*, 2007).

260. The limited impact of iron fortification programmes on markers of iron status may be due to a number of factors including: widespread use of elemental iron powders which are poorly absorbed; insufficient intakes of the fortified food; or inadequate level of fortification. The impact of iron fortification will also depend on the proportion of anaemia in the population that is due to iron deficiency.

Supplements

261. Many non-haem iron supplements are available over the counter from chemists, supermarkets and health food shops. The most common forms are ferrous sulphate, ferrous fumarate, ferrous gluconate, ferrous glycine sulphate and iron polysaccharide (Fairweather-Tait & Teucher, 2002). The bioavailability differs but all are generally better absorbed than slow-release capsules or multivitamin/multimineral supplements (Dawson *et al*, 1998).

262. Iron supplements are usually used as a short-term measure to provide extra iron when iron levels are low. Commercially available prophylactic doses used to prevent deficiency usually range between 7-50 mg/day. Supplemental intakes above the Guidance Level of 17mg/day[12] are not advised in the UK (EVM, 2003). During pregnancy, iron supplements are recommended for women with haemoglobin concentrations outside the normal UK range for pregnancy (i.e. 110g/L during the first trimester and 105g/L at 28 weeks) (NICE, 2008).

263. Information on the contribution made by supplements to iron intake in the UK can be found in section 9.

The effect of vegetarian diets on iron status

264. Vegetarian diets contain, almost entirely, non-haem iron and generally higher quantities of inhibitors of iron absorption, such as phytate (associated with legumes, including soy, and whole-grain cereals).

265. Several, mostly small, cross-sectional studies, have compared the iron intake and/or the iron status markers of vegetarians with, otherwise broadly similar, non-vegetarians (see Annex 2, Table 5.3). Results from these studies show that dietary iron intakes of vegetarians are on average similar, or sometimes higher, than those of non-vegetarians. In the European Prospective Investigation of Cancer and Nutrition (EPIC)-Oxford cohort estimated iron intakes among women (n=43 582) were 12.6, 12.8, 12.6, and 14.1 mg/d for meat-eaters, fish-eaters, lacto-ovovegetarians, and vegans respectively (Davey *et al*, 2003).

266. A comparison of the haemoglobin concentrations of vegetarians and non-vegetarians shows that they are similar in the two diet groups, though in several studies mean values are slightly lower in vegetarians. Serum ferritin concentrations are consistently significantly lower in vegetarians compared to non-vegetarians; they are, however, usually within the reference ranges compatible with health.

267. An intervention study (Hunt and Roughead, 1999) compared the effect of consuming a lacto-ovovegetarian or non-vegetarian diet for 8 weeks on serum ferritin concentrations of 21 women (20-42y) in a random crossover design (see previous paragraph 225). Type of diet had no effect on serum ferritin concentrations at the end of each 8-week dietary period.

Summary and conclusions

268. Iron is present in foods as haem or non-haem iron compounds. Haem iron is found mainly in foods of animal origin as haemoglobin and myoglobin. Non-haem iron is

[12] The Guidance level is the amount that would not be expected to cause any adverse effects in the majority of people.

found in animal and plant tissues. Other sources of non-haem iron are foods fortified with iron and supplements.

269. The efficiency of intestinal absorption of iron from food is strongly influenced by systemic iron needs. More iron is absorbed from the diet in a state of iron deficiency and less is absorbed when iron stores are replete. The homeostatic control of non-haem iron is more pronounced than that of haem iron.

270. The bioavailability of dietary iron, i.e., the amount available for systemic utilisation, is affected by the chemical form of iron. The absorption of haem iron from the diet is more efficient than the absorption of non-haem iron.

271. Evidence from single-meal studies suggests that a number of dietary components modulate non-haem iron bioavailability by increasing or reducing the amount of non-haem iron absorbed from the diet. The main enhancers of non-haem iron absorption are meat, and ascorbic acid found in fruit and vegetables; the main inhibitors of non-haem iron absorption are calcium, phytates in cereals and legumes, and phenolic compounds found in tea, coffee, and other beverages.

272. The individual effects of dietary factors which influence iron absorption may be reduced when they are consumed as part of a whole diet. Evidence from whole diet studies, over a number of days or weeks, suggest that the overall effect of enhancers and inhibitors on iron absorption is considerably less than predicted from single meal studies.

273. The effects of dietary modulators of non-haem iron absorption on markers of iron status (usually haemoglobin and serum ferritin concentration) are difficult to ascertain in cross-sectional and prospective studies. An important consideration is the difficulty of obtaining accurate exposure data because of the quality of dietary assessments, limited food composition data for some modifiers of iron absorption, and interactions between enhancers and inhibitors of iron absorption. Observational studies are also affected by a number of confounding factors such as disease which can raise serum ferritin concentrations. Such studies usually assume a direct relationship between markers of iron status and risk of iron deficiency or excess, however markers of iron status are also influenced by homeostatic metabolic responses, menstrual blood losses and genetic polymorphisms.

274. Cross-sectional and longitudinal data suggest that most dietary inhibitors and enhancers of iron absorption do not substantially influence iron status. The only dietary factor consistently associated with iron status in observational studies is meat or haem iron.

275. Long term intervention studies of enhancers and inhibitors of iron absorption have also, overall, not shown a corresponding change in iron status parameters. A measurable effect of dietary modulators may only be observed in individuals with inadequate iron status and as a consequence, higher absorptive capacity. Most intervention studies were carried out in iron replete western populations who are less likely to have a physiological response to additional dietary iron. It is also possible that the lack of effect on serum ferritin concentration is because of relative insensitivity of serum ferritin concentration to changes in iron depots.

276. The lack of effect of enhancers and inhibitors on iron status raises uncertainties regarding the importance of iron bioavailability for meeting iron requirements in the UK and of dietary advice regarding enhancers and inhibitors of iron absorption. With regard to calcium, which has been shown to inhibit iron absorption in single meal studies, dietary advice to reduce consumption would be inappropriate due to its importance in skeletal development and maintenance.

277. The main determinant of the amount of dietary iron absorbed by the body is the systemic need for iron. Absorption of dietary iron will also be influenced by the absolute amount of iron in the diet and the overall composition of the meal rather than a single food item that enhances or inhibits iron absorption.

278. UK and western diets include a broad range of foods containing iron. They also include various modulators of iron absorption which may lead to complex interactions between enhancers and inhibitors of iron absorption. Consequently the bioavailability of dietary iron may have little influence on iron status of the UK population.

279. The effects of enhancers and inhibitors of iron absorption may be more important in developing countries where populations are at greater risk of iron intakes which are insufficient to meet requirements as diets are plant-based, more limited and monotonous, contain higher levels of inhibitors, lower levels of enhancers, and less haem iron. Under these circumstances, the imbalance between requirements and absorption may lead to iron deficiency.

280. In the UK, addition of iron to white and brown wheat flour is mandatory. A number of foods, including breakfast cereals, are fortified with iron on a voluntary basis. The iron compounds used for fortification vary in their relative bioavailability and their potential to cause unfavourable sensory changes to the food vehicle. Elemental iron powders, which have the lowest bioavailability, are widely used for flour fortification programmes because they do not cause organoleptic problems during storage and are relatively inexpensive. Evidence suggests that they may be of little practical use in improving iron status.

281. The inter-relationship between the different factors that affect iron absorption (individual iron requirements, inter-individual variability in absorption; iron intake and dietary type, i.e. haem or non-haem) will influence iron status.

6. CONSEQUENCES OF IRON DEFICIENCY

Physiological consequences of iron deficiency

Metabolic response to iron deficiency

282. Synthesis of haemoglobin and myoglobin is decreased in iron deficiency which results in impaired peripheral distribution of oxygen, and reduced activities of the iron-dependent enzymes (see Table 2.1, section 2). Many metabolic and biochemical processes and pathways, especially oxidative metabolism, are affected which may lead to suboptimal physiological function and ill health. Of particular concern are effects on physical work capacity, reproductive efficiency, cognitive and psychomotor development and immune function. The effects of iron deficiency on immune function and infection are considered in section 8.

Development of iron deficiency

283. The development of iron deficiency can be considered in three stages (Charlton & Bothwell, 1982) corresponding to the sequential effects on the systemic iron stores, the supply of iron to the tissues, and the subsequent impairment of iron dependent functions. As iron deficiency becomes more severe relative to the systemic needs, the tissue ferritin depots become depleted and there is a concomitant and an increased risk of defective functional outcomes.

284. Depletion of cellular iron depots leads to mobilisation of iron depots from macrophages or hepatocytes, upregulation of iron absorption, decreased serum ferritin concentration, and reduced stainable iron in bone marrow smears**.**

285. Impaired supply of iron to tissues results in a decrease in serum iron concentrations, a progressive fall in serum transferrin saturation and an increase in serum transferrin concentration, in numbers of transferrin receptors on tissue surfaces, and in concentrations of serum transferrin receptor. Decreases in transferrin saturation below 16% leads to defective erythropoiesis (Bainton & Finch, 1964).

286. Progressive iron deficiency leads to a decrease in haemoglobin concentrations and in circulating numbers of precursor red cells (reticulocytes) and iron-dependent functions are affected (Hercberg & Galan, 1989).

Causes of iron deficiency and anaemia

287. Globally, anaemia is one of the most prevalent nutritional deficiency diseases. Iron deficiency is one possible cause of anaemia. Iron deficiency is not necessarily caused by an inadequate dietary intake of iron or reduced dietary availability of iron. However, in the literature, anaemia is often equated with iron deficiency without full characterisation of the anaemia or assumed iron deficiency.

288. Other causes of iron deficiency and anaemia include impaired absorption, and increased blood losses due to menstruation or gastrointestinal blood loss caused by gastrointestinal disease (Rockey & Cello, 1993). Gastrointestinal blood loss, associated with use of non-steroidal anti-inflammatory drugs such as aspirin, may be an important cause of iron deficiency in older people (Fleming *et al,* 2001). In many parts of the world intestinal helminthiasis is a major cause of anaemia because it can lead to significant blood loss from the intestine (Roche & Layrisse, 1966; Crompton &

Nesheim, 2002). Anaemia caused by intestinal helminthiasis is not of relevance in the UK.

289. Although iron deficiency anaemia may respond to iron supplements, these do not treat the underlying cause and may not treat the entire nutritional deficit. However, this is the nutritional state in which the adverse effects attributed to iron deficiency are studied.

HEALTH CONSEQUENCES OF IRON DEFICIENCY

Iron and physical work capacity

290. Physical work capacity has been reported to be impaired by iron deficiency.

291. Iron is required for oxidative energy production. It is proposed that anaemia (all-causes) and iron deficiency affect work capacity by two separate mechanisms: anaemia (low haemoglobin concentration) reduces the oxygen transport capacity of the circulation, which impairs aerobic capacity; iron deficiency reduces tissue oxidative capacity, which impairs endurance capacity and energetic efficiency (Davies *et al*, 1984). These impairments could lead to reductions in work productivity and voluntary activity, with economic and social implications.

292. A number of studies in animals and humans have investigated the relationship between iron deficiency and work capacity. In many of these studies, iron deficiency is poorly characterised; anaemia is often assumed to be caused by iron deficiency without corroborative data (measurement of markers of iron deficiency or sufficiency, such as serum ferritin concentration). There are also difficulties in identifying thresholds of iron deficiency associated with adverse outcomes because most data are categorised and discontinuous. This problem is evident in all the health consequences of iron deficiency considered in this section.

293. The evidence for a causal relationship between iron deficiency and physical work capacity has been systematically reviewed by Haas and Brownlie (2001); both animal (9 studies) and human (20 studies: 5 cross-sectional studies/15 trials) were evaluated. Five aspects of work capacity were examined: aerobic capacity, endurance capacity, energetic efficiency, voluntary activity and work productivity. The human studies were divided into *laboratory studies* and *field studies* of workers in Africa, China, Indonesia, Venezuela, and Sri Lanka. Findings from this review are summarised below.

Aerobic capacity

294. Aerobic capacity is assessed using the maximum oxygen consumption (VO_2 max) test which measures oxygen uptake at maximum physical exertion, usually on a treadmill or cycle ergometer. The Harvard step test is commonly used in the field and involves measuring heart rate responses to a fixed workload.

Animal studies

295. Studies in animal models have demonstrated an association between haemoglobin concentration and aerobic capacity, with severity of anaemia directly proportional to the degree of impairment in aerobic capacity. Perkkio *et al* (1985a) found that a decrease in haemoglobin concentration (from 140 to 80g/L) was accompanied by a

linear decline (of about 16%) in aerobic capacity, however a much steeper decline was observed at haemoglobin concentrations below 70g/L, suggesting a threshold below which aerobic capacity cannot be sustained. Iron supplementation of iron deficient rats was found to return aerobic capacity and haemoglobin concentration to control values after 3 days whilst endurance and muscle oxidative enzymes returned to control values after 5 days (Davies *et al* 1982). In another study (Davies *et al*, 1984), inducing severe iron deficiency anaemia (haemoglobin <80g/L) reduced aerobic capacity by 50%, which was restored to control values after iron supplementation. Findings from these studies suggest that haemoglobin is the primary determinant of aerobic capacity.

Human studies

296. Phlebotomy studies in humans suggest that decreases in haemoglobin concentrations are associated with impaired aerobic capacity, which is proportional to the severity of haemoglobin reduction. Woodson *et al* (1978) reported that an acute reduction in mean haemoglobin concentration from 150g/L to 104g/L reduced aerobic capacity by 16% and that the decrease was proportional to the haemoglobin reduction. In another study (Celsing *et al*, 1986), reduction in mean haemoglobin concentration from 146g/L to 110g/L was associated with an 18% reduction in aerobic capacity. Iron deficiency without anaemia was not associated with impaired aerobic capacity (Klingshirn *et al*, 1992; Li 1993; Lukaski *et al*, 1991; Newhouse *et al*, 1989; Zhu and Haas, 1998b).

297. Findings from two randomised double blind placebo controlled trials (Gardner *et al*, 1975; Ohira *et al*, 1979) reported improvements in various measures of aerobic activity after iron treatment (intramuscular or intravenous injection of iron dextran) of individuals with low haemoglobin (60-80 g/L). The studies did not assess iron deficiency without anaemia.

Endurance capacity

298. Endurance capacity is the maximum length of time an individual can sustain a given workload and is dependent on both oxygen delivery and oxygen use capacities of the working muscle. It is assessed by progressively increasing exercise intensity during fixed intervals of long duration and measuring time to exhaustion or by using shorter tests with higher workloads. Another test measures submaximal work on a cycle ergometer in which resistance and number of revolutions are fixed but pedal speed is at the subject's discretion (Zhu and Haas, 1998b; Hinton *et al*, 2000).

Animal studies

299. All of the animal studies observed a significant association between haemoglobin concentration and endurance capacity. Edgerton *et al* (1972 &1977) and Ohira *et al* (1981) reported a significant correlation between run time to exhaustion and haemoglobin concentration. Perkkio *et al* (1985b) showed that endurance capacity was correlated with cytochrome c concentration and that the relationship became stronger with decreasing concentration, supporting the suggestion that reduced oxidative capacity mediates the impairments in endurance.

Human studies

300. Four trials considered the effect of iron deficiency without anaemia (haemoglobin >120g/L) on endurance capacity (Celsing *et al*, 1986; Rowland *et al*, 1988; Klingshirn et al, 1992; Zhu & Haas, 1998). Celsing *et al* (1986) reported a significant decrease

in endurance capacity of 47% following induction of moderate iron deficiency anaemia (decrease in haemoglobin concentration from 146 to 110g/L; decrease in serum ferritin concentration from 60 to 7 µg/L). In contrast to findings from animal studies, only one study (Rowland *et al*, 1988) reported that improving iron status (increase in serum ferritin concentration from 8.7 to 26.6 µg/L) significantly increased endurance capacity.

301. Hinton *et al* (2000) observed that iron supplementation (20 mg/d for 6 weeks) of non anaemic (haemoglobin >120 g/L) iron deficient (serum ferritin<16 µg/L) women (n=42) for 4 weeks increased endurance capacity (time to complete a 15km cycle ergometer test) compared to the placebo group; however, all participants had also undertaken an aerobic training regimen during the trial and endurance capacity was significantly improved in both supplemented and placebo groups compared to baseline.

Energetic efficiency

302. Energetic efficiency is the amount of energy required to perform a given amount of external work. Energy expenditure is usually measured by calorimetry and external work is assessed at the same time by physical work on a cycle ergometer or a treadmill. In field studies, energy expenditure is measured by monitoring heart rate and work output is measured by practical items of output, for example, the amount of tea picked.

Human studies

303. A cross-sectional study observed no difference in work output at different work levels on a cycle ergometer between iron deficient (serum ferritin<12 µg/L) and non iron deficient (serum ferritin>12 µg/L) women (Zhu and Haas, 1997).

304. A double-blind randomised trial (Zhu and Haas, 1998) of marginally iron deficient (haemoglobin >120 g/L, serum ferritin<16µg/L) women (n=37) reported that iron supplementation (27mg/d for 8 weeks) significantly reduced total energy expended during a fixed-distance cycle ergometer test and that energetic efficiency was significantly related to serum ferritin concentration.

305. In a trial of Chinese female cotton mill workers (n=80) with iron deficiency (haemoglobin≥120g/L; serum ferritin<12 µg/L) and iron deficiency anaemia (haemoglobin<120g/L; serum ferritin<12 µg/L), earnings per unit of energy expended over 8 hours of work were significantly improved in the group supplemented with iron (60 or 120 mg/day for 12 weeks) compared to the placebo group, resulting in an increase in production efficiency of 17% (Li *et al,* 1994). The iron supplemented group also reported an increase in time engaged in leisure activities and an increase in energy expended during those activities.

Voluntary activity

306. Iron deficiency may affect voluntary activity by causing fatigue. Voluntary activity is assessed by activity wheels in animal studies and by time allocation questionnaires and heart rate monitoring in human studies.

Animal studies

307. Two animal studies assessed the relationship between iron deficiency and voluntary activity (Edgerton *et al* 1972 and Hunt *et al* 1994). Both reported significant

reductions in voluntary activity of rats after inducing iron deficiency; greater reductions were observed with increasing severity of iron deficiency, however the level of voluntary activity did not increase with iron repletion (Edgerton *et al*, 1972).

Human studies

308. Edgerton *et al* (1979) observed that voluntary activity of Sri Lankan female tea plantation workers (n=18) was increased after 2-3 weeks of iron supplementation (40 mg/day). In the study of female cotton mill workers by Li et al (1994) (see paragraph 305), the iron supplemented group reported an increase in time engaged in leisure activities compared to the placebo group.

Work productivity

309. Productivity has been measured in jobs that involve producing a commodity that can be easily quantified over a specified time. Studies which evaluated the effects of iron deficiency on economic productivity were all conducted in developing countries and include studies of rubber tree tappers, tea pickers, cotton mill workers and cigarette rollers.

310. An important problem with these studies is that productivity is influenced by a number of factors; for example, productivity can be influenced by motivation which is strongly affected by production incentives which could, in turn, have an effect on the effort expended. Weaker people could achieve the same productivity as stronger ones by spending more energy at work. Type of labour can also affect the mechanism by which iron affects productivity: physically demanding work, requiring high aerobic capacity, could be impaired by anaemia, while less strenuous work could require greater endurance and might be affected by iron deficiency. Impaired productivity during shorter more physically demanding work may be easier to assess than longer less strenuous tasks which may be affected by motivation.

311. Cross-sectional studies have reported significantly higher work productivity in female cigarette rollers (n=230) with haemoglobin concentrations above120 g/L compared to those with haemoglobin less than 120 g/L (Untoro *et al*, 1998) and in female jute factory workers (n=92) with haemoglobin concentration >110g/L compared to those with haemoglobin < 110g/L (Scholz *et al*, 1997).

312. A trial in Indonesia (Basta *et al,* 1979) reported a 17% increase ($p<0.05$) in work output of anaemic (haemoglobin<130g/L) rubber tree tappers (n=302) receiving iron (100 mg/d for 60 days) compared to those receiving a placebo. In another study of anaemic (haemoglobin 102-114 g/L) female tea pickers in Sri Lanka (Edgerton *et al*, 1979), significantly more tea was picked by workers who had been supplemented with iron (40 mg/day for 30 days) compared to those who had received placebo. The study of Chinese female cotton mill workers by Li *et al* (1994) (see paragraph 305), reported a 17% significant increase ($p<0.01$) in the production efficiency (productivity/energy expended) of Chinese female cotton mill workers (n=80) after 12 weeks of iron supplementation (60 mg/day if haemoglobin 1020 g/L, serum ferritin<12 µg/L; 60 mg/day if haemoglobin 100-120g/L; 120mg/day if haemoglobin<100g/L, serum ferritin<12 µg/L) compared to the change in the placebo group, however there were no differences in productivity; although the women were paid by the quantity and quality of yarn produced, productivity was limited by the speed of the machines used in the mill. Another placebo controlled study of female tea pickers (n=199; haemoglobin, 102-114 g/L) in Sri Lanka (Edgerton *et al*, 1979),

found that iron supplementation (40 mg/d for 30 days) had little effect on the amount of tea picked.

Other studies

313. Studies since the review by Haas and Brownlie (2001) have mainly examined the effect of iron deficiency on endurance capacity during aerobic exercise training. In a double-blind randomised controlled trial (Brutsaert *et al,* 2003), iron supplementation (20 mg/d for 6 weeks) of iron depleted (haemoglobin>110 g/L; serum ferritin<20 µg/L) women (n=20) was associated with a significant improvement (p=0.01) in muscle fatigue resistance in the iron supplemented group but not in the placebo group; however there was no improvement in measures of iron status. In another randomised controlled trial (Brownlie *et al*, 2004) iron depleted women (haemoglobin>120g/L; serum ferritin<16 µg/L; n=41) were supplemented with either iron (16 mg/d) or placebo for 6 weeks; significant treatment effects of iron supplementation were observed in participants with baseline transferrin receptor concentration >8.0 mg/L. Hinton and Sinclair (2007) examined the effect of iron supplementation (30 mg/d for 6 weeks) compared to placebo on iron deficient men (haemoglobin>130g/L; serum ferritin<16 µg/L; n=3) and women (haemoglobin>120g/L; serum ferritin<16 µg/L; n=17) with previous aerobic exercise training. Iron supplementation significantly increased serum ferritin concentration (p=0.01) and endurance capacity (p=0.01) compared to placebo treatment.

314. Gera *et al* (2007) systematically reviewed randomised placebo controlled trials investigating the effect of iron supplementation/iron fortified milk or cereals on physical performance (assessed by heart rate, treadmill endurance time, blood lactate levels or oxygen consumption) in children and adolescents. The pooled analysis showed beneficial effects on blood lactate levels and treadmill endurance time. The findings were not considered conclusive due to the limited data: only 3 trials (n=106) were included in the analysis; details were not available about other factors affecting physical performance, e.g. energy adequacy or illness; and participants in one of the trials were adolescent athletes who had received one month of physical training, which is also likely to improve physical performance.

Summary and conclusions

315. Evidence from animal studies suggests that decreases in haemoglobin concentration are associated with impairments in various aspects of physical work capacity, including aerobic capacity, endurance capacity, and voluntary activity.

316. Studies in humans suggest that iron deficiency anaemia (haemoglobin<120g/L plus an additional indicator of iron deficiency) is associated with reduced aerobic capacity although there is no clear threshold of haemoglobin concentration for this effect. There is no clear evidence that iron deficiency in the absence of anaemia (haemoglobin>120g/L plus an additional indicator of iron deficiency) has adverse effects on work capacity.

317. Although there is evidence that iron deficiency might impair endurance capacity in animal studies, this needs further substantiation in humans.

318. There is limited evidence suggesting that iron deficiency impairs voluntary activity but this needs further exploration.

319. Very few studies have examined the effects of anaemia (all-cause and iron deficiency anaemia) on work productivity and all have been conducted in developing countries in subjects with haemoglobin concentrations below those usually observed in the UK. Overall, there are insufficient data to assess the effects of iron deficiency on work productivity.

320. There are a number of uncertainties and difficulties in interpreting the data examining the relationship between iron status and physical work capacity. Most field studies have been carried out in developing countries where populations are associated with multiple deprivations (nutritional, social, and economic) which can all affect work capacity. Studies have also used different criteria to classify iron deficiency/iron deficiency anaemia, treatment duration and dose has varied considerably, a number of different test protocols have been used and sample sizes were very small in most studies.

Maternal iron status and pregnancy outcome

321. During pregnancy, iron is needed to support fetal growth and development, the placenta, expansion of maternal red cell mass and to cover the iron lost in blood during delivery (see section 3). In normal pregnancy, plasma volume increases steadily until 32-34 weeks causing a fall in maternal haemoglobin concentration although red cell mass actually expands during the second and third trimesters. Separate cut-off points have been established to diagnose anaemia during pregnancy because of the changes in the relative balance between plasma volume and red cell mass (see Table 3.2, section 3).

Epidemiological studies

322. Epidemiological studies have suggested a relationship between maternal haemoglobin concentration during pregnancy and birth outcome, i.e., birth weight, gestation length, and neonatal/perinatal mortality. Haemoglobin concentrations at either the low or high end of the distribution have been associated with increased risk of low birth weight (i.e. small for gestational age, <2.5kg; or preterm, <37 weeks gestation) and perinatal mortality (Rasmussen, 2001).

323. Due to the range of physiological changes associated with pregnancy (plasma volume expansion and the corresponding haemodilution), markers of iron metabolism are difficult to interpret during this time. Birth outcome is also affected by a wide array of health behaviours, as well as demographic, socio-economic and nutritional factors. These confounders are rarely fully accounted for or considered in epidemiological studies investigating the effect of maternal iron deficiency on fetal and early infant development (US Preventive Services Task Force, 1993).

324. Unusually high haemoglobin concentrations during pregnancy are not caused by high intakes of dietary or supplemental iron but are more likely to be associated with other pathophysiological processes which in turn cause a smaller plasma volume expansion and poor reproductive outcomes (Koller *et al*, 1979; Lu *et al*, 1991). Inadequate plasma volume expansion is associated with restricted fetal growth and low birth weight (Dunlop *et al*, 1978) or preterm birth (Forest *et al*, 1996). Failure of plasma volume expansion has also been associated with a greater than threefold increase in the incidence of pre eclampsia in pregnancy (Murphy, 1986). In developed countries it is likely that high haemoglobin concentrations are themselves not the cause of low birth weight and preterm labour but are the result of reduced plasma volume secondary to other causes of failed pregnancies (Steer, 2000).

325. Garn *et al* (1981) analysed pregnancy outcome data in the USA (n=50,000) and reported that low birth weight, preterm birth, and fetal death were minimal at maternal haemoglobin concentrations of 110-120g/L for Caucasians and 90-100g/L for African Americans; the incidence of unfavourable pregnancy outcomes (live born, term) was increased at or below haemoglobin concentrations of 90 g/L and at or above 130 g/L. In the UK, Murphy *et al* (1986) analysed haemoglobin concentration at first antenatal attendance (n= 54,382); the highest rates of low birth weight, preterm birth, and perinatal mortality were associated with maternal haemoglobin concentrations below 104 g/L or above 132 g/L irrespective of whether first antenatal attendance was in the first or second trimester. An analysis of 153,602 pregnancies in the UK (Steer *et al*, 1995), reported highest birth weight at lowest maternal haemoglobin concentrations of 85-95g/L. The incidence of low birth weight and preterm labour was minimal at

lowest haemoglobin concentrations between 95-105 g/L, however this observation is limited as haemoglobin concentrations were not measured at a consistent gestational period.

326. A prospective study of pregnant women (n=829) in China (Zhou *et al*, 1998) observed an association between haemoglobin concentration in early pregnancy (second gestational month) and rates of low birth weight and preterm birth. Compared to women with haemoglobin concentration between 110-119 g/L, the relative risk of low birth weight was significantly increased in women with haemoglobin concentrations between 100-109 g/L (RR=2.73; 95% CI, 1.01-7.39) and 90-99 g/L (RR, 3.27; 95% CI, 1.09-9.77); the relative risk of preterm birth was also significantly increased in women with haemoglobin concentrations between 90-99 g/L (RR, 2.63; 95% CI, 1.17-5.90) and <90g/L (RR, 3.73; 95% CI, 1.39-10.23). The relative risks of preterm birth associated with haemoglobin concentrations <100g/L in the first trimester were not significant in the second trimester (fifth gestational month) or third trimester (eighth gestational month) but the relation with low birth weight remained significant in the second and third trimester. The minimum risk of low birth weight was at haemoglobin concentrations of 110-119g/L.

327. The epidemiological studies have a number of limitations which complicate their interpretation. In most populations women who are anaemic also have other factors associated with a risk of adverse pregnancy outcomes which can be difficult to control for (see paragraph 323). Another difficulty is that the physiological changes that occur during pregnancy obscure the usual relationship between haemoglobin concentration and other markers of iron status. Additionally, haemoglobin concentrations in early pregnancy will differ from those in the second trimester (when plasma volume expansion is at its peak and haemoglobin concentration is therefore reduced) and the third trimester (when plasma volume stops expanding and red cell mass increases). There is little consistency between studies in the time point at which haemoglobin concentration was assessed. This is particularly problematic when the lowest measurement of haemoglobin concentration is used because this occurs at different times during pregnancy. Another difficulty with interpreting data is that many older studies did not discriminate between small for gestational age and preterm infants as causes of low birth weight.

328. It is not possible to identify upper or lower thresholds for haemoglobin concentrations which have been associated with adverse birth outcomes in observational studies as these have varied across studies and have been measured at different stages of pregnancy.

Intervention trials of iron supplementation in pregnancy

329. A Cochrane Review of 40 trials (Pena-Rosas & Viteri, 2006) evaluated the effects of iron supplementation (alone or in combination with folic acid) during pregnancy on haematological parameters and pregnancy outcomes (low birth weight and preterm delivery). Most trials were carried out in industrialised countries. The authors observed that the majority of trials focused on haematological outcomes, data on pregnancy outcomes were limited, and there was significant heterogeneity between studies. Women receiving daily iron supplements (alone or in combination with folic acid) had higher haemoglobin concentration at term than women who did not receive supplements; haemoconcentration (haemoglobin >130 g) at term as well as during the 2nd and third trimester was also associated with daily iron supplementation.

However the authors advise caution in the interpretation of the results due to substantial heterogeneity between treatment effects. The review concluded that there were not enough data to determine whether iron supplementation has any beneficial or harmful effects on low birth weight or preterm delivery.

Summary and conclusions

330. Epidemiological data suggest that maternal haemoglobin concentrations at either the low or high end of the distribution during pregnancy are associated with increased risk of adverse birth outcomes, i.e. low birth weight, preterm birth, perinatal mortality. However these are not necessarily related to a causal relationship with iron supply or nutrition. Physiological changes which occur during pregnancy make it difficult to interpret markers of iron metabolism at this time: haemoglobin concentrations fall during early pregnancy due to plasma volume expansion. High haemoglobin concentrations during pregnancy are usually caused by inadequate plasma volume expansion which is also associated with adverse birth outcomes.

331. Intervention studies of routine iron supplementation during pregnancy have not shown beneficial or adverse effects on pregnancy outcomes. Most trials were conducted in industrialised countries where iron deficiency in early pregnancy is less prevalent than in developing countries so any potential benefits may not be apparent.

332. As recommended by NICE (2008) iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks.

Cognitive, motor, and behavioural development in children

Introduction

333. There is an extensive body of research considering the relationship between iron deficiency anaemia and cognitive, motor and behavioural development in children. While most researchers conclude that iron deficiency anaemia causes poor cognition in school aged children, the effect on younger children remains controversial. Some researchers consider iron deficiency anaemia causes poor child development (e.g. Walker *et al*, 2007) while others have concluded that there is inadequate evidence of an effect (e.g. Sachdev *et al*, 2005). Many studies share common design flaws which limit their interpretation. Iron deficiency anaemia is associated with a large number of socio-economic and biomedical disadvantages that can themselves affect children's development but are difficult to control for in observational studies or uncontrolled treatment trials. Double blind randomized controlled trials of iron supplementation, treating or preventing iron deficiency anaemia provide the most accurate way of determining whether iron deficiency is a cause of poor cognitive, motor and behavioural development.

334. Many factors are associated with both iron deficiency anaemia and cognitive function. These include: low socio-economic status (Owen *et al*, 1971), poverty (Czajka-Narins *et al,* 1978), poor quality of stimulation in the home (de Andraca *et al,* 1990), lack of maternal warmth, low levels of maternal education (de Andraca *et al,* 1990; Idjradinata and Pollitt, 1993) and IQ (Lozoff *et al,* 1991), maternal depression (de Andraca *et al*, 1990), absent fathers, low birth weight (<2.5kg), early weaning (Lozoff *et al,* 1991), parasitic infection (Ramdath *et al*, 1995), elevated blood lead levels, and undernutrition.

335. Based on data from the UK National Diet and Nutrition Survey (NDNS) of children aged 1.5 to 4.5 years (n=1859) (Gregory *et al*, 1995), Thane *et al* (2000) reported that the parents of children with haemoglobin concentrations below 110g/L[13] were more likely to be receiving benefits, be unemployed, and have lower educational attainment levels and incomes than non anaemic children. Iron deficient children were less likely to have been breast fed and more likely to be poor feeders. A greater proportion of children with low serum ferritin concentrations (<10 µg/L) were also from homes whose household heads had poorer occupational status. Both groups consumed more cows' milk and less meat and fruit. Blood specimens were not available for young children in the Low Income Diet and Nutrition Survey (Nelson *et al*, 2007) due to a low response rate.

336. Smaller surveys indicate that children of Asian origin are also at high risk of anaemia (haemoglobin<100 g/L) (Grindulis *et al*, 1986; Lawson *et al*, 1998).

Iron in the brain

337. The amount of iron in the brain increases throughout childhood and early adult life: about 10% of the brain iron content at 20 years of age is present at birth and 50% is present at 10 years of age. Although iron is distributed throughout the brain, the highest concentrations are in the basal ganglia (caudate-putamen, globus pallidus and the substantia nigra) which effect fine control and integration of movement. The

[13] For children aged 6 months to 6 years, haemoglobin concentrations below 110g/L are defined as anaemia by the WHO.

iron content (13-21 mg/100mg) in these areas is comparable to that of the liver (Hallgren and Sourander, 1958).

338. Mechanisms for the uptake and transfer of iron into the brain, and for the regulation of the processes involved, are still being identified (Moos *et al,* 2007). Receptor mediated uptake of iron transferrin occurs at the capillary brain barriers. The mechanisms for the differential distribution of iron in the brain have not been characterised but the overall processes involved are similar to those of other organs (Patel *et al*, 2002). It has been suggested that because transferrin is quickly saturated in cerebrospinal fluid, neurones might acquire some iron in the form of low-molecular weight citrate and ascorbate iron II complexes, thereby increasing the risk of free radical damage in some circumstances (Sipe *et al*, 2002).

339. In the brain, iron is found predominantly in the oligodendrocytes which are the cells responsible for producing myelin, the lipid sheath that insulates nerve cells. Defective myelination is thought to be one mechanism by which iron deprivation might impair neurological function. Additionally iron is a catalytic element in the synthesis of neurotransmitters such as dopamine and serotonin (Thompson *et al*, 2001).

340. Neuronal metabolism is also impaired by iron deficiency. Iron deprivation in young rats, induced by feeding their mothers iron deficient diets during gestation and lactation, has been associated with reduced cytochrome oxidase activity in the hippocampus (which processes information to create memory) and other areas of the brain (Ungria *et al*, 2000), and with an altered neurochemical profile indicative of altered energy metabolism and neurotransmission (Rao *et al*, 2003). The hippocampus is thought to be important for spatial navigation. Persisting difficulty with spatial navigation has been observed in rodent models of early life iron deficiency, which is analogous to observations of impaired spatial memory in adolescents who were iron deficient as infants (Lozoff, 2000).

Mechanism

341. There are several biologically plausible ways in which iron deficiency could affect cognitive development; most directly, changes may occur to the structure and function of the central nervous system (CNS).

Brain development

342. Animal research has provided evidence of changes to the brain in iron deficiency; there is also some evidence of changes to the CNS in children. Burden *et al* (2007) found that the development of event related potentials, which measure transient changes in electrical activity of the brain in response to a stimulus and are associated with attention and recognition memory tasks, were delayed in 9 and 12 month old infants with iron deficiency anaemia[14].

343. Other studies used auditory brain stem responses (ABRs), which provide a measure of the activation of the auditory pathway from the distal part of the acoustic nerve to the brain; the central conduction time (CCT) is an indicator of CNS development. Roncagliolo *et al* (1998) found that the CCT was prolonged in infants (aged 6m) with

[14] Haemoglobin ≤105 g/L and ≥ 2 other abnormal iron status measures: mean corpuscular volume <74 fL, red cell distribution width >14%, zinc protoporphyrin/haem ratio >69 µmol/mol of haem, ferritin <12 µg/L, and transferrin saturation <12%. The comparison group of infants with IS had haemoglobin 115 g/L and 1 abnormal iron indicator.

iron deficiency anaemia[15] compared to infants without iron deficiency anaemia; the difference was larger 6 and 12 months after iron treatment. The authors suggested that the prolonged CCT may have been caused by impaired myelination, which has been observed in iron deficient animals (Yu *et al*. 1986). Peirano *et al* (2001) reported that children who had iron deficiency anaemia in infancy[16] also had longer latencies in visual evoked potentials (VEPs) at 3 to 5 years of age. One study of infants who had iron deficiency anaemia [17] in infancy found that both ABRs and VEPs remained abnormal at age 4 years (Algarin *et al* 2003). However, another study (Sarici *et al* 2001) reported no differences in ABRs of infants with iron deficiency anaemia (not defined) compared to controls. There is also evidence of changes in the autonomic nervous system that cause sleep disturbances (Peirano *et al* 2001 & 2007, Kordas *et al* 2008).

344. These observations provide a plausible mechanism for causality. They also raise the possibility that there may be periods when the developing brain might be particularly sensitive to iron deprivation and suggest why it might not be possible to compensate early deficits by subsequent iron replenishment.

345. During the immediate postnatal period in the rat, a rapid increase occurs in the brain content of transferrin and iron. In young animals, however, iron seems to be preferentially distributed to the erythron over other organs, including the brain. Thus in early life it is conceivable that iron deficiencies might exist in tissues, such as the brain, in the absence of iron deficiency anaemia (Allen, 1997; Lozoff, 2000).

Behaviour

346. It has been hypothesised that the link between iron deficiency anaemia and poor cognitive development can be explained by functional isolation (Levitsky and Strupp, 1995). Anaemic children explore and move around their environment less than non-anaemic children and, in response, caretakers are less stimulating. Both behaviours may delay their acquisition of new skills.

347. Compared with non-anaemic (haemoglobin>110-120g/L) children in the first two years, iron deficient anaemic (haemoglobin, 100-110g/L and 2 markers of potential iron deficiency) children are more fearful (Lozoff *et al*, 1982a; Lozoff *et al*, 1996), withdrawn, unreactive to usual stimuli (Lozoff *et al*, 1982a), unhappier (Lozoff *et al,* 1996; Walter *et al*, 1983), make fewer attempts at tasks, less playful and have poorer attention (Lozoff *et al*, 1998a). These behaviours were found to persist after treatment.

348. It remains possible that these behaviours could be due to deprived environments rather than iron deficiency anaemia. A study of undernourished children with similar behaviour found that the behaviour was changed with stimulation alone (Grantham-McGregor *et al*, 1989).

[15] IDA defined as: haemoglobin≤100g/L and ≥ 2 iron measures – mean cell volume <70fl, erythrocyte protoporphyrin>1.7 µmol/L, serum ferritin<12 µg/L.

[16] Haemoglobin≤100g/L at 6 months and <110g/L at 12 and 18 months; and 2 out of 3 measures in the iron deficient range: mean cell volume<70 fL, erythrocyte protoporphyrin >1.77 µmol/L, serum ferritin <12 µg/L..

[17] Iron deficiency anaemia definition as above.

Review of evidence

349. There are several reviews on the relationship between iron and cognitive development (Grantham-McGregor and Ani, 2001; Martins *et al*, 2001; Sachdev *et al*, 2005; McCann *et al,* 2007; Lozoff, 2007).

350. Evidence from correlational and case-control studies suggests an association between iron deficiency and iron deficiency anaemia and cognitive and/or motor and/or behavioural development. Longitudinal studies have indicated that these effects may continue into primary school age. Findings from a longitudinal study with 19 years of follow-up suggest that children from poor backgrounds may be more vulnerable to the long-term effects of iron deficiency anaemia (haemoglobin≤100g/L; serum ferritin≤12 µg/L and either erythrocyte protoporphyrin>1.77 µmol or transferrin saturation≤10%) on cognitive deficits than those from more affluent backgrounds (Lozoff, 2006).

351. While observational studies consistently show a relationship between iron and cognitive development they suffer from a number of limitations including lack of control for social determinants and other confounding variables which may also affect child development (see paragraphs 334-335). Definitions of anaemia and iron deficiency also varied in most studies. Observational data are not considered further in this section as there is a large evidence base of intervention studies which have examined the relationship between iron and cognitive development.

Measures of child development

352. Most studies examining the relationship between iron and cognitive development in young children used the Bayley Scales of Infant Development (BSID) to assess outcome. The BSID consists of two age-standardised sub-scales, the Mental Development Index (MDI) and the Psychomotor Development Index (PDI); these indices give little indication of specific deficits. The ability of the BSID to predict future development in the first year of life is extremely limited, but increases in the second year (Colombo, 1993). However, in nearly all the studies considered in the following sections, the BSID were sensitive to differences between anaemic and non anaemic groups. Studies in older children have generally used tests of intelligence, specific cognitive functions, or school achievement.

Intervention studies

353. A Cochrane review of randomised controlled trials examining the effects of iron supplementation on psychomotor development and cognitive function in children under 3 years of age with anaemia (defined as haemoglobin<105 g/L in most studies) or iron deficiency anaemia (defined as haemoglobin<105 g/L and at least 2 abnormal measures of iron status in most studies) (Martins *et al,* 2001) identified 5 trials (n=180) of 5-11 days and 2 trials (n=160) of more than 30 days. The findings of the review suggest that short term iron treatment had no effects on psychomotor development; the effect of longer term treatment was unclear. Another systematic review (Sachdev *et al*, 2005) of 17 trials (duration 1 week to15 months) in children (aged under 2 months to 15 years) reported that iron supplementation significantly improved mental development of children ≥ 8 years ($p<0.001$) but not children under 27 months ($p=0.21$) and had no effect on motor development.

354. The key intervention studies considered in this section are treatment trials in iron deficient children (divided into short and long term treatment), trials in populations

with mixed iron status, and preventive treatment trials with iron replete children. The treatment trials are further divided by age of subjects (under and over 3y). The majority of trials were carried out in developing countries. Details of the studies considered, including country, sample size, allowance for confounding factors, iron status, and specific cut-offs used to define iron deficiency/iron deficiency anaemia are provided in Annex 3 (Tables 6.1-6.6). The tables are based on those included in the systematic review by Grantham-McGregor and Ani (2001).

Treatment trials in children ≤ 3 years

Short term trials in children with iron deficiency or iron deficiency anaemia (Table 6.1)

355. Seven early trials assessed short-term effects (less than 2 weeks) of iron supplementation in young children (aged 6-26 months) with iron deficiency anaemia (Oski and Honig, 1978; Lozoff *et al*, 1982b; Oski *et al,* 1983; Walter *et al*, 1983; Driva *et al*, 1985; Lozoff *et al*, 1987; Walter *et al*, 1989). The findings suggest that short-term treatment does not have beneficial effects on mental or motor development of children with iron deficiency or iron deficiency anaemia. However the hypothesis has not been well tested; sample sizes were small in all the studies and none reported their statistical power to show differences. It is also unlikely that the type of skills measured by the BSID would improve substantially in two weeks.

Longer term trials in children with iron deficiency or iron deficiency anaemia (Table 6.2)

356. Of the 10 longer term trials (2-12m) of children (aged 12-30m), 7 were not randomised; 5 of these trials used non-anaemic iron replete groups as controls (Lozoff *et al*, 1987; Walter *et al*, 1989; Lozoff, 1996; Harahap *et al*, 2000; Akman *et al* 2004). This was based on the idea that the anaemic group would have an initial deficit and would catch up with iron treatment. However, evidence suggests that iron deficiency anaemia children in poor circumstances do not develop similarly to non-anaemic children but actually increase their deficits over time (Lozoff *et al*, 2006); so even maintaining the same degree of deficit could be a treatment effect.

357. The treated anaemic groups did not catch up with the non-anaemic groups in 3 of the 5 studies (Walter *et al*, 1989; Lozoff *et al*, 1987; Lozoff, 1996) and caught up in 2 (Harahap *et al,* 2000; Akman *et al,* 2004). In the study by Akman *et al* (2004) non-anaemic iron deficient (haemoglobin>110g/L; serum ferritin<12 µg/L) children were randomised to iron treatment or no treatment, an iron deficiency anaemia group (haemoglobin<110g/L; serum ferritin<12 µg/L), received iron treatment, and an iron replete non-anaemic control group (haemoglobin>110g/L; serum ferritin>12 µg/L) received no treatment. The groups with iron deficiency anaemia and non-anaemic iron deficiency began with poorer development scores than the iron replete non-anaemic group; after treatment the iron replete non-anaemic, the non-anaemic iron deficient and the iron deficiency anaemia groups were all similar. Although the non-anaemic iron deficient children were randomised, "intent to treat analysis" was not reported.

358. Hasanbegovic *et al* (2004) compared the effects of iron supplementation in children with haemoglobin concentration less than 95g/L (A) or between 95-110g/L (B). Initially group A had lower mental and motor development scores than group B; the deficit increased after treatment with only group B showing some improvement. The authors suggested that children with haemoglobin concentrations below 95g/L had irreversible deficits.

359. The differences in failure of anaemic children to catch up with the non-anaemic children in these trials are not readily explained by duration of treatment or severity of anaemia. The poorer backgrounds of the anaemic children could explain some of the failure to catch up. Few trials comprehensively controlled for environmental factors.

Longer term randomised trials in children with iron deficiency or iron deficiency anaemia (Table 6.3)

360. Only 3 of the 10 treatment trials were double blind randomised controlled trials (Aukett *et al*, 1986; Idjradinata and Pollitt, 1993; Stoltzfus *et al,* 2001). Idjradinata and Pollitt (1993) reported a significant effect in both mental and motor development after 4 months of treatment. Stoltzfus *et al* (2001) reported that iron supplementation for 12 months significantly improved language milestones regardless of initial haemoglobin concentration and improved motor milestones in children with initial haemoglobin concentrations below 90mg/L; however, the predictive validity of these developmental milestones are not well-established. In contrast, Aukett *et al* (1986) did not find a significant treatment effect although there was a suggestion of an effect in post hoc analyses. However the Denver test which was used in this study is not sensitive to small differences.

Longer term randomised trials with children of mixed iron status (Table 6.4)

361. In these trials (6-12m) iron status of children (aged 6-11m) varied from non-anaemic iron sufficient to anaemic iron deficient. As it is unlikely that any benefits of iron treatment would be observed in iron replete children, the mean effect size would be expected to be small and studies would require larger sample sizes. Three randomised controlled trials were identified (Black *et al*, 2004; Lind *et al*, 2004; Olney, 2006); a fourth randomised controlled trial was reported in abstract only (Black *et al*, 2002) and is not included.

362. Lind *et al* (2004) reported a significant treatment effect on motor but not mental development. Olney *et al* (2006) examined only time to begin walking and reported that the treated group walked sooner than the untreated group; however the treatment group also received folic acid with the iron.

363. Black *et al* (2004), examined the effect of 6 months treatment with different micronutrients including iron alone, zinc alone, iron+ zinc, or a micronutrient mix (containing 16 vitamins and minerals including iron and zinc) in a groups of infants aged 6m; 68% of infants were anaemic (haemoglobin<110 g/L). The 4 groups were compared with a group receiving riboflavin. PDI scores declined in all groups, however the decreases were significantly smaller in infants who received iron and zinc together or with other micronutrients. The group receiving only iron declined less than the riboflavin group but the difference was not significant. It is unlikely that this study had sufficient statistical power to detect small differences.

Preventive trials with non-anaemic children (Table 6.5).

364. These studies are based on the theory that if the placebo group develops iron deficiency anaemia and iron deficiency is prevented in the treated group, it should be possible to determine the effect of iron deficiency anaemia. However, the design is only valid if a sufficient proportion of the placebo group become iron deficient, otherwise the impact would be expected to be small or absent.

365. Eight trials were identified (Heywood *et al*, 1989; Walter *et al*, 1989; Moffatt *et al*, 1994; Morley *et al*, 1999; Williams *et al*, 1999; Lozoff *et al*, 2003, Friel *et al,* 2001, 2003). The age of the children ranged from 2 to 9 months and duration of treatment was 5 to 13 months. Four of these studies are not included in Table 6.5 (Annex 3) (Heywood *et al,* 1989; Walter *et al*, 1989; Morley *et a*l, 1999; Friel *et al*, 2001). The trial by Friel *et al* (2001) lacked internal validity as no differences were found in haemoglobin concentration between babies who received formula fortified with 20.7mg/L or 13.4mg/L. The presence of malaria confused the results in the study by Heywood *et al* (1989) and analysis was not reported according to the original trial design in the study by Walter *et al* (1989). Morley *et al* (1999) found no benefits of treatment but as data on initial and final haemoglobin concentrations were lacking it is not possible to determine how many children, if any, were initially iron deficient or if there was any difference in iron status at the end of the study.

366. The four remaining studies were randomised trials (Moffatt *et al*, 1994; Friel *et al*, 2003; Williams *et al*, 1999; Lozoff *et al*, 2003) and some beneficial response to iron treatment was found in all of them. Williams *et al* (1999) reported beneficial effects of iron treatment; however, since one group was given fortified formula while the other group was given money to buy cow's milk, participants were not blind to treatment. It is possible that other constituents in the formula could have been responsible for the observed benefits or cows' milk could have reduced the absorption of other nutrients.

367. Lozoff *et al* (2003) reported that although iron supplementation had no effect on PDI, MDI, or a test of recognition memory, there were improvements in speed of information processing, behaviour, and age of crawling. However, as supplementation procedures were changed half way through the study, and cows' milk was given to some of the control group, it is not possible to infer with confidence that iron treatment caused these differences.

368. Moffatt *et al* (1994) reported that children given iron fortified formula from 2 months of age for 13 months had significantly higher PDI scores than the placebo group at 9 and 12 months. The benefit was no longer significant at 15 months; however, the difference between the groups in iron status measures was limited and was smallest at 15 months. There was no significant effect on MDI. Friel *et al* (2003) reported that iron replete infants supplemented at 1 month of age for 5 months had significantly higher PDI but not MDI scores at 12 months of age. The power of this study was limited because there was little difference between groups in iron status measures and sample sizes of the groups were small.

369. A study of very low birth weight infants (<1301g) randomised to early (mean 14 days) or late (mean 61 days) iron supplementation reported that the early supplemented children had slightly better (but not significant) neurological, cognitive, achievement and disability outcomes at 5 years of age (Steinmacher *et al,* 2007).

Summary of findings from supplementation trials with children ≤ 3 years

370. There is no clear evidence that short-term (les than 2 weeks) iron treatment benefits psychomotor and mental development of anaemic children aged 3 years or under, however this has not been rigorously tested.

371. Longer term (3-6m) iron treatment trials that were not randomised are difficult to interpret because of differences in the development of non-anaemic and anaemic children who are usually from poorer social backgrounds.

372. Of 3 randomised long term (2-12m) trials with anaemic children two showed benefits in motor and mental or language development (Idjradinata and Pollitt, 1993; Stoltzfus *et al*, 2001).

373. Of the 3 randomised trials (6-12m) of children with mixed iron status the two studies reported significant benefits to motor development only (Lind *et al*, 2004; Olney *et al*, 2006). The third reported beneficial effects on motor development from iron and zinc combined but only a non-significant benefit from iron alone (Black *et al*, 2004).

374. Of the 5 preventive trials (5-13m), 2 (Moffatt *et al*, 1994; Friel *et al*, 2003) reported beneficial effects on motor development but sample sizes were small and differences in iron status were limited, reducing the chances of finding other benefits.

Randomised Controlled Trials in Children >3 years of age (Table 6.6)

375. Fourteen iron treatment trials with anaemic or iron deficient children over 3 years of age were identified. Duration of treatment was 2-6 months; the age of the children ranged from 3 to 18y, although 2 studies included children aged 3y or younger (Deinard *et al*, 1986; Metallinos-Katsaras *et al*, 2004). Two trials were not randomised (Pollitt *et al*, 1983; Pollitt *et al*, 1986), in one the method of assignment was not clear (Lynn and Harland, 1998), one reported no statistical analysis (Soemantri, 1989) and one had no placebo group (Seshadri and Gopaldes, 1989; study 1).

376. Nine of the studies were double blind randomised controllede trials (Soemantri *et al*, 1985; Deinard *et al,* 1986; Seshadri and Gopaldes, 1989 [studies 2-4]; Pollitt *et al,* 1989; Soewondo *et al*, 1989; Bruner *et al*, 1996; Metallinos-Katsaras *et al*, 2004). .

377. Seven of the 9 double-blind randomised controlled trials used cognitive tests as outcome measures. Seshadri and Gopaldes (1989) (study 2) reported that the children in the iron treated group were significantly better than the control group in verbal and performance tasks, however children were given folic acid with iron, which may have had an independent benefit. In study 3 (Seshadri and Gopaldes, 1989), the treated group improved significantly in most of the cognitive tests while the placebo group did not. In study 4 (Seshadri and Gopaldes, 1989), iron treated children improved more than the placebo group in 2 of 4 tasks. Only study 4 (Seshadri and Gopaldes, 1989) reported difference between the groups in change of scores but analysis was restricted to anaemic children.

378. Two studies reported significant improvements with iron treatment of children with iron defiency anaemia in speed of processing (Metallinos-Katsaras *et al*, 2004) and with non-anaemic iron deficient girls in memory (Bruner *et al*, 1996) but not in other tests. No significant treatment effects were reported in two other trials (Deinard *et al*, 1986; Soewondo *et al*, 1989).

379. Two randomised controlled trials examined school achievement (Soemantri *et al*, 1985; Pollitt *et al,* 1989). A significant improvement with iron treatment was reported in the study by Soemantri *et al* (1985) but not in the study by Pollitt *et al* (1989). The

criteria for iron deficiency anaemia was higher in the study by Pollitt *et al* (1989) (haemoglobin<120 g/L compared to <110g/L) and haemoglobin concentration of the placebo group improved, reducing the difference in haemoglobin concentration between the treatment and placebo groups. The quality of schooling may also play a role.

Summary of findings from supplementation trials with children >3 years

380. Out of 8 randomised controlled trials in which cognitive tests were outcome measures, two reported clear benefits, 4 reported findings suggestive of benefits but did not report an analysis of change in scores between treated and placebo groups, and two reported no effects of iron treatment.

381. One of two randomised controlled trials assessing school achievement found benefits with iron treatment.

Limitations of supplementation trials

382. Common problems with the intervention studies were that they were often not double blind and some were not randomised. Other studies were confounded with substances such as infant formula, cows' milk or additional micronutrients. Many studies lacked statistical power because of small sample sizes or due to little difference between the groups in iron status measures even after treatment. Early studies often failed to conduct analyses by intention to treat or to control for initial scores. When batteries of tests were used, several different scores from each test were often examined increasing the possibility of observing spurious significant effects. Events in early childhood may show immediate or delayed effects that are transient or sustained, however few trials followed the children after treatment ceased. Lastly, there is a paucity of data on high-risk children (for example, those with low birthweight, undernourished) as they were excluded from most studies.

Cut off levels at which haemoglobin concentration affects development

383. Few investigators have determined the threshold levels of haemoglobin concentration which may be associated with developmental decline. Deficits in motor development have been reported at haemoglobin concentrations below 105-110 g/L and declines in mental development have been reported at concentrations below 100-110 g/L, but the actual thresholds of haemoglobin concentration at which these deficits occur have not been identified.

384. Response to iron treatment of children with different haemoglobin concentrations provides some help with assessing the haemoglobin concentrations below which children's development is affected. However, it is possible that deficits are irreversible. There are currently no consistent findings from such trials.

385. Improvements in motor development following iron treatment were reported at haemoglobin concentrations below 80 g/L and benefits in language development at all concentrations (Stolzfus *et al*, 2001); however, these children had a high prevalence of malaria which reduces haemoglobin concentrations independent of iron and other nutritional deficiencies. Another trial (Bruner *et al*, 1996) found benefits (but only in one of many measures) in non-anaemic (haemoglobin>120g/L) girls aged 13-18y with reduced iron depots (serum ferritin <12µg/L). Three smaller trials, however, did not find benefits of iron supplementation in non-anaemic,

potentially iron deficient children aged over 3 years (Pollitt *et al,* 1989; Soewondo *et al,* 1989; Pollitt *et al,* 1985) or under 3 years (Idjradinata and Pollitt, 1993). In one study (Hasanbegovic *et al,* 2004), children with haemoglobin concentrations below 95 g/L had lower mental and motor development scores than those with concentrations between 95-110 g/L and both were lower than non-anaemic children (haemoglobin>110g/L) suggesting a relation between severity of iron deficiency and development.

386. Based on current data, it is difficult to derive thresholds of iron deficiency measures at which cognitive, motor and behavioural development might be at risk. However, the evidence suggests that at haemoglobin concentrations above 100-110 g/L the risks of such deficits is less than at concentrations below this range, although the deficits may not be solely attributable to iron deficiency.

Summary and conclusions

387. Evidence from observational studies suggests iron deficiency and iron deficiency anaemia are usually associated with many psychosocial, economic and biomedical disadvantages, which can independently affect development. It remains possible that measured and unmeasured environmental variables could explain these findings.

388. Evidence from randomised controlled trials of iron supplementation suggests that iron deficiency anaemia is associated with poor motor development in children in the first 3 years of life. The long-term implications of these finding are unknown.

389. There are insufficient rigorous randomised controlled trials to assess whether iron deficiency or iron deficiency anaemia affects cognitive or language development in children ≤ 3y. The relatively short duration of follow up in the trials may explain the lack of effect. Also, in the trials of children with mixed iron status and the preventive trials there was generally very little difference in iron status measures between groups at the end of treatment.

390. There is evidence for a beneficial effect of iron treatment on cognitive development in anaemic older children. However, none of the trials reported long term follow-up of children to determine whether any benefits were sustained. There is insufficient evidence to determine the effect of iron treatment on school achievement.

391. Based on current evidence, it is not possible to derive thresholds of iron deficiency at which cognitive, motor and behavioural development might be at risk.

7. HEALTH CONSEQUENCES OF HIGH IRON INTAKE AND HIGH IRON BURDEN

Recommended upper intake levels for iron

UK

392. In May 2003, the Expert Group on Vitamins and Minerals (EVM) reported on the safety of vitamin and mineral supplements and recommended maximum advisable levels of intake. Safe Upper Levels (SULs) were established when supported by sufficient data; the SUL represents an intake that can be consumed daily over a lifetime without significant risk to health. A Guidance Level (GL) on the safe level of intake was set when the evidence base was inadequate to establish an SUL. GLs represent an approximate indication of levels that would not be expected to cause adverse effects; since GLs are derived from limited data, they are less secure than SULs.

393. The EVM concluded that there were insufficient data to establish an SUL for iron, but set a GL for supplemental iron intake of 17 mg/day[18]. Iron supplements at doses of 50-220 mg/d and above are associated with gastrointestinal effects, including constipation, nausea, diarrhoea, and vomiting (EVM, 2003).

USA

394. In the USA, the Institute of Medicine (IOM) set a Tolerable Upper Intake Level[19] (UL) for iron of 45 mg/day[20] for adults ≥19 years (National Academy of Sciences, 2001) based on gastrointestinal effects. There were insufficient data to determine the UL based on the other effects that were considered (impaired zinc absorption, increased risk of cardiovascular disease and cancer, systemic iron overload).

Europe

395. In Europe, the Scientific Panel on Dietetic Products, Nutrition and Allergies concluded that the available data were insufficient to establish a UL for iron (EFSA[21], 2004). Adverse gastrointestinal effects reported after short-term oral dosage of supplemental iron (50-60mg) were not considered a suitable basis to establish a UL for iron from all sources. A UL was not established based on increased risk of chronic diseases (cardiovascular disease, diabetes, cancer) due to lack of convincing evidence or on iron overload due to a poor correlation between iron intake and biochemical indicators of iron status.

Physiological consequences of high iron Intakes and overload

396. Iron can exert a range of acute and chronic adverse effects by facilitating oxidative reactions or by competing with other transition metals of nutritional importance (e.g. copper, zinc) in a number of physiological processes.

397. Theoretically, iron may exert adverse effects under a number of conditions. It may have detrimental effects on the gut through direct interaction with the intestinal mucosa or on other tissues if regulation of iron absorption from the intestine is

[18] The GL is not applicable to those with a susceptibility to iron overload associated with haemochromatosis (see section x, chapter 2).
[19] The Tolerable Upper Intake Level represents the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population.
[20] The UL does not apply to individuals with haemochromatosis.
[21] European Food Safety Authority

impaired or overloaded, if iron deposition in tissues is impaired, or if iron is inappropriately released from tissue depots (see section 2).

Interaction with zinc and copper

398. Since iron, zinc, and copper have similar physical and chemical characteristics and share similar absorption and transport mechanisms, they are thought to compete for uptake and transport pathways (Sandström, 2001). Consequently iron supplementation can impair the uptake and use of zinc and copper by the body. Antagonistic interactions between these minerals may have negative consequences on functional outcomes such as growth and development in populations with higher requirements such as infants, adolescents, and pregnant and lactating women (Sandström, 2001).

Interaction with zinc

399. Sandström *et al* (1985) examined the effect of iron on zinc absorption in humans (n=55). Zinc absorption was determined from measurement of whole body retention 14 days after consumption of test solutions or meals labelled with a zinc radioisotope (^{65}Zn). Addition of iron to an aqueous solution of zinc (40 µmol/2.6mg) in a 1:1 molar ratio reduced zinc absorption from 74% to 58%; increasing the iron:zinc molar ratio to 2.5:1 had no further effect, however at a ratio of 25:1, zinc absorption was significantly reduced to 34%. Addition of iron to test meals at the same molar ratios of iron to zinc did not decrease zinc absorption (25, 23, and 22% respectively).

400. A review of randomised controlled trials, assessing the interactive effects of iron and zinc supplementation on markers of iron (serum ferritin and haemoglobin concentration) and zinc (serum zinc concentration) in children under 5y and women of child bearing age, reported that, in most studies, iron supplementation did not affect serum zinc concentration (Fischer Walker *et al*, 2005); however, these interactions are confounded by other factors, particularly the diet, and plasma zinc concentration may not be an adequate indicator of zinc status. There were insufficient data to assess the effects of joint iron and zinc supplementation on growth.

Interaction with copper

401. Studies using Caco-2 cells have shown that iron and copper directly compete for uptake across the apical membrane (Tandy *et al*, 2000; Arredondo *et al*, 2006) and studies in animal models have shown adverse effects of high levels of dietary iron on copper metabolism (Johnson & Hove, 1986; Storey & Greger, 1987). There are limited data from human studies on iron and copper interactions. Haschke *et al* (1986) reported that infants (n=7) fed formula containing low concentrations of iron (2.5 mg/L) absorbed significantly more copper ($p<0.01$) than when they were fed formula with high iron concentration (10.2 mg/L). In another study Lönnerdal and Hernell (1994) compared infants (n not specified) fed formula containing 4 mg/L of iron with those receiving formula containing 7 mg/L from 1.5 to 6 months of age; both formulas contained 0.04 mg/L of copper. Although serum copper concentrations were similar at the start of the study, infants fed the formula with the highest iron concentrations had significantly lower ($p<0.05$) serum copper levels at 6 months. Morais et al (1994) reported that serum copper concentrations in iron deficient (serum ferritin=3.9 µg/L; haemoglobin=102g/L) children (n=31; median age 32 months) after 2 months of iron therapy (5mg/kg/day) were significantly lower ($p=0.010$) than they were before iron therapy. In a study of low birth weight infants

(n=55) randomly assigned to receive daily supplements of either 13.8 mg iron, 7 mg iron or no elemental iron (iron edetate) from age 28 d to 20 wks, activity of the copper dependent enzyme, superoxidase dismutase, was significantly lower in infants given 13.8 mg iron compared to those in the group given 7 mg/d ($p<0.05$) or none ($p<0.01$) (Barclay *et al,* 1991).

The role of iron as a pro-oxidant

402. The ability of iron to gain or lose single electrons makes it an efficient catalyst for free-radical reactions. Fe^{2+} (ferrous form) and Fe^{3+} (ferric form) which have 4 and 5 unpaired electrons in each configuration respectively, and Fe^{4+} (ferryl species), can exist in biological systems. Ferryl species are generated when certain haem moieties react with hydrogen peroxide (H_2O_2).

403. Haemoglobin and myoglobin contain haem iron and undergo oxidation to form superoxide and Fe^{3+} protein; both can initiate damage when they react with peroxide. Haemoglobin and myoglobin will degrade to haem and iron ions, which can in turn stimulate lipid peroxidation, protein oxidation and DNA oxidation. Haem iron can also catalyse the decomposition of pre-existing lipid peroxides to alkoxy and/or peroxyl radicals, causing cellular damage and leading to cell death (McCord *et al*, 1998).

404. Cells have evolved protective mechanisms to remove free radicals. These include superoxide dismutase which converts superoxide to H_20_2 and catalase, peroxidase and glutathione peroxidase that reduce peroxides and H_20_2. Under normal conditions these defence mechanisms are effective at scavenging free radicals generated by iron-dependent and iron-independent mechanisms.

405. Free radical defence systems are impaired in certain diseases and it has been proposed that this is an important factor in pathogenic processes. Concentrations of antioxidants in animals fed an iron-rich diet have been shown to be lower than those in animals receiving less dietary iron (Kuratko, 1998). Evidence to support the role of antioxidant nutrients in humans is mainly associative. Intervention trials have shown either no beneficial effect or adverse effects of antioxidant nutrients on cancer risk (WCRF, 2007).

Iron overload

406. Iron overload occurs when excess iron accumulates in the body. It can be caused by increased absorption of dietary iron or by parenteral iron loading (repeated blood transfusions) as there is no mechanism to excrete excess iron (see section 2).

Increased iron absorption

407. Iron absorption is increased with: the haemochromatoses (see Table 2.2, section 2); rare genetic disorders of iron metabolism which include atransferrinaemia, aceruloplasminaemia; and sub-Saharan dietary iron overload (see paragraph 58).

408. Chronic liver disease (e.g. alcoholic cirrhosis) and porphyria cutanea tarda (associated with homozygosity for HFE C282Y gene) may also be associated with iron loading. Ineffective erythropoiesis is associated with increased iron absorption in severe thalassaemia disorders, e.g., β thalassaemia major and intermedia; sideroblastic anaemias (either inherited or acquired).

Parenteral iron loading

409. The rate of iron loading through regular transfusions is considerably greater than the maximum possible through increased iron absorption. One unit of red blood cells delivers approximately 200 mg iron, which means that individuals with transfusion-dependent anaemias are at significant risk of iron overload. With congenital anaemias, such as β-thalassaemia major, this can lead to the accumulation of up to 100 g iron by 20 years of age, by which time most patients would have died from toxic effects of the excess iron (Modell, 1979). Complications associated with iron overload include cardiac arrhythmias and heart failure, diabetes, delayed onset of puberty, and cirrhosis (Pippard, 1994).

Health consequences of high iron intakes

410. It has been hypothesised that high tissue iron concentrations carry an increased risk of: neoplasia (Nelson, 1992); atherosclerotic disorders (Sullivan, 1981; Kent & Weinberg, 1989); infection (Kent & Weinberg, 1989); neurodegenerative disorders (Thompson *et al*, 2001); and inflammatory conditions (Halliwell & Gutteridge, 1984).

411. This report focuses on the relationship of iron intakes and iron status with cancer and cardiovascular disease (CVD) risk as these were considered to be the two main issues of public health concern in the UK. Studies of colorectal cancer and meat intake, the main source of haem iron, are also considered. Other conditions that have been associated with high intakes/status of iron are only considered briefly.

412. Slightly higher mean concentrations of serum ferritin and serum transferrin saturation have been observed in individuals heterozygous for the C282Y and H63D mutations of the HFE gene. Although these markers are still within the accepted reference range, this population might provide useful information on whether higher body burdens of iron are associated with increased risk of ill health.

Epidemiological studies of iron and chronic disease

413. The majority of data on the relationship between iron and chronic disease are based on epidemiological studies which have a number of important limitations. These include the difficulty of obtaining reliable estimates of iron exposure (intake) and accurate measures of iron burden as well as confounding by other dietary and lifestyle factors that have been associated with chronic disease risk.

414. Assessments of iron intakes in epidemiological studies are not very reliable because of errors in estimated intakes of foods and supplements, because values in food composition tables may not be accurate, and because of continually changing exposure to iron caused by the constant introduction or removal of foods voluntarily fortified with iron. There is considerable variation in the haem iron content (22-80% of total iron) depending on the food (Carpenter and Clark, 1995), however most studies assume haem iron content to be 40% of total iron. There are also difficulties in classifying and assessing intakes of meat (see paragraphs 448-451), which is an important source of iron. Serum-based measures of iron depots, distribution, and use, are more objective but there are problems with their interpretation (see section 4) and serum samples need to be collected some years before diagnosis of the disease.

415. Measures of iron intake and iron status are subject to confounding by both dietary and non-dietary variables. For example, high iron intakes in western populations

may be due to high intakes of meat and could therefore be associated with other nutritional factors related to meat, e.g., saturated fats. Associations between meat consumption and cancer risk might also be affected by other differences in diet, such as variations in fibre, fruit, and vegetable consumption. Serum markers of iron metabolism are affected, for example, by blood loss (e.g. menstruation, or gastro-intestinal disease), diurnal variation, infection, and inflammation.

416. In this respect, data on disease risk among heterozygotes for hereditary haemochromatosis are potentially useful because individuals with this genotype have a marginally elevated mean body iron load (see paragraph 412), which is not associated with lifestyle factors. However, even if heterozygosity for hereditary haemochromatosis is associated with increased risks for chronic diseases this does not mean that dietary iron has the same effect. Results from these studies were, therefore, not given as much weight in the overall evaluation of possible adverse effects of high intakes of dietary iron.

Iron and cancer

417. Although it is uncertain whether, or how, iron might be carcinogenic, evidence suggests that oxygen free radical formation catalysed by iron might play a role in this process (Toyokuni, 1996; Okada, 1996). Two main pathways have been suggested: increased DNA damage induced either directly or indirectly by impeding DNA repair; and modulation of nuclear redox sensitive transcriptional regulators through signal transduction mechanisms (Galaris and Evangelou, 2002). Haem iron, but not inorganic iron, also increases production of N-nitroso compounds in the lumen of the gastrointestinal tract (Cross *et al*, 2003) which have been shown to be human and animal carcinogens (IARC[22], 1978). In addition, iron is a limiting nutrient for the growth and replication of cancer cells in the human body (Weinberg, 1984).

418. Animal studies have shown that iron is essential for proliferation of neoplastic cells (Siegers, 1991). Iron supplementation was found to enhance the rate of tumour growth in mice with chemically induced colonic neoplasia (Siegers *et al*, 1992); the increase in tumour rate was dependent on the iron concentration in the diet. Hann *et al* (1988) observed that tumour growth in mice inoculated with colonic adenoma-carcinoma cells was decreased after they were fed an iron deficient diet.

419. Attention has mainly focused on the relationship between iron and colorectal cancer, based on the hypothesis that high intakes of iron might increase colorectal cancer risk by intraluminal (Graf and Eaton, 1985) or systemic effects (Stevens and Kalkwarf, 1990). Since most dietary iron is not absorbed, luminal exposure to excessive dietary iron may result in direct oxidative damage to the colorectal lumen. Higher concentrations of body iron may increase cancer risk by increasing oxidative stress to cells and by providing iron for growth and replication of cancer cells.

Epidemiological studies of iron and colorectal cancer

420. The relationship between dietary iron and cancer was considered by COMA in their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), which reviewed studies on iron and cancer published up to 1996. Based on the available evidence at that time, COMA concluded that high iron stores may be related to increased risk of colorectal cancer. However, it was noted that iron stores may not be related directly

[22] International Agency for Research on Cancer

to iron intake and further data from prospective studies would be required to confirm whether iron intake has a role in colorectal cancer.

421. The World Cancer Research Fund (WCRF) considered 4 cohort studies on iron intake and colorectal cancer (Wurzelmann *et al,* 1996; Glynn *et al*, 1996; Kato *et al*, 1999; Konings *et al*, 2002) and 1 cohort study on haem iron intake and colorectal cancer (Lee *et al*, 2004) (WCRF, 2007). Four studies suggested an increased risk of colorectal cancer among people with the highest iron intake compared to those with the lowest intake, which was statistically significant in 2 studies (Wurzelmann *et al*, 1996; Lee *et al*, 2004). The report concluded there was limited evidence that foods containing iron are a cause of colorectal cancer and commented that the evidence was "*sparse, of poor quality, and inconsistent*".

422. In this report, consideration of the relationship between iron and colorectal cancer is based on prospective studies published since 1996[23] which have examined the relationship between colorectal cancer and dietary iron (including supplements) and serum ferritin concentration (however, serum ferritin concentrations do not necessarily represent the amount of iron to which the colorectal mucosa has been exposed). Studies of individuals heterozygous for hereditary haemochromatosis were also considered. Details of these studies, including sample size, duration of follow-up, and allowance made for confounding factors, can be found in Annex 4 (Tables 7.1-7.4).

423. Five prospective studies (Table 7.1) have reported on dietary iron and colorectal cancer; most studies did not include iron intake from dietary supplements. Six out of the 7 relative risks reported were above one; of these, 1 was significant. The median relative risk was 1.08.

424. Four prospective studies have reported on dietary haem iron and colorectal cancer risk (Table 7.2). Two of the studies (Lee *et al*, 2004; Larsson *et al*, 2005) assumed haem iron content from all types of meat to be 40% while in the other two studies (Balder *et al*, 2006; Kabat *et al*, 2007) haem iron content was estimated according to the type of meat; Kabat *et al* (2007) found similar results using the 2 different methods. Five of the 6 relative risks were above 1; none were significant. The median relative risk was 1.26.

425. Two small prospective studies (Kato *et al*, 1999; Cross *et al*, 2006) have reported on serum ferritin concentration and colorectal cancer risk (Table 7.3). Both observed a significant reduction in risk with increasing serum ferritin concentrations. In the study by Kato *et al* (1999) the authors suggest that low ferritin concentrations might be caused by blood loss associated with preclinical colorectal cancer because the average time between blood donation and cancer diagnoses was only 4.7 years. Cross *et al* (2006) suggest that low serum ferritin concentrations might: indicate that less iron was absorbed and therefore more was present in the gut lumen where it could exert direct oxidative damage, be due to increased iron requirement for tumour growth; or, although all cases were diagnosed at least 5 years after blood collection, that bleeding from a tumour could explain the lower serum ferritin concentrations because of the long induction period (5-10 years) associated with colorectal cancer.

[23] In their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), COMA considered studies published up to 1996.

Hereditary haemochromatosis and cancer

426. Hepatocellular carcinoma, the main form of liver cancer, is very strongly associated with haemochromatosis. Bradbear *et al* (1985) first quantified the excess risk for hepatocellular carcinoma as 200-fold in patients with genetic haemochromatosis. Subsequent studies have confirmed this strong association (Hsing *et al,* 1995; Fracanzani *et al*, 2001) and indicated that the increased risk generally follows the development of cirrhosis.

427. The risk for cancers other than hepatocellular carcinoma in patients with hereditary haemochromatosis has also been investigated in several small studies. Bradbear (1985) reported no excess of non-hepatocellular cancers, based on 8 cancers in a cohort (n=208) of hereditary haemochromatosis patients. Fracanzani *et al* (2001) reported that the risk for non-hepatic cancers among 230 patients with hereditary haemochromatosis compared to 230 patients with non iron–related chronic liver disease was 1.8 (95% CI, 0.8-4.0). Geier (2002) reported that, among 59 patients with hereditary haemochromatosis, there were 13 non-hepatocellular cancers and a standardized incidence ratio of 1.40 ($p<0.04$).

Heterozygosity for type 1 genetic haemochromatosis and colorectal cancer

428. Seven studies have assessed the association between heterozygosity for genetic haemochromatosis and colorectal cancer (Table 7.4).

429. Six out of the 8 relative risks reported were greater than one; 1 of these was statistically significant (Nelson *et al*, 1995). The median relative risk was 1.05.

430. In the study which reported a significantly increased risk of colorectal cancer associated with C282Y heterozygosity (Nelson *et al*, 1995), colorectal cancer risk was assessed from postal questionnaires sent to individuals homozygous for genetic haemochromatosis regarding the health histories of their parents (who were assumed to be heterozygotes). Their spouses were asked to complete questionnaires on the health histories of their parents (who were assumed not to be heterozygotes). HH heterozygosity was not confirmed by DNA analysis and colorectal cancer was not confirmed by hospital records.

431. Robinson *et al* (2005) reported that although C282Y or H63D heterozygosity was not associated with colorectal cancer risk, individuals who were compound heterozygotes (C282Y/H63D) were at increased risk of colorectal cancer compared with those with a single mutation (OR, 3.03; 95% CI, 1.06-8.61; p=0.038); however this finding did not reach statistical significance after adjustment for multiple *post hoc* testing.

Meat and colorectal cancer

432. There are a number of plausible biological mechanisms for an association between meat and colorectal cancer. Meat, particularly red meat, contains high levels of haem iron, which is proposed to catalyse the production of free radicals. Consumption of red and processed meat, but not white meat or fish, is associated with increased endogenous production of potentially carcinogenic N-nitroso compounds (Bingham *et al*, 2002). Heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons, which are formed when foods are cooked at very high temperatures have also been proposed to increase the risk of colon cancer (Sugimura, 2000). The fat contained in meat may also affect colorectal cancer risk

by increasing production of secondary bile acids which have been associated with promoting colon cancer (Narisawa *et al*, 1978). The potential mechanisms are considered further in Annex 5. In addition, people eating diets high in meat may eat less fruit and vegetables, foods which might be protective against cancer risk (WCRF, 2007).

433. Several studies have examined the association between red and processed meat intake and colorectal cancer risk. There are a number of difficulties in interpreting the results from these studies including lack of consistency in definitions of red and processed meat, adequacy of dietary assessment methods, and variability in quantification of intakes. These limitations are discussed in further detail in paragraphs 446-454.

434. The relationship between meat intake and colorectal cancer was previously considered by COMA in their report, *Nutritional Aspects of the Development of Cancer* (DH, 1998), which reviewed epidemiological studies of meat and colorectal cancer risk published up until 1996. The report concluded that evidence from cohort studies was: inconsistent for an effect of total meat consumption on risk of colorectal cancer; moderately consistent for a positive association between consumption of red or processed meat and risk of colorectal cancer; moderately consistent that poultry (white meat) and fish were not associated with colorectal cancer risk. COMA recommended that average consumption of red and processed meat (90g/day cooked weight or 8-10 portions/wk which was current at that time) should not increase and that higher consumers (above 140 g/day or 12-14 portions/week) should consider reducing their intakes.

435. A meta-analysis of 13 cohort studies investigating the relationship between meat and colorectal cancer risk (Sandhu *et al*, 2001) reported that an increase in intake of 100 g/day of all meat significantly increased risk of colorectal cancer 12-14%, 100 g/day red meat significantly increased risk by 13-17%, and 25 g/day increase in intake of processed meat was associated with an increased risk of 49%.

436. A subsequent meta-analysis considered epidemiological studies of meat and colorectal cancer risk published between 1973-1999 (Norat *et al*, 2002); 23 studies of red meat (9 cohort; 14 case-control) and 23 studies of processed meat (7 cohort; 16 case-control) were included in the meta-analysis. Compared to lowest level of intake, highest level of red meat consumption was associated with a significant increase in colorectal cancer risk: RR, 1.35 (95% CI, 1.21-1.51) for cohort studies and 1.36 (95% CI, 1.17-1.59) for case-control studies; the RR for an increase of 120g/day of red meat was 1.24 (1.08-1.41) based on cohort and case control studies and 1.22 (95% CI, 1.05-1.41) based only on the cohort studies. Processed meat was also associated with a significant increase in risk: RR, 1.39 (95% CI, 1.09-1.76) for cohort studies and 1.29 (95% CI, 1.09-1.52) for case-control studies; the RR for an increase of 30g/day of processed meat was 1.36 (1.15-1.61) based on cohort and case control studies and 1.54 (95% CI, 1.10-2.17) based just on the cohort studies. No significant association was found between total meat consumption and colorectal cancer risk.

437. Another meta-analysis (Larsson, 2006) included prospective studies published up to March 2006 (15 studies of red meat, 14 studies of processed meat). The relative risk of colorectal cancer for individuals in the highest category of red meat consumption compared with those in the lowest category was 1.28 (95% CI, 1.15-1.42); an

increase of 120 g/day of red meat was associated with a relative risk of 1.28 (95% CI, 1.18-1.39). The relative risk of colorectal cancer for individuals with the highest compared to the lowest processed meat intake was 1.20 (95% CI, 1.11-1.31) and an increase of 30g/d of processed meat intake was associated with a relative risk of 1.09 (95% CI, 1.05-1.13).

438. The relationship between red and processed meat intake and colorectal cancer risk was also considered by the WCRF (2007). Sixteen cohort studies on red meat intake and colorectal cancer risk published between 1975 and 2005 were identified; all showed an increased colorectal cancer risk for highest v lowest intake, which was significant in 4 studies. Meta-analysis of 7 studies that measured red meat intake in times/week and 3 studies that measured g/day reported relative risks of 1.43 (95% CI, 1.05-1.94) per time/day and 1.29 (95% CI, 1.04-1.6) per 100 g/day. Fourteen cohort studies on processed meat and colorectal cancer risk, published between 1990 and 2005, were considered. Twelve studies showed an increased colorectal cancer risk for highest versus lowest intake, which was statistically significant in 3 studies. A meta-analysis of 5 of these studies (which assessed consumption in g/day) reported a relative risk of 1.21 (95% CI, 1.04-1.42) for 50g/day of processed meat intake.

439. The WCRF concluded that red and processed meat *"is a convincing cause of colorectal cancer"* and recommended that the population average consumption of red meat[24] should be no more than 300g per week and that very little if any should be processed. On an individual basis, the report recommended that people who eat red meat should not consume more than 500g/week, with very little or any processed meat.

440. The WCRF report also noted that there was insufficient evidence to draw any conclusions on the association between poultry (white meat) and colorectal cancer risk and limited evidence that eating fish protects against colorectal cancer.

Prospective studies of red and processed meat intake and colorectal cancer risk

441. In this report prospective studies published after August 1996[25], on the association between red meat and processed meat intake and colorectal cancer, are considered in more detail (Annex 4, Tables 7.5-7.6).

Red meat intake and colorectal cancer risk (Table 7.5)

442. Twenty-one prospective studies have been published since 1996 (including some updated analyses from previously published cohorts). Two of these studies (Balder *et al,* 2006; Sato *et al*, 2006) considered the risk of colorectal cancer with total meat consumption rather than only red meat. Twenty-one out of the 25 relative risks reported were greater than one, 3 significantly so; the median relative risk for highest vs lowest red meat intake was 1.17. The increased relative risk was statistically significant in 1 out of the 4 largest studies (Wei *et al*, 2004; Chao *et al*, 2005; Norat *et al*, 2005; Cross *et al*, 2007); the trend was statistically significant in 2 of the 4 studies and close to significance in 1 study.

[24] Defined as beef, pork, lamb, goat, including that contained in processed foods
[25] The COMA report, '*Nutritional Aspects of the Development of Cancer'* considered cohort studies up to August 1996.

443. Significantly increased colorectal cancer risk was associated with red meat intakes of: ≥ 114 g/d for men, ≥ 80 g/d for women (Chao *et al*, 2005); ≥ 94 g/day (Larsson *et al*, 2005); and 62.7 g per 1000 kcal (equivalent to 145 g/d for men and 102 g/d for women[26]) (Cross *et al*, 2007). In all the studies which reported a significantly increased risk of colorectal cancer, processed meat was included under the category of red meat.

Processed meat intake and colorectal cancer risk (Table 7.6)

444. Fourteen prospective studies have been published since 1996 (including some updated analyses from previously published cohorts). Thirteen out of the 18 relative risks for highest compared to lowest processed meat intake were greater than one, 5 significantly so; the median relative risk was 1.16. The relative risk was statistically significant in 3 out of the 4 largest studies (Wei *et al*, 2004; Chao *et al*, 2005; Norat *et al*, 2005; Cross *et al*, 2007) and the trend was statistically significant in all the studies.

445. Significantly increased colorectal cancer risk was associated with processed meat intakes of: ≥ 20.3 g/day (Oba *et al*, 2006); ≥ 29 g/d (English *et al*, 2004), ≥ 80 g/day (Norat *et al*, 2005); 22.6 g per 1000 kcal (equivalent to 52.3 g/d for men and 36.9 g/d for women[27]) (Cross *et al*, 2007) and 5 times per week or more (Wei *et al*, 2004).

Limitations in interpreting the results from prospective studies on red and processed meat intake and colorectal cancer risk

446. The majority of prospective studies published after 1996, suggest that high intakes of red and processed meat are associated with an increased risk for colorectal cancer. Although the increased risk was not statistically significant in most studies, this would be expected if the studies were not adequately powered to detect a significant association.

447. There are a number of methodological inconsistencies between the different studies which make comparisons difficult. These include: adequacy of the dietary assessment methods to obtain reliable estimates of red and processed meat intake; lack of consistency in the categorisation of red and processed meat; and variability in the reporting of quantities of red and processed meat intake.

448. In most studies red meat and processed meat intake is based on a single dietary assessment at the start of the study. This does not take account of changes in dietary patterns over a number of years and could therefore be an unreliable estimate of intake over the specified follow-up period. The relevant period between dietary intake and development of cancer is also uncertain and dietary intakes estimated at baseline may not be the relevant period for dietary assessment in relation to cancer risk.

449. There are also considerable inconsistencies between studies in categorisation and definition of red and processed meat. Some studies have collected detailed information of the foods included under the red and processed meat categories, while others have used very broad classifications (e.g., beef, pork, lamb). In addition, some studies have separated red and processed meat categories and have only

[26] Based on average total energy intake of 2313 kcal/day for men and 1632 kcal/day for women reported in the 2000/1 NDNS survey (Henderson et al, 2003).
[27] Based on average total energy intake of 2313 kcal/day for men and 1632 kcal/day for women reported in the 2000/1 NDNS survey (Henderson et al, 2003).

included fresh or untreated red meat in the red meat category while other studies have also included processed meat under the red meat category.

450. Another difficulty is the variability in reporting quantities of red and processed meat intake. While most studies compared highest versus lowest red and processed meat intake in grams per day/week/month, some studies reported intake as g/1000 kcal, frequency of intake per week/month or servings/day. There are also large differences in the quantiles of intake between different studies so that the amounts in lowest quantiles described in some studies are higher than the top quantiles in other studies: for red meat, intakes in the highest quantiles ranged from more than 40-158 g/d and in the lowest quantile from 10-61 g/d; for processed meat, intakes in the top quantiles ranged from 16.3 g/d to 80 g/d or more and intakes in the lowest quantiles ranged from 0-12 g/day.

451. Some studies also analysed the association between colorectal cancer risk and red/processed meat intake for men and women combined although intake in the highest and lowest quantiles differed by sex (Brink *et al*, 2005; Chao *et al*, 2005). In the study by Chao *et al* (2005), a significant association was found for men and women combined in the highest compared to the lowest quintile of red meat intake, however intakes of men in the highest quintile were above 114 g/d whilst for women they were above 80 g/d.

452. Although results from prospective studies of dietary fibre and colorectal cancer are inconsistent, it has been suggested that higher intakes of foods containing fibre may protect against colorectal cancer risk (WCRF, 2007; Bingham *et al,* 2003). However only 8 out of the 21 studies on red meat intake and colorectal cancer risk and 5 out of the 14 studies on processed meat intake and colorectal cancer risk adjusted for fibre intake.

453. There are also a number of other factors that have been associated with colorectal cancer risk. These include genetic predisposition, high total fat intake, low fruit and vegetable intake, low fibre intake, low physical activity, and meat preparation and cooking methods. Studies have varied in the adjustments made for all these factors, so the effects of confounding cannot be excluded.

454. All these factors make it difficult to quantify a level of red or processed meat intake that may be associated with colorectal cancer risk. The effect of a recommendation to reduce the consumption of red meat and processed meat intake on iron and zinc intakes in the UK is considered in section 10.

455. Toxicological data examining whether differences in colorectal cancer risk between white, red, and processed meat can be explained on the basis of heterocyclic amines, N-nitroso compounds, or haem iron can be found in Annex 5.

Epidemiological studies of iron and cardiovascular disease

456. Premenopausal women have a lower incidence and mortality from coronary heart disease (CHD) compared to men and to postmenopausal women (Wingard *et al*, 1983; Lerner & Kannel, 1986). It has been proposed ('iron hypothesis') that this is due to higher levels of stored iron in men and postmenopausal women (Sullivan, 1981). The iron hypothesis suggests that menstrual iron loss protects against CHD

and that depletion of body iron stores though blood donation would also be protective against CHD risk (Sullivan, 1991).

457. Two prospective studies reported that, after adjustment for other cardiovascular disease (CVD) risk factors, blood donation was associated with a reduced risk of CVD (Meyers *et al*, 1997; Salonen *et al*, 1998). Meyers *et al* (1997) reported that the occurrence of CVD events in a cohort of men and women (n=3855) followed for 5-8 years was significantly lower only in non-smoking male blood donors compared to non-donors (OR, 0.67; 95% CI, 0.45-0.99). Salonen *et al* (1998) reported a significantly reduced risk of acute myocardial infarction in blood donors compared to non-donors (relative hazard=0.12, 95% CI, 0.02-0.86; p=0.035) in a cohort of men (n=2862) followed for 9 years. However, a much larger prospective study of men (n=38,244) followed for 4 years found no association between blood donation and risk of myocardial infarction or fatal CHD (Ascherio *et al,* 2001).

458. A meta-analysis of 12 prospective studies assessed the association between CHD and markers of iron status (serum ferritin, transferrin saturation, TIBC, serum iron concentration) (Danesh *et al*, 1999). In 5 studies which assessed CHD and serum ferritin concentration, the combined risk ratio for individuals with serum ferritin ≥200 µg/L compared to those <200 µg/L at baseline was not significantly different from unity (RR, 1.03; 95% CI, 0.83-1.29). Combined analysis of three studies comparing total dietary iron intake of individuals in the top third of intake with those in the bottom third did not find an increased CHD risk (RR, 0.83; 95% CI, 0.66-1.03).

459. The mechanism by which iron could affect heart disease risk is not clear. Based on evidence from cell and animal studies, it has been suggested that iron might play a role in the development of atherosclerosis by promoting oxidation of low density lipoprotein cholesterol through catalysing the formation of free radicals or promoting free radical mediated myocardial damage following an ischaemic event (de Valk & Marx, 1999).

460. Details of the studies considering the association between iron and CVD risk are provided in Annex 4 (Tables 7.7-7.11). Limitations associated with epidemiological studies of iron and chronic disease are considered in paragraphs 413-415.

461. Nine prospective studies have reported on total dietary iron and risk of CHD (Table 6.7). Three out of the 10 relative risks comparing highest vs lowest iron intake or by incremental increases in intake were greater than 1, of which one RR was significant; the median relative risk was less than one (0.91).

462. Five prospective studies have reported on the association between haem iron intake and CVD risk (Table 7.8). Only 2 out of the 5 studies explained the method used to calculate the haem iron content of foods containing iron. Four out of the 5 relative risks for highest vs lowest haem iron intake were greater than one and all were significant; the median RR was 1.48. It is possible that the increased risk could be due to other components of meat (the main source of haem iron) associated with increased CVD risk, such as saturated fats or an unmeasured component of meat. Although all the studies adjusted analyses for saturated fat intake, the possibility of residual confounding by saturated fats cannot be excluded because of unreliable estimates of both haem iron and saturated fat intakes. The observed association could also be due to unidentified dietary or lifestyle factors associated with meat intake.

463. Fifteen prospective studies have reported on the association between serum ferritin and CVD risk (Table 7.9). Of the 17 relative risks comparing individuals with serum ferritin measurements in the top quantiles with those in the bottom, with serum ferritin above 200 µg/L compared to those below 200 µg/L or the effect of incremental increases in serum ferritin concentration, 8 were above one, two significantly so; the median relative risk was 1.00.

Heterozygosity for genetic haemochromatosis and cardiovascular disease

464. Studies examining the association between heterozygote carriers of genetic haemochromatosis and CVD risk are very diverse and have considered various CVD endpoints or early and late onset disease. Although there are some prospective studies, the majority are case control studies.

465. A meta-analysis of studies examining the relationship between HFE mutations and CHD reported that C282Y or H63D heterozygotes were not at increased risk for CHD (van der A *et al*, 2006). Seven prospective studies, 11 case-control studies and 1 cross-sectional study were included in the meta-analysis of the C282Y mutation and 2 prospective studies, 8 case-control studies and 1 cross-sectional study were included in the meta-analysis of the H63D mutation. The pooled estimate was1.02 (95% CI, 0.94-1.11) for C282Y heterozygotes and 1.03 (95% CI, 0.96-1.11) for H63D heterozygotes.

466. Eight prospective studies have reported on C282Y heterozygosity and CVD risk (Table 7.10). Six of the 9 relative risks reported were greater than one; 2 of these were significant. The median relative risk was 1.25.

467. Four prospective studies have reported on H63D heterozygosity and CVD risk (Table 7.11). Two of the 5 relative risks reported were greater than one; none were significant. The median relative risk was 0.98.

Other potential adverse effects of high exposures to iron

Negative effects of iron supplements on growth

468. Some studies have reported an adverse effect of iron supplements on growth in iron replete children (Idjradinata *et al*, 1994; Dewey *et al*, 2002; Majumdar *et al*, 2003).

469. A study from Indonesia reported that among iron replete infants (haemoglobin>120g/L; serum ferritin>12 µg/L) aged 12-18 months (n=47), randomised to receive iron supplement (3 mg/kg per day) or placebo for 4 months, the rate of weight gain was significantly lower in those receiving iron supplements compared to those receiving placebo (Idjradinata *et al*, 1994). No difference was found in linear growth.

470. Results from a double-blind randomised controlled trial suggest a negative effect of iron supplements on linear growth and head circumference during infancy (Dewey *et al*, 2002). Full-term infants in Sweden (n=101) and Honduras (n=131) were randomly assigned at 4 months of age to 3 interventions groups: iron supplement (1 mg/kg per day) from 4-9m; placebo 4-6m and iron 6-9m; placebo from 4-9m. All infants were exclusively or near exclusively breast-fed (≤15 ml/d of foods/fluids other than breast

milk and no iron-fortified foods) until 6 months of age. Among the Swedish infants, gains in length and head circumference were significantly lower in those receiving iron supplements compared to those receiving placebo from the age of 4-9m, particularly for the 6-9 month period. In Honduras, the negative effect of iron supplements was observed on linear growth but only from 4-6m among those with initial haemoglobin concentration 110 g/L or above. There were no differences in head circumference among treatment groups.

471. A randomised controlled trial in India (Majumdar *et al*, 2003) of infants aged 6-24m (n=150) reported that, compared to the placebo group, weight gain and linear growth was significantly decreased in iron replete (haemoglobin>110 g/L; serum ferritin>12 µg/L) children (n=50) who were supplemented with iron (2 mg/kg/d); a significant improvement in weight gain and physical growth was seen with iron supplementation (6 mg/kg/d) of iron deficient (haemoglobin=50-110 g/L; serum ferritin<12 µg/L) children compared to placebo.

472. It is possible that iron supplementation of iron replete children inhibits absorption of other essential nutrients required for growth, such as zinc (see paragraphs 398-401). In the study by Dewey *et al* (2002), no differences were found in plasma zinc concentration between the iron treated and placebo treated groups; however plasma zinc concentration is not be an adequate indicator of marginal zinc deficiency.

Neurodegenerative conditions

473. Iron acts as a catalyst in the biosynthesis of the neurotransmitters dopamine and serotonin and is also important in the formation of myelin by oligodendrocytes (Thompson *et al*, 2001). Abnormally high amounts of iron have been detected in brain material from individuals with neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Qian and Shen, 2001).

474. There are no epidemiological data to support a role for dietary iron in neurodegenerative conditions. When regulation of systemic iron is under normal homeostatic control, it is unlikely that excess dietary iron will have any effects on cognitive function or neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. It is possible that local iron homeostasis is disrupted by disease processes associated with neurodegenerative disorders rather than iron *per se* being a primary cause of the pathogenesis.

Arthritis

475. Arthropathy is a common and early clinical manifestation of haemochromatosis. There is, however, little available data regarding iron overload or the frequency of heterozygosity for haemochromatosis in arthritis patients (Worwood, 2002).

476. Rheumatoid Arthritis (RA) is a chronic inflammatory disease that predominantly affects the small joints of the body. It is associated with disturbed metabolism of iron as manifest by the anaemia of chronic disorders. Iron accumulates in the rheumatoid synovial membrane and is largely present within ferritin and haemosiderin; these are labile and the associated iron can have a catalytic oxidant capability leading to a cascade of oxidative damage to lipid, protein and DNA (Halliwell & Gutteridge, 1985).

477. There are limited data on dietary risk factors associated with RA and the existing evidence is inconclusive (Choi, 2005). A prospective nested case-control study in

the UK reported that patients who developed inflammatory polyarthritis (n=88) had a significantly higher median intake of red meat (p=0.04) and red meat combined with meat products (p=0.02) than controls (n=176); individuals with the highest intakes of total red meat combined with meat products were at increased risk of inflammatory polyarthritis compared to those with the lowest intakes (OR, 2.3; 95% CI, 1.1-4.9). However no association was found with iron intake (Pattison *et al*, 2004). A prospective study in Denmark (n=57,053) found no associations between risk of RA and intakes of iron or meat (Pedersen *et al*, 2005). Another prospective study in the USA (n=82,063) also found no significant associations association between RA and intakes of total dietary iron, iron from foods, iron from supplements, haem iron, red meat, or total meat (Benito-Garcia *et al,* 2007).

Diabetes Mellitus

478. Diabetes Mellitus (type 2 diabetes) is a common clinical manifestation of haemochromatosis which has led to the suggestion that high iron depots may increase the risk of developing diabetes. A review (Saudek and Charache, 1992) concluded that the prevalence of haemochromatosis among diabetic patients was low (1%). Results from later studies have been inconsistent. An analysis of diabetic (type 1 & 2) patients (n=727) in the UK reported that the prevalence of haemochromatosis was 0.1% (Turnbull *et al* (1997). Another study in Italy (Conte *et al,* 1998) compared the prevalence of haemochromatosis in diabetic (type 1 & 2) patients (n=894) with matched controls (n=467) and reported an overall prevalence of 1.34% in the diabetic patients compared to 0.2% in the controls; the odds ratio for haemochromatosis in association with diabetes was 6.3 (95% CI, 1.1-37.7). When only patients with type 2 diabetes were considered, the prevalence of haemochromatosis was 1.54% and the odds ratio of haemochromatosis with type 2 diabetes was 7.3 (95% CI, 1.3 to 41.9); the authors concluded that haemochromatosis was underdiagnosed in patients with diabetes.

479. Studies have also examined whether iron depots in individuals without haemochromatosis are associated with diabetes. A prospective study in Finland (Salonen, 1998) which followed 1038 men over a period of 4 years, reported that men with high iron depots (transferrin receptors to ferritin ratio <9.4 µg/ µg) were 2.4 times more likely to develop diabetes (95% CI, 1.03-5.5; p=0.04) than men with lower iron values. A larger prospective study of women (n=32, 826) followed for 10 years (Jiang *et al*, 2004a) reported that, after adjustment for other risk factors for diabetes and for inflammation, those with plasma ferritin concentration in the highest quintile (≥107 µg/L) compared to the lowest quintile (<21 µg/L) were at significantly increased risk of developing diabetes (RR, 2.61; 95% CI, 1.68-4.07; p<0.001).

480. A subsequent prospective study of men and women (n=15,792) followed for 8 years (Jehn *et al*, 2007) reported that the risk of diabetes was significantly increased in those with plasma serum ferritin concentration in the top quintile (355 µg/L) compared to those in the bottom quintile (20 µg/L) after adjustment for age, ethnicity, smoking, and alcohol intake (hazard ratio [HR], 1.74; 95% CI, 1.14-2.65; p<0.001). However, after adjustment for factors associated with metabolic syndrome (HDL cholesterol, waist circumference, hypertension, glucose level, triglyceride level), no relation was found between plasma serum ferritin concentration and diabetes risk (HR, 0.81, 95% CI, 0.49-1.34; p trend=0.87).

481. Another prospective study examined whether dietary iron intake or blood donation was related to diabetes risk in a cohort of men (n=38,394) followed for 12 years (Jiang *et al*, 2004b). After adjustment for other risk factors for diabetes, no association was found between total iron intake and risk of diabetes, however men in the highest quintile of haem intake (median, 1.9 mg/d) compared to those in the lowest quintile (median, 0.8 mg/d) were at increased risk of diabetes (RR, 1.28; 95% CI, 1.02-1.61; p trend=0.045). After further adjustment for red meat intake, no association was found between haem iron and diabetes risk (RR, 0.96; 95% CI, 0.74-1.23; p trend = 0.58). Haem iron intake from sources other than red meat (such as chicken and fish) was not associated with diabetes risk. No association was found between blood donation and diabetes risk.

Summary and conclusions

482. In the UK, the Guidance Level (GL[28]) for supplemental intake has been set at 17 mg/day for adults (EVM, 2003). The GL is based on adverse gastrointestinal effects associated with intakes of supplemental iron (50-220 mg/day).

483. Epidemiological studies of iron and chronic disease have a number of limitations. The most important considerations are reliable assessments of dietary intake and iron status and confounding of iron intake or iron status by other dietary and lifestyle factors.

484. Limited evidence from epidemiological studies on the relationship between iron intake and colorectal cancer risk suggest that increased dietary intakes of total or haem iron are associated with increased risk of colorectal cancer; the increased risk was not significant in most studies but this may be because they may not have had sufficient power to detect an association. Inaccuracies in assessment of total and haem iron could lead to over- or underestimation of risk. Confounding by other dietary and lifestyle factors is also possible. Limited epidemiological data suggest that high iron stores are not associated with increased colorectal cancer risk. Overall, there are insufficient data on the association between intakes of total dietary iron, haem iron, or iron status and colorectal cancer risk to reach clear conclusions.

485. Limited evidence from epidemiological studies suggests that heterozygosity for hereditary haemochromatosis is associated with increased risk of colorectal cancer although this was not significant in most studies; there are insufficient data to reach clear conclusions.

486. Meat, particularly red meat, is the main source of haem iron. Epidemiological studies suggest an increased risk for colorectal cancer in association with increasing intakes of red and processed meat. Although the increased risk is not statistically significant in most studies, this may be due to lack of statistical power to detect an association. Overall, the available evidence suggests that red and processed meat intake is *probably* associated with increased colorectal cancer risk. However, since the evidence is based on prospective observational studies, the effects of confounding by other factors associated with increased colorectal cancer risk cannot be excluded. It is not possible to identify if there is a dose response or a threshold level of red or processed meat associated with increased colorectal cancer risk because of a

[28] The GL is based on limited data and represents an approximate indication of intakes that would not be expected to produce adverse effects.

number of limitations in the available data, including lack of consistency in categorisation and in reporting quantities of red and processed meat intake.

487. Evidence from observational studies of total iron intake or status and CVD do not suggest an association. Limited evidence from prospective studies suggests that high intakes of haem iron increase CVD risk. It is possible that the increased risk could be due to other components of meat (which is the main source of haem iron) associated with CVD risk, such as saturated fats, or dietary and lifestyle factors associated with meat intake. Further long term prospective studies, with more accurate and reliable measures of haem iron intake, are required to confirm this finding.

488. Studies of HFE heterozygosity and CVD risk suggest that C282Y heterozygotes, but not H63D heterozygotes, may be at increased CVD risk; however, there are insufficient data to reach clear conclusions.

489. Limited evidence suggests that iron supplementation may have detrimental effects on the physical growth of iron replete infants and children. Further studies are required to confirm this effect.

490. A number of common neurodegenerative conditions (e.g. Parkinson's disease and Alzheimer's disease) are associated with iron accumulation in the brain. There is no evidence to show dietary iron is associated with these conditions.

491. Evidence for an association between iron intake and rheumatoid arthritis is limited and inconclusive.

492. Overall there is insufficient evidence to suggest an association between iron stores or iron intake and diabetes mellitus in the general population. The power of different analyses vary but, overall, data suggest that being homozygous or heterozygous for either HFE C282Y or HFE H63D variants of hereditary haemochromatosis is not associated with an increased risk of diabetes.

8. EFFECT OF IRON DEFICIENCY AND EXCESS ON IMMUNITY AND INFECTION

493. A role of iron in immunity and infection is supported by a large body of literature from animal studies investigating the effects of iron deficiency and excess on immune cell functions and whole body immune responses (Kuvibidila and Baliga, 2002).

The immune response

494. A wide range of cells are involved in the immune response. A key role of neutrophils and monocytes is to engulf bacteria by the process of phagocytosis; they are then destroyed primarily by reactive oxygen species produced in the respiratory (also called oxidative) burst. Once a pathogen is engulfed by an antigen presenting cell (primarily dendritic cells, monocytes and macrophages), antigenic peptides derived from the digested pathogen are presented to antigen-specific T lymphocytes. This causes activation of cell-mediated immunity.

495. The precise phenotype of T lymphocytes that are stimulated by antigen presenting cells depends upon the nature of the antigen (e.g. if it is bacterial or viral in origin). Bacterial antigens typically lead to stimulation of helper T lymphocytes while viral antigens stimulate cytotoxic T lymphocytes. The process of T lymphocyte proliferation increases the number of antigen-specific T lymphocytes following infection.

496. Activation of antigen presenting cells and T lymphocytes results in increased production of peptide mediators, termed cytokines, which act to regulate the activities of various cell types and to co-ordinate the overall response. The principal cytokine involved in regulating T lymphocyte proliferation is interleukin-2 (IL-2). However, the precise phenotype of T cells that develop is determined by the mixture of cytokines produced by the antigen presenting cell and the T lymphocyte. Interferon-γ (IFN-γ) produced by type-1 helper T lymphocytes activates macrophages to induce bacterial killing and activates B lymphocytes to induce production of some classes of antibodies (immunoglobulins) that will be specific for the original antigen. Many other cytokines are involved in regulating the functions of immune cells and in coordinating the immune response.

Effects of iron deficiency on immune function

497. A number of studies have assessed the impact of iron deficiency anaemia (typically haemoglobin<100g/L plus 1 or more measure of iron deficiency including serum iron, total iron binding capacity, transferrin saturation) on different aspects of immune function. Results from these studies have shown decreases in: the proportion of T lymphocytes in the blood (Swarup-Mitra & Sinha, 1984; Kemahli *et al*, 1988); the lymphocyte proliferative response to antigens (Swarup-Mitra & Sinha, 1984; Ahluwalia, 2004); and secretion of IL-2 (Galan *et al*, 1992; Thibault *et al*, 1993). Neutrophil function (respiratory burst, bacterial killing) is also impaired (Yetgin *et al*, 1979; Walter *et al*, 1986). B cell functions (antibody responses) seem little affected by iron deficiency (MacDougall *et al*, 1975; Bagchi *et al*, 1980; Prema *et al,* 1982; Krantman *et al,* 1982). There are a limited number of studies on the impact of iron deficiency on the functions of monocyte/macrophages, no studies of antigen presentation, and few studies of cytokines other than IL-2.

498. Several studies have reported that immune impairments in iron deficient subjects were restored to control values after iron therapy (Bhaskaram & Reddy, 1975; MacDougall *et al*, 1975; Sawitsky *et al,* 1976; Yetgin *et al*, 1979; Swarup-Mitra & Sinha, 1984; Walter *et al,* 1986; Chwang *et al*, 1988), suggesting a pathogenic role of iron deficiency.

499. Most of these studies involved small numbers of participants and were mainly conducted in children, often from developing countries, where confounding factors might include the presence of infections and the co-existence of other or multiple nutrient deficiencies. Findings from these studies may not be relevant to UK populations. Additionally, many of these studies were carried out when immunological knowledge and techniques were less well developed which limits the conclusions that can be drawn from their findings.

Effects of iron overload on immune function

500. There are fewer studies on the effects of iron overload on immune function and most have been carried out in patients with hereditary haemochromatosis or with transfusional iron overload associated with conditions such as ß-thalassaemia.

501. Patients with iron overload due to multiple transfusion generally have reduced proportions of T lymphocytes (Gugliemo *et al*, 1984; Kaplan *et al*, 1984; Dwyer *et al*, 1987) and reduced lymphocyte proliferative responses (Hernandez *et al*, 1980; Munn *et al*, 1981; Dwyer *et al*, 1987; Escalona *et al*, 1987). The effect of hereditary haemochromatosis on T cell proliferative responses is not clear (Bryan *et al*, 1984, 1991).

502. Limited data indicate that circulating B cell numbers (Bryan *et al*, 1991) or immunoglobulin concentrations (Bryan *et a*l, 1984) are not affected in haemochromatosis patients but are increased in patients with ß-thalassaemia and sickle cell disease (Glassman *et al*, 1980; Dwyer *et al*, 1987; Escalona *et al,* 1987). There are reports of impaired phagocytosis of bacteria by monocytes in haemochromatosis patients (van Asbeck *et al*, 1982, 1984); in patients with ß-thalassaemia, phagocytosis was found to be normal but bacterial killing was impaired (Van Asbeck *et al*, 1984; Ballart *et al*, 1986). Phagocytosis and bactericidal capacity by neutrophils is also impaired in patients with ß-thalassaemia (Waterlot *et al*, 1985; Cantinieaux *et al*, 1987, 1990).

Iron status and infection

503. A consequence of impaired immunity may be an increased susceptibility to infectious pathogens. Iron is required for both the host immune response and by pathogens for growth and replication. The acute phase recompartmentation of iron in response to inflammation and stress (see section 2) might be a host defence strategy to deprive invading pathogens of iron. It is therefore possible that iron supplements might exacerbate infections by providing iron to pathogens.

504. The immune response to infections is often pathogen specific and the components of the host response will have different susceptibilities to iron deprivation. Additionally pathogens have different dependencies on endogenous iron containing enzymes and/or on host iron-dependent functions.

505. Some studies have suggested that iron supplementation to manage iron deficiency in populations where malaria is a risk increases susceptibility to malaria and other infections (Murray *et al*, 1978; Oppenheimer *et al*, 1986a, b; Smith *et al*, 1989; Gordeuk, 1992a, b; van den Homberg *et al*, 1996). However, several studies have not reported an increased incidence of malaria (Harvey *et al*, 1989; Menendez *et al*, 1997; Berger *et al*, 2000) or other infections (Harvey *et al*, 1989; Berger *et al*, 2000) with oral iron supplementation in countries where malaria is prevalent.

506. A systematic review of 28 randomised controlled trials of the effects of iron (supplements, fortified foods and beverages, parenteral) on incidence of infectious illness in children (Gera and Sachdev, 2002), including 5 trials assessing malaria incidence, reported that iron supplementation did not affect the incidence rate of malaria (incidence rate ratio [IRR], 1.07; 95% CI, 0.94-1.24; p=0.35).

507. This finding was confirmed by 2 further trials. In Kenya, Verhoef *et al* (2002) reported that malaria risk was not increased in anaemic (haemoglobin concentration<100g/L) children (n=328; aged 2-36m) given iron supplements (6 mg/kg/wk) for 12 weeks compared to those given placebo. A trial in Zanzibar (Mebrahtu *et al*, 2004) assessed the effect of long-term iron supplementation (10 mg/d for 12m) on malaria infection in children aged 4-71m (n=614); 94% of the children were anaemic (haemoglobin concentration <110 g/L). No difference was found in the prevalence of malarial infection between the iron supplementation and placebo groups.

508. The systematic review by Gera and Sachdev (2002) (see paragraph 506) also reported that iron supplementation did not increase the risk of non-diarrhoeal (IRR, 0.97; 95% CI, 0.95-1.06, p=0.99), respiratory tract (IRR, 0.98; 95% CI, 0.90-1.06; p=0.54), lower respiratory tract (IRR, 0.97; 95% CI, 0.83-1.23; p=0.93), or other (IRR, 1.04; 95% CI, 0.98-1.11; p=0.20) infections in children. However, risk of developing diarrhoea was significantly increased (IRR, 1.11; 95% CI, 1.01-1.23; p=0.04).

509. A trial of breast-fed infants in Sweden (n=101) and Honduras (n=131), found no difference in diarrhoea risk of infants supplemented with iron (1 mg/kg body weight/day) from 4-6 or 4-9 months of age compared to infants not receiving supplements (Dewey *et al*, 2002). However, for infants with haemoglobin concentration less than 110 g/L at 4 months, diarrhoea was less common among those supplemented with iron compared to those who received placebo (OR, 0.21; 95% CI, 0.04-0.95; p=0.04). Infants supplemented with iron, with initial haemoglobin concentration above 110 g/L at 4 months, had a significantly greater risk of diarrhoea (OR, 2.4; 95% CI, 1.0-5.8; p=0.046).

510. A study in Bangladesh of infants aged 6 months (n=799) who were supplemented with iron (20 mg/wk, alone and in combination with 20 mg/wk of zinc) for 6 months reported no differences in risk of diarrhoea or acute lower respiratory infection between the iron supplemented and control groups (Baqui *et al*, 2003). However, among infants that were less well-nourished (weight-for-age Z score below -1) the iron plus zinc supplemented group were at significantly decreased risk of severe diarrhoea (IRR, 0.70; 95% CI, 0.54-0.92) and severe acute lower respiratory infection (IRR, 0.60; 95% CI, 0.40-0.90).

511. Two parallel randomised controlled trials assessed morbidity and mortality effects of iron (12.5 mg/day) and folic acid (50 µg/d) supplementation (with and without zinc) on infants (aged 1-35m; n=24,076) in Zanzibar (Sazawal *et al*, 2006) where malaria

transmission is high and occurs all year, and infants (aged 1-36m; n=26,250) in Nepal (Tielsch *et al*, 2006) where malaria incidence is low; average follow-up was for approximately 1 year. The trial in Zanzibar reported that, compared to the placebo group, infants supplemented with iron and folic acid were at significantly increased risk of adverse events (RR, 1.12; 95% CI, 1.02-1.23; p=0.02) and hospital admission (RR, 1.11; 95% CI, 1.01-1.23; p=0.03). Mortality risk was also increased but this was not significant (RR, 1.15; 95% CI, 0.93-1.41; p=0.19). The trial in Nepal reported no differences in mortality or morbidity outcomes between the supplemented and placebo groups.

512. Another trial of children aged 0.5-15y (n=855) in Peru (Richard *et al*, 2006) assessed the effect of daily supplementation (for 7 months) with iron (15 mg/d) alone or combined with zinc (20mg/d) on morbidity outcomes. Compared with placebo, iron supplementation significantly increased malaria incidence in children aged 5 years and above (IRR, 1.76; 95% CI, 1.14-2.70; p=0.010); iron combined with zinc did not affect malaria incidence. Among children aged 9 years or over, iron (alone and in combination with zinc) significantly increased diarrhoea risk (iron: IRR, 1.72; 95% CI, 1.06-2.79; p=0.03/iron + zinc: IRR, 1.99; 95% CI, 1.18-3.34; p=0.009). Iron supplementation (alone and in combination with zinc) had no effect on incidence of acute lower respiratory infection.

513. Following publication of the studies by Sazawal *et al* (2006) and Tielsch *et al* (2006), WHO and UNICEF (2006) issued a joint statement advising that iron and folic acid supplementation should be targeted only to those children who are anaemic and at risk of iron deficiency and that they should receive concurrent protection from malaria and other infectious diseases.

Iron and human immunodeficiency virus (HIV) infection

514. Iron enhances transcription of HIV *in vitro*, probably by increasing oxidative stress leading to activation of the transcription factor, nuclear factor kappa B (Sappey *et al*, 1995; Boelaert *et al*, 1996a, b). Conversely, iron chelation with deferoxamine[29] (DFX) in cell culture inhibits HIV replication and decreases apoptosis of helper T lymphocytes.

515. A cross-sectional study in Zimbabwe (Friis *et al*, 2003) examined the association between serum ferritin concentration and HIV infection in pregnant women (n=526). After controlling for serum alpha-1-antichymotrypsin concentration (an acute phase reactant), mean viral load was lowest in women with serum ferritin concentrations below 6 µg/L and increased with increasing serum ferritin concentrations (p=0.08). Another cross-sectional study of HIV-infected women (n=483) in Malawi found no association between serum ferritin concentration and HIV progression or viral load (Semba *et al*, 2001).

516. A retrospective analysis of HIV infected patients (n=348) in the USA found that the risk of death was greater in those with the highest bone marrow macrophage iron stores[30] compared to those with normal or low iron stores (hazards ratio, 2.1; 95% CI, 1.3-3.5; p=0.003) (de Monye *et al*, 1999). Infections caused by *Candida spp*, *Pneumocystis carinii* and *Mycobacterium spp* were also more common in patients

[29] Deferoxamine is a widely used chelating agent used to remove excess iron in thalassaemia patients with transfusional iron overload
[30] Bone marrow macrophage iron stores were graded on a scale of 1-5; patients with grades 4-5 iron stores (high) were compared to those with grade 0-2 iron stores (normal or low).

with the highest bone marrow macrophage iron stores than in those with low or normal stores (p≤0.006).

517. A study in France (Costagliola *et al*, 1994) reported that the rate of progression of HIV disease among patients (n=64) with thalassaemia major was inversely proportional to DFX dose (p<0.002).

518. A prospective study in the USA reported a significant protective relationship between increasing intakes of iron and development of AIDS in HIV infected men (n=296) followed over 6 years (Abrams *et al*, 1993). After adjustment for age, smoking, energy intake, health status at baseline, men with iron intakes of 23-33 mg/d were significantly associated with an increased progression to AIDS compared to men with iron intakes ≥ 49 mg/d (HR, 2.0; 95% CI, 1.2-3.5; p trend=0.02).

519. A small trial of HIV infected adults in Kenya (n=32), found no effect of 60 mg iron/twice weekly for 4 months on HIV-1 viral load (Olsen *et al*, 2004). Kupka *et al* (2007) followed a cohort of HIV-infected pregnant Tanzanian women (n=584) from 12-27 week gestation to 30 weeks postpartum. All the women received 120mg/day of iron during their pregnancy. Serum ferritin concentration was not associated with HIV-related mortality or HIV progression.

520. Haptoglobin (Hp), a plasma protein which removes free haemoglobin from the circulation (Langlois & Delanghe, 1996), exists as three major phenotypes (Hp 1-1, Hp 2-1, Hp 2-2). In men, the Hp 2-2 phenotype has been shown to be associated with increased serum ferritin concentrations and with increased macrophage iron accumulation (Langlois *et al*, 2000; Delanghe & Langlois, 2002). Delanghe *et al* (1998) reported that HIV infected males with the Hp 2-2 phenotype in Belgium (n=493) and Luxembourg (n=160) had higher plasma HIV levels (p=0.03), a greater increase in plasma HIV level over one year (p=0.003), higher mortality (risk ratio, 1.78; 95% CI, 1.25-2.54; p=0.0001) and shorter median survival time compared to those with the Hp 1-1 or 2-1 phenotypes. Friis *et al* (2003) reported that the viral load of HIV infected pregnant Zimbabwean women with the Hp 2-2 phenotype was twice that of women with Hp 1-1 phenotype (95% CI, 1.4-4.0; p=0.002).

Iron and tuberculosis (TB)

521. *Mycobacterium tuberculosis* grows within macrophage phagosomes. The mycobacterium appears to evade host defence by preventing phagosome acidification and lysosome fusion and to acquire iron from host endosomal holotransferrin (Boelaert *et al*, 2007).

522. Iron has been shown to enhance the growth of *Mycobacterium tuberculosis* both *in vitro* (Cronje *et al*, 2005) and in animal studies (Lounis *et al*, 2001). *In vitro* studies have shown that e*xcess* iron impairs the anti-microbial cytotoxic effects of macrophages against pathogens (Moyo *et al*, 1997).

523. There are limited human data on the relationship between iron status and TB. Iron overload due to excessive dietary intakes has been associated with increased risk of progression and death from TB. Dietary iron overload is common in rural populations of southern Africa and is caused by consumption of traditional alcoholic beverages which are brewed in iron drums and cans (Bothwell *et al*, 1964) (see paragraph 58).

In southern Africa, a high incidence of iron overload has been reported in patients dying from TB (Moyo *et al*, 1997; Lounis *et al*, 2001).

524. A prospective study of TB patients (n=98) and controls (n=98) in Zimbabwe examined whether previous dietary exposure to iron was a risk factor for active TB. Patients were followed from 1 week to 9 months after start of treatment. After controlling for HIV status and liver function, increased dietary iron (defined as an estimated lifetime consumption of >1000 L of traditional beer) was significantly associated with a 3.5-fold increase in the odds of developing active TB (95% CI, 1.4-8.9; p=0.009) (Gangaidzo *et al*, 2001). However, increased dietary iron in this population was from an alcoholic beverage and alcoholism has also been associated with increased TB risk (Feingold, 1976).

525. A study in Indonesia reported no difference in distribution of Hp phenotypes (see paragraph 520) in TB patients (n=97) and healthy controls (n=126) (Grange *et al*, 1985). Kasvosve *et al* (2000) reported that the distribution of Hp phenotype of TB patients in Zimbabwe (n=98) were not significantly different from healthy controls (n=98); however during 18 months of follow-up after start of treatment the odds of dying from tuberculosis were 6-fold higher in patients with Hp 2-2 phenotype compared to those with Hp 1-1 phenotype (95% CI, 1.04-35.1; p=0.04).

Summary and conclusions

526. Iron deficiency anaemia (typically defined in most studies as haemoglobin <100 g/L plus 1 or more measure of iron deficiency including serum iron, total iron binding capacity, transferrin saturation) and iron overload (due to multiple blood transfusions in ß-thalaessemia patients) impairs some aspects of immune function. The functional consequences of these impairments on morbidity are unclear.

527. It has been suggested that iron supplementation may decrease resistance to infectious pathogens by providing them with a supply of iron which is required for their growth and replication. In human studies, the effect of iron supplementation on morbidity and mortality from infections is uncertain. Most studies have been carried out in children and in developing countries. The evidence suggests that iron supplementation does not increase the risk of non-diarrhoeal or respiratory tract infections in children but may increase diarrhoea risk.

528. Studies which have examined whether iron supplementation increases malaria risk are inconsistent. It is also not clear whether iron supplementation increases risk of infectious diseases in areas where malaria incidence is high.

529. There is currently insufficient evidence to draw conclusions on the relationship between iron supplementation and HIV or TB.

530. Most studies on iron and infection have been conducted in developing countries where there are multiple nutrient deficiencies which may also affect resistance to infection. All these factors are not taken into account in most studies.

531. The relevance of most of these studies to the UK may be limited. On balance, there is no evidence to suggest that improving iron status in the UK would have any impact on infectious disease incidence or morbidity. Some evidence suggests that iron

supplementation to improve iron status may have adverse effects in some sub-groups of the population, e.g., those with HIV and children at risk of diarrhoea.

9. DIETARY IRON INTAKES AND IRON STATUS OF THE UK POPULATION

532. Nationally representative data on iron intakes and iron status in the UK were obtained from the *National Diet and Nutrition Survey* (NDNS) series: children aged 1½-4½ years in 1992/3 (Gregory *et al*, 1995); people aged 65 years and over in 1994/5 (Finch *et al*, 1998); young people aged 4-18 years in 1997 (Gregory *et al*, 2000); and adults aged 19-64 years in 2000/1 (Henderson *et al*, 2002; Henderson *et al*, 2003; Rushton *et al*, 2004). An earlier survey of adults aged 16-64 years was conducted in 1986/7 (Gregory *et al*, 1990). No nationally representative data are available for infants and children under 1½ years or for pregnant or lactating women.

533. Nationally representative data for iron intakes and iron status of low income populations (2 years and above) were obtained from the *Low Income Diet and Nutrition Survey* (LIDNS) (Nelson *et al*, 2007a, b).

Assessment of iron intakes

534. In the NDNS series, diet was assessed by weighed records of all foods consumed over: 7 consecutive days for adults aged 19-64 years and young people aged 4-18 years; 4 consecutive days for adults over 65 years and children aged 1½-4½ years. In the LIDNS, diet was assessed by 24h recall on 4 non-consecutive days including, where possible, a weekend day.

535. Iron intake data from these surveys were compared against the DRVs for iron intake (see Table 3.1).

536. Difficulties associated with the use of dietary surveys to assess the adequacy of nutrient intakes against DRVs include reliability of food composition tables and misreporting of food consumption, which reduces confidence in the accuracy of population mean intakes and can affect the distribution of measured intakes. Participants may forget or purposely omit to record some items consumed so their intake is under-reported; over-reporting can also occur if, for example, food left on the plate is not taken into account. Additionally, judgements of dietary adequacy of iron intakes against DRVs only take limited account of the amount of iron absorbed from the diet. Iron absorption of 15% was assumed in the derivation of the UK DRVs. This was based on the consumption of a diverse diet and may not apply to sub-groups of the population with different dietary patterns (DH, 1991); this assumption also does not take account of absorptive adaptation according to systemic iron needs.

Assessment of iron status

537. In this report, internationally accepted reference values for iron sufficiency published by the WHO (2001) (Table 9.1) were used to evaluate the haemoglobin and serum ferritin concentrations of the UK population. Haemoglobin concentrations below the WHO thresholds are used to define anaemia and serum ferritin concentrations below the WHO thresholds are used to define iron deficiency or to identify a risk of iron deficiency because of low ferritin depots of iron. Haemoglobin and serum ferritin concentrations were measured for all age groups of the population in the NDNS and LIDNS; where age bands overlapped with more than one WHO age band, both thresholds were used (Table 9.2).

538. Combinations of biochemical indicators have been proposed and used to define iron deficiency (see section 4) including: ferritin + transferrin saturation + erythrocyte protoporphyrin + mean cell volume; and transferrin saturation + erythrocyte protoporphyrin. Although many of these measurements were included in the NDNS and LIDNS, multiple indices were not used in this report because of the lack of internationally agreed cut-off levels.

Table 9.1: WHO (2001) thresholds for adequate haemoglobin and serum ferritin concentration (WHO, 2001)

Age/sex	Haemoglobin g/L	Serum ferritin µg/L
Children 0.5-4.99 years	110	12
Children 5-11 years	115	15
Children 12-14 years	120	15
Non-pregnant women (>15y)	120	15
Men (>15y)	130	15

Table 9.2: Thresholds of haemoglobin and serum ferritin concentration used to assess adequacy of iron status of the UK population according to age bands in the National Diet and Nutrition Survey and the Low Income Diet and Nutrition Survey

Age/sex	Haemoglobin g/L	Serum ferritin µg/L
Children 1½-4½y	110	12
Children 4-6y	110 & 115	12 & 15
Children 7-10y	115	15
Children 11-14 years	115 & 120	15
Non-pregnant women (>15y)	120	15
Men (>15y)	130	15

IRON INTAKES OF THE UK POPULATION

539. See Annex 6 (Tables 9.3-9.10).

Dietary sources of iron

540. In the NDNS (Table 9.3), cereals and cereal products were the main dietary source of iron (45% of total intake) for adults aged 19-64y; the major sources within this group were breakfast cereals and white bread. Substantial contributions were also made by meat and meat products (19% in men; 15% in women) and vegetables, including potatoes (16% in men, 19% in women). The contribution made by cereals to iron intakes was greater in younger and older age groups (47-53%) while that from meat and meat products was lower in younger children (14%). Milk and milk products provided approximately 5-6% of iron intakes for children aged 1½-4½y and adults aged 65y and over, living in institutions, but less for other age groups.

541. In the LIDNS (Table 9.4), cereals and cereal products were the main source of iron intakes in adults over 19y (41% of total) and children aged 2-18 years (51% of total). Other sources were meat and meat products. The contribution made by meat and meat products to overall iron intakes was greater for adults (21%) than for children (17%) and for men (23%) compared to women (21%). Vegetables, including potatoes, were also major contributors to iron intakes of adults and children (19%).

Iron intake from all sources

542. In the NDNS (Table 9.5), mean intakes of iron from all sources (food and supplements) for adults 19y and over were 10-15 mg/d for men and 8-13 mg/d for women. Average intakes tended to be at the higher end of the range for adults aged 25-64y and at the lower end for adults aged under 25y and over 65y. Average iron intakes of children aged 1½-4½y were 5-6 mg/d; intakes of young people aged 4-18y were 8-13 mg/d for boys and 7-9 mg/d for girls. Differences in mean iron intake between the sexes and at different ages were less apparent after correction for energy intake, indicating that the iron density of the diet was similar in males and females at all ages (about 1.25 mg/MJ/d or 0.3 mg/1000kcal/d).

543. Adults aged 65y and over living in institutions had lower iron intakes than free-living adults (8.6 mg/d vs 10.0 mg/d) which were largely related to lower energy intakes.

544. In the LIDNS (Table 9.6) average intakes from all sources were between 10-12 mg/day for men (19y and over) and about 9 mg/day for women (19y and over). Average intakes were 8-9 mg/d for children aged 2-10y and 9-11 mg/d for older children aged 11-18y. In all age groups, males had higher mean intakes of iron than females.

Intake of haem iron

545. Haem iron, which is present in meat and fish, is absorbed more efficiently than non-haem iron and is considered more bioavailable (see section 5). Haem iron contributed approximately 3-6% of total iron intake in the NDNS (Table 9.7) and 4-8% in the LIDNS (Table 9.8). This is lower than the amount usually cited (10%-12%) for a diet with substantial amounts of red meat (Hunt *et al,* 1999).

546. The distribution of haem iron intakes in the LIDNS was skewed, with median intakes 9-28% lower than mean intakes depending on age and sex.

Iron intake from supplements

547. In the NDNS, iron from supplements contributed a relatively small proportion of total daily iron intake in most age groups. As a consequence, average iron intakes from all sources were similar to intake from food sources. The exception was for adult women: supplements contributed 21% of total iron intake for women aged 35-49y, 12% for women aged 19-24y and 11% for women aged 50-64y.

548. Supplements made a greater contribution to iron intakes of men, but not women, in the 2000/1 adult survey than in the 1986/7 survey. For men, supplements contributed 6% of total iron intake in 2000/1 compared to 2% in 1986/7; for women supplements contributed 15% of total intake in 2000/1 compared to 14% in 1986/7.

549. In the LIDNS, supplements contributed 4% and 7% of total iron intakes for men and women respectively.

Intake of fortificant iron

550. The main sources of fortificant iron in the UK diet are white and brown wheat flours, which are fortified with iron on a mandatory basis, and a number of other foods, particularly breakfast cereals, which are fortified with iron on a voluntary basis. The

contribution of fortificant iron is included in the iron content of specific food items in food composition databases, and to the estimation of total iron and non-haem intakes. Values in food composition tables may not be accurate, however, because of the continuing introduction and withdrawal of food products voluntarily fortified with iron. The NDNS and LIDNS do not provide information on the contribution of iron from fortified foods to total iron intakes, however cereals and cereal products provide approximately 50% of average daily iron intakes for all population groups.

Iron intakes of sub-groups of the population

551. The NDNS is not of sufficient size to obtain representative data on sub-groups of the population. However, in additional analyses of NDNS data from the surveys of children aged 1½-4½y and young people aged 4-18y , a vegetarian diet or avoiding meat were not associated with lower than average total iron intakes (Thane *et al*, 2003; Thane and Bates, 2000); girls aged 11-18y who smoked had lower total iron intakes than those who did not (Thane *et al*, 2003). There was no evidence of associations between ethnicity and total iron intakes among children aged 4-18y, but children aged 1½-4½ y in Caucasian or Black households had lower iron intakes than those from Asian households.

552. In the LIDNS, sample sizes for ethnic groups were small and should be interpreted with caution. For adults 19y and over, mean iron intakes were not significantly different by ethnic group. Mean intakes were 12.2 mg/d for Asian men (n=35) compared to 11.0 mg/d for Caucasian men (n=876). Mean iron intakes were 9.5 mg/d for Asian women (n=58), 7.9 mg/d for Black women (n=49) and 8.7 mg/d for Caucasian women (n=1713). For girls and boys aged 2-18 years and black men, sample sizes were too small (<30) to compare intakes by ethnic groups.

Comparison of iron intakes with dietary reference values

Comparison of mean daily intakes as percentage of the reference nutrient intake (RNI)

553. In a normal population, the RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population (DH, 1991). This means that most individuals in a population will have a requirement that is below the RNI.

554. In the NDNS (Table 9.9), average daily intakes of iron were above the RNI, or above 90% of the RNI, for all age groups except: children aged 1½-3½ y (73-81% of RNI), girls aged 11-18y (about 60% of RNI), and women aged 19-49y (66-87% of RNI).

555. In the LIDNS (Table 9.10), average daily iron intakes were above the RNI for males in all age groups. For females, only girls aged 2-10y and women 65y and over had daily intakes at or above the RNI; mean daily intakes were 63% of the RNI for girls aged 11-18y and approximately 60% for women aged 19-49y.

Iron intakes below the lower reference nutrient intake (LRNI)

556. The LRNI represents the amount of a nutrient that is likely to meet the needs of only 2.5% of the population (DH, 1991). This means that in a normal population, 2.5% would be expected to have requirements below the LRNI. Intakes below this level are almost certainly inadequate for most individuals. Low intakes of iron would be of public health concern when the prevalence of intakes below the LRNI exceeds 5%.

557. In the NDNS (Table 9.9), population groups with substantial proportions with iron intakes below the LRNI were children aged 1½-3½ (12-24%), girls aged 11-18y (44-48%) and women aged 19-49y (25-40%).

558. In the LIDNS (Table 9.10), a higher proportion of females aged 11-49y (39% of girls aged 11-18y; 50% women aged 19-49y) had iron intakes below the LRNI compared to women aged 50y and over (13%, 50-64y; 5%, 65y and over) and males (7% aged 2-18y; 3-5% aged 19y and over). A higher proportion of boys aged 11-18y (14%) had intakes below the LRNI compared to those aged 2-10y (2%).

559. Both the NDNS and LIDNS indicate that substantial proportions of some population groups in the UK have iron intakes that are below the LRNIs set for iron. The high proportion of toddlers and females with intakes of iron below the LRNI suggests that the DRVs for iron set for these population groups may be too high. The DRVs for iron are derived from limited data (see section 3); they also assume that only 15% of iron is absorbed from the diet which does not take account of absorptive adaptation to increased systemic iron needs.

IRON STATUS OF THE UK POPULATION (EVIDENCE OF ANAEMIA, IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA)

560. See Annex 6 (Tables, 9.11-9.16).

561. Data on haemoglobin and serum ferritin concentration of children aged 2-18y in LIDNS are not included in this report since the response rate for blood specimens in this age group was low suggesting that the results may not be representative.

Haemoglobin concentration

562. In the NDNS (Table 9.11), groups which had the highest proportions with haemoglobin concentrations below WHO cut-offs for anaemia were adults aged 65y and over in institutions (39% of women; 52% of men), free-living adults aged 75y and over (13-38%), and girls aged 4-6y (15% based on 115g/L cut-off; 9% based on 110 g/L cut-off). For adults aged 19-64y, the prevalence of anaemia was higher in women (7-9%) compared to men (0-4%). In adults aged 65y or over the prevalence was greater in men (16-38%) than women (13-16%). For adults aged 65-74y the proportion with haemoglobin concentrations below the WHO cut-offs for anaemia was similar for men (7%) and women (6%).

563. In the LIDNS (Table 9.12), the highest proportions of adults with haemoglobin concentrations below WHO cut-offs for anaemia were among men aged 65y and above (20%); prevalences were much lower for men aged 19-64y (0-5%). In contrast, prevalence of anaemia was higher in younger women aged 19-49y (12-18%) than women aged 50-64y (6%) and women 65y and over (9%).

Serum ferritin concentration

564. In the NDNS (Table 9.13), highest proportions with ferritin concentrations below the WHO cut-offs indicating iron deficiency were found in children aged 1½-4½y (25-34%), girls aged 11-14y (12%) and 15-18y (24%), women aged 19-24y (16%) and 35-49y (13%), and free-living women 75y and over (12-14%).

565. In the LIDNS (Table 9.14), the highest proportions with serum ferritin concentrations below WHO cut-offs were found in women aged 19-34y (21%) and 35-49y (14%); the proportion was lower in women aged 50y and over (4-5%). For men aged 19y and over, the proportion with serum ferritin concentrations below WHO cut-offs ranged between 0-5%.

Haemoglobin and serum ferritin combined (iron deficiency anaemia)

566. Based on the WHO thresholds of haemoglobin and serum ferritin concentrations used to define iron deficiency and anaemia, the prevalence of iron deficiency anaemia in the NDNS (Table 9.15) was 5% or above for children aged 1½-2½y (6%), females aged 15-18y (5%) and 35-49y (5%), free-living adults aged over 85y (6%) and men aged 65y or over living in institutions (5%).

567. The prevalence of iron deficiency anaemia for these population groups is not clearly consistent with their mean iron intakes which were similar for all children aged 1½-4½y (5-6 mg/d) and slightly higher for females aged 35-49y (13 mg/d) than for females aged 19-34y (10-12 mg/d) and 50-64y (12 mg/d). The findings are consistent with the groups having high proportions with intakes below the LRNI for children aged 1½-2½y (24%) and girls aged 15-18y (48%). They are not consistent for women aged 35-49y who had a lower proportion with intakes below the LRNI (25%) than women aged 25-34y (40%); however the proportion of women with iron deficiency anaemia in the 25-34y age group was only 2%. They are also not consistent for free-living adults aged 85y + (4-10% below LRNI) or men aged 65y or over living in institutions (4-5% below LRNI).

568. In the LIDNS (Table 9.16) the prevalence of iron deficiency anaemia was above 5% for women aged 19-49y (9-11%) and 65y and over (6%).

569. The prevalence of iron deficiency anaemia in women aged 19-49y and 65y and over is also not clearly consistent with the mean intakes which were similar for all women aged 19-65y and over (9 mg/d). The findings are consistent with the proportions with intakes below the LRNI for women aged 19-49y which was highest for this group (50%). They are not consistent for women aged 65y and over as the proportion in this age group with intakes below the LRNI was 5%.

570. Findings from the NDNS and LIDNS broadly show that men (under 85y) in the UK are not at risk of iron deficiency anaemia and that some groups of women of reproductive age (15-50y), particularly those from low income groups, may be at increased risk. This is consistent with increased blood losses due to menstruation in this age group making them more vulnerable to iron deficiency anaemia. Iron deficiency anaemia observed in some adult groups aged 65 years and over (free-living men and women 85y and over; men aged 65y and over living in institutions) is consistent with blood loss due to gastrointestinal disease or medication (e.g. aspirin) in older people (see paragraph 288).

Relationship between iron status markers and iron intakes

571. Few significant associations were observed between haemoglobin and serum ferritin concentrations and intakes of total iron, non-haem iron or haem iron in any of the surveys in the NDNS series. This would be expected in a population which has an adequate iron supply.

572. The relationship between dietary iron intake and biochemical markers of iron status may also be complicated by a number of confounding factors which affect both iron absorption and biochemical markers of iron status (see paragraphs 210-211). There are also problems associated with obtaining reliable estimates of dietary iron intake in dietary surveys (see paragraph 209). Additionally, measurement of only one blood sample will not take account of diurnal and day-to-day variability in haematological markers of iron status.

573. Correlation coefficients were statistically significant in a few age and sex categories. Significant positive correlations were observed between haemoglobin concentration and dietary intakes of haem, non-haem and total iron in children aged 1½-4½y ($p<0.05$) and in boys ($p<0.01$) and girls ($p<0.05$ for total and non-haem iron; $p<0.01$ for haem iron) aged 4-18y. These relationships were apparent when all ages were combined but rarely reached significance when the age groups were considered separately. In adults aged 19-64y, there was a significant positive association between haemoglobin concentration and haem iron intake for women ($p<0.05$) but not for men. Intakes of non-haem iron and total iron were negatively correlated with haemoglobin concentration in men aged 19-24y ($p<0.05$) but positively correlated with haemoglobin concentration of men aged 25-34y ($p<0.01$). In free-living adults aged 65y and over, positive correlations were found between haemoglobin concentration and haem iron intake for men ($p<0.01$) and with total, haem and non-haem iron for women ($p<0.01$). In adults aged 65y and over living in institutions no significant relationship was observed between haemoglobin concentration and iron intake for men but positive associations were observed with total and non-haem iron for women ($p<0.05$).

574. There were no significant associations between serum ferritin concentrations and iron intakes in children aged 1½-4½y. In young people aged 4-18y, serum ferritin concentration was positively associated with intakes of total ($p<0.05$), haem ($p<0.01$) and non-haem ($p<0.05$) iron in boys, but only with haem iron intakes in girls ($p<0.01$). For adults aged 19-64y, serum ferritin concentration was positively correlated with haem iron intake in men aged 19-24y ($p<0.05$) and 35-49y ($p<0.01$) and in women aged 50-64y ($p<0.01$), but no correlations were observed for total iron or non-haem iron. In the free-living adults aged 65y and over, ferritin concentration was positively associated ($p<0.05$) with haem iron intake in both men and women, and also with total and non-haem iron in women ($p<0.01$). No significant associations were observed between serum ferritin concentration and iron intakes of adults aged 65y and over living in institutions.

575. Correlation coefficients for haemoglobin and serum ferritin concentrations with dietary intakes of iron are not reported in the LIDNS. Mean intakes of iron were lowest for boys aged 11-18y and men aged 65y and over. These findings are consistent with prevalence of haemoglobin concentrations below cut-off points, which were highest in these groups, but are not consistent with serum ferritin concentrations. In women, low reported iron intakes (as a percentage of the RNI) amongst those aged 19-34y and 35-49y were consistent with the high prevalence of serum ferritin concentrations below 15 µg/L and haemoglobin concentrations below 120 g/L in these age groups although not to the extent suggested by the dietary data.

Influence of vitamin C intakes on iron status markers

576. In the NDNS, relationships between haemoglobin and serum ferritin concentrations and intakes of vitamin C were considered for children aged 1½-4½y, children aged 4-18y, and adults aged 65y and over.

577. No significant associations were observed between haemoglobin concentration and vitamin C intake for children aged 1½-4½y or adults aged 65y and over. In young people aged 4-18y, a significant positive association was found for boys ($p<0.01$) and girls ($p<0.05$), however when age groups were considered separately, the association was significant only for boys aged 4-6y ($p<0.01$) and 7-10y ($p<0.05$).

578. No significant associations were observed between ferritin concentration and vitamin C intakes in children aged 1½-4½y or 4-18y, but positive correlations ($p<0.01$) were observed for free-living adults aged 65y and over.

Further analysis of specific age-groups in the NDNS series

579. Further analyses of NDNS data in relation to risk factors for poor iron status have been carried out for children aged 1½-4½y (Thane *et al,* 2000; Thane & Bates, 2000), children aged 4-18y (Thane *et al*, 2003) and older people aged 65y and over (Doyle *et al*, 1999).

580. Thane *et al* (2000) reported that among children aged 1½-4½y, significantly higher proportions with haemoglobin concentrations <110g/L were found in those: whose head of household was unemployed; with low household incomes; whose mothers had low educational attainment; and those who had never been breast-fed. The proportion of children with ferritin concentrations <10 µg/L was significantly greater in those whose head of household did manual work, had never worked, or was in the armed forces. The proportion of children with both haemoglobin concentrations <110g/L and ferritin concentrations <10 µg/L was greater in households receiving benefits and in those whose mothers had low educational attainment.

581. The proportion of children with either haemoglobin concentrations <110 g/L or serum ferritin concentrations <10 µg/L were those in the lowest quintile of meat and fruit intake and those consuming large quantities of milk and milk products (Thane *et al*, 2000). Serum ferritin concentrations were lower in children who were vegetarian, and were significantly lower in children under 3y (Thane & Bates, 2000). A greater proportion of girls aged 11-18y who did not eat meat had haemoglobin concentrations below 115g/L (11-12y) or 120g/L (13y and over) and serum ferritin concentrations <15 µg/L (Thane *et al*, 2003).

582. Doyle *et al* (1999) reported that the haemoglobin concentration of adults aged 65y was negatively associated with intakes of dairy foods, calcium and tea and positively associated with intakes of meat, poultry, vegetables, fibre and alcohol although these associations were not significant in all age and sex groups. A significant positive association was reported between serum ferritin concentrations and vitamin C and vegetable intake.

IRON INTAKE AND STATUS IN INFANTS AND YOUNG CHILDREN UP TO 18 MONTHS

583. National data on the nutritional adequacy of the diet of infants up to 18 months of age are very limited. In the UK, the only nationally representative survey of food and nutrient intakes in infants aged 6-12 months was conducted in 1986 (Mills and Tyler, 1992). This survey reported that mean iron intake was 8.1 mg/d (above the RNI of 7.8 mg/d), median intake was 7 mg/d and 15% of infants had intakes below the LRNI (4.2 mg/d). Average daily intakes of iron were significantly lower in infants aged 9-12 months (6.7mg) than infants aged 6-9 months (9.3mg). For most infants, commercial baby foods, including infant formulas, represented the major source of iron; older infants obtained a large proportion of iron from 'family foods'. Most iron consumed was non-haem rather than haem iron.

584. Findings from the Infant Feeding Survey (DH, 2002) suggest considerable changes in infant feeding practices since the survey by Mills and Tyler (1992) which collected data in 1986. For example, between 1995 and 2000 there was a significant increase in the incidence of breast feeding[31] in the UK: in 2000, 30% of mothers did not breast feed at all and gave infant formula as the sole source of nutrition from birth, compared to 34% who did no breast feed in 1995; the proportion of mothers who gave their babies cows' milk in some form by 9 months of age significantly decreased from 61% in 1995 to 54% in 2000; and in 2000, 24% of mothers introduced solid foods by 3 months of age compared with 56% in 1995. The effects of these changes on nutrient intake and status of infants is unknown.

585. A number of small studies have assessed the iron status of infants; iron intakes were usually not reported in these studies.

586. A longitudinal prospective study (Taylor *et al*, 2004) which followed infants (n=198) from before 4 months to 24 months of age reported that mean daily iron intakes met the RNI at 4 and 8 months (except for non-meat eaters); by 12 months however, mean intakes were below the RNI in all groups. At 4, 12, and 24 months, haemoglobin concentrations were below the WHO threshold for anaemia (110 g/L) for 34, 23, and 13% of infants respectively. At 12 and 24 months, 4.2% and 2.8% of infants had low serum ferritin concentrations (<10 µg/L).

587. In another UK study (Sherriff *et al*, 1999), the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC), there was little variation in haemoglobin concentrations of infants (n=1175) from 8 to 18 months. However, mean serum ferritin concentrations changed rapidly over this period: 36.6 µg/L at 8 months, 32.8 µg/L at 12 months, and 27.5 µg/L at 18 months. The authors of the study suggested that ferritin concentration as an indicator of iron status should be used with caution.

588. A longitudinal study in Ireland (n=76) reported that haemoglobin concentration <110g/L was strongly associated with early introduction of cows' milk. An increase in the proportions of infants with haemoglobin <100g/L from 12 to 24 months was attributed to the change from a fortified to an unfortified diet (Freeman *et al*, 1998).

[31] Incidence of breast feeding is defined as the proportion of babies who were breast fed initially. This includes all babies who were ever put to the breast, even if this was on only one occasion.

Iron status of ethnic groups

589. The first nationally representative survey of infant feeding practices in families of Asian (Bangladeshi, Indian or Pakistani) origin living in England was conducted between 1994 and 1996 (DH, 1997). Haemoglobin and serum ferritin concentrations were analysed when the children (n=1057) were aged 2 years (Lawson *et al*, 1998). Mean haemoglobin concentrations were 116 g/L for Bangladeshi and Indian children and 114 g/L for Pakistani children compared to 120 g/L for children aged 1½-2½ y in the NDNS. Twenty-nine per cent of Pakistani children, 25% of Bangladeshi, and 20% of Indian children had haemoglobin concentrations <110 g/L compared with 12% of children aged 1½-2½y in the NDNS. The dietary factors found to have a significant negative impact on haemoglobin and serum ferritin concentrations included early introduction (at 9 months) of cows' milk as a main drink, the quantity of cows' milk consumed, and the prolonged use of bottle feeding which has been associated with higher milk consumption to the detriment of other foods.

590. A number of smaller-scale local studies conducted in England have reported a high prevalence of anaemia (haemoglobin<110g/L) in young Asian children (Grindulis *et al*, 1986; Duggan *et al*, 1991) and a higher prevalence in Asian children compared with white children living in the same area (Erhardt 1986; Warrington & Storey, 1988; Morton *et al,* 1988; Mills 1990; Marder *et al*, 1990). In contrast, a study (n=150) investigating the relationship between anaemia (haemoglobin<110 g/L) at 14 months and risk of anaemia at 2 years in children attending a deprived inner city practice found no significant difference in the prevalence of anaemia between ethnic groups (James *et al*, 1995).

591. It is possible that the agreed thresholds set by the WHO (2001) for haemoglobin concentration are not appropriate for Asian children so a direct comparison of Asian and non-Asian children may not be valid.

Summary and conclusions

592. The major dietary sources of iron are cereals, including bread, which account for about half of the iron intake of most of the UK population. Meat/meat products and vegetables (including potatoes) also make substantial contributions to dietary iron intakes.

593. Assessing the adequacy of iron intakes of the population using dietary reference values is limited by a number of factors, including the accuracy of dietary measurements, reliability of food composition data, and the amount of iron absorbed from the diet.

594. In the general population, groups with substantial proportions below the LRNI are children aged 1½-3½y (12-24%), girls aged 11-18y (44-48%) and women aged 19-49y (25-40%). In low income populations, females aged 11-49y have the highest proportions with intakes below the LRNI (39% of girls 11-18y; 50% women, 19-49y).

595. Groups with the highest prevalence of haemoglobin concentrations below WHO thresholds for anaemia in the general population were adults aged 65y and above living in institutions (39-52%), free-living adults aged 75y and over (13-38%), and girls aged 4-6y (15% based on higher threshold of 115g/L; 9% based on lower threshold of 110 g/L). In low income groups the highest proportions of adults with

haemoglobin concentrations below the WHO cut-offs were men aged 65y and above (20%).

596. Measures of serum ferritin concentrations, an indicator of body iron stores, suggest that in the general population, children aged 1½-4½y (25-34%), girls aged 11-18y (12%), women aged 19-24y (16%), 35-49y (13%), and free-living women 75y and over (12-14%) may be at risk of iron deficiency. In low income groups, women aged 19-49y (14-21%) are at greatest risk of iron deficiency.

597. The prevalence of iron deficiency anaemia (both haemoglobin and serum ferritin concentrations below WHO thresholds) in the general population ranged between 0-6% according to age and sex. Population groups with the highest prevalence of iron deficiency anaemia (5-6%) were children aged 1½-2½y, girls aged 15-18y, women aged 35-49y, men 65y and over living in institutions, and free-living adults aged 85y and over. In low income groups, the prevalence of iron deficiency anaemia was highest for women aged 19-39y (9-11%) and 65y and over (6%).

598. The prevalence of iron deficiency anaemia in the different population groups is not clearly consistent with their dietary iron intakes. However the NDNS and LIDNS both broadly show that adult men (under 65y) in the UK are not at risk of iron deficiency anaemia and that women aged 15-50y are at increased risk. This is consistent with increased iron losses in this age group due to menstrual blood loss. Iron deficiency anaemia observed in some adults aged 65y and over is consistent with blood loss due to gastrointestinal disease or medication in older age groups.

599. Although data from NDNS and LIDNS indicate that iron intake and iron status may be of public health concern for some population groups in the UK, this is dependent on the confidence placed on the dietary reference values for iron intake which are based on limited data and on iron status criteria which are not associated with functional outcomes.

600. The disparity between the high proportions of toddlers and females aged 11-50y with intakes below the LRNI and the relatively low prevalence of females with iron deficiency anaemia in the general population suggest that the DRVs set for these groups may be too high. As well as being based on limited data, the DRVs do not take account of absorptive adaptation to increased iron needs.

601. In the NDNS, few consistent associations were observed between markers of iron status and intakes of total iron, non-haem iron, haem iron, or vitamin C. This would be expected in a population with an adequate supply of dietary iron.

602. Data on iron intakes and status of infants up to 18 months of age and of minority ethnic groups are limited. Findings from the only nationally representative survey of infants up to 18 months suggest that 15% of infants aged 6-12 months have intakes below the LRNI. The effect of changes in infant feeding practices since the survey was carried out in 1986 is unknown.

603. Data from a nationally representative survey suggest that the prevalence of haemoglobin concentration <110 g/L is 20-29% in Asian infants at 2 years of age. It is possible that the WHO cut-offs for haemoglobin concentration may not be appropriate for Asian children.

10. THE POTENTIAL IMPACT OF REDUCING TOTAL RED MEAT CONSUMPTION ON INTAKES OF IRON AND ZINC

604. Red meat is a rich source of iron (0.5-3.0 mg/100 g cooked red meat) and zinc (1.7-9.0 mg/100 g cooked red meat). A recommendation to reduce consumption of red and processed meat, in order to decrease colorectal cancer risk, could therefore have a negative impact on intakes of these nutrients in the UK.

605. The impact of dietary recommendations to reduce consumption of total red (including processed) meat on the iron and zinc intakes of adults was investigated by modelling intake data from the 2000/1 NDNS of adults aged 19-64y (Henderson *et al*, 2002). The effects of reducing red meat consumption was not modelled for adults aged 65y and over as the data were collected in 1994/5 and may be out of date.

606. The purpose of the modelling exercise was to explore the effect of reducing total red meat intakes of red meat consumers on:
    - mean intakes of iron and zinc;
    - the proportion of adults with intakes below the LRNIs for iron and zinc.

607. The potential effects of reducing red meat consumption (of red meat consumers) were assessed for the following maximum daily intakes: 180g, 120g, 100g, 90, 80g, 70g, 60g, 50g, 0g. The intakes of consumers exceeding each maximum level were reduced to the maximum level; intakes of those consuming below each maximum level were left unchanged.

608. The modelling exercise also explored the effect of reducing total red meat consumption on vitamin D intakes as red meat contains small amounts of vitamin D (0.3-1.0 µg/100 g cooked red meat). The impact of total red meat reduction on vitamin D intake is described in Annex 7.

Methods and assumptions

Categorisation of red meat

609. Epidemiological data vary in their definition and categorisation of red and processed meat. Red meat usually refers to beef, goat, lamb, pork and processed meat refers to meat (usually red) that has been preserved by smoking, curing, salting, or addition of preservatives (WCRF, 2007). In the modelling exercise red meat was not separated on the basis of whether it was processed or unprocessed because toxicological data suggest no clear evidence to explain an association between colorectal cancer risk and presence of preservatives (see Annex 5).

Estimates of total red meat consumption

610. There are no accurate estimates of total red meat consumption in the UK because the NDNS only provides estimates of total meat and meat dishes consumed (204 g/d for men; 135 g/d for women). However, composite meat dishes (e.g. lasagne, pies), which also contain non-meat components, are reported as total amount of meat consumed, resulting in an overestimation of meat consumption. To obtain more realistic estimates of total red meat consumption, the actual amount of red meat within composite meat dishes was quantified (see Annex 7).

Estimates of total iron and zinc intakes from red meat and products

611. Current iron and zinc intakes from total red meat were assessed from estimates of typical iron and zinc content of each meat type together with consumption estimates of each meat type (see Annex 7).

612. Further details of the methods and the assumptions used to estimate current intakes of iron and from total red meat consumption of adults (19-64y) are described in Annex 7.

Results of modelling exercise

Current intakes of total red meat, iron, and zinc

Table 10.1: Current total red meat consumption[32]

	Mean (95% confidence interval	Median	97.5 percentile	Maximum
Total red meat (g/d)				
Men	88 (84.3-91.1)	81	209	323
Women	52 (49.7-54.4)	48	133	249
Iron from total red meat (mg/d)				
Men	1.5 (1.5-1.6)	1.3	4.5	8.9
Women	0.9 (0.8-1.0)	0.8	2.7	7.5
Zinc from total red meat (mg/d)				
Men	3.3 (3.2-3.4)	3.0	7.8	12.8
Women	2.0 (1.9-2.1)	1.8	5.8	10.5

613. It can be seen from Table 10.1 that for consumers of red meat, current mean total red meat consumption is approximately 88g/d for men and 52g/d for women (70 g/d for men and women combined)[33]. The previous estimate for average total red meat intake of 90 g/d for the UK population reported by COMA (DH, 1998) is higher than the current estimate because it included non-meat components of composite dishes, which would overestimate red meat consumption.

614. Mean intake of iron from total red meat consumption is estimated to be 1.5 mg/d for men and 0.9 mg/d for women; therefore the amount of iron obtained per gram of total red meat is approximately 0.017 mg.

615. Mean intake of zinc from total red meat consumption is estimated to be 3.3 mg/d for men and 2.0mg/d for women; therefore the amount of zinc obtained per gram of total red meat is approximately 0.038 mg.

Effects of reducing total red meat consumption

616. The effect of reducing total meat consumption on mean intakes of iron and zinc are shown in Table 10.2 and the impact on the proportion of the population with intakes below the LRNI for iron and zinc is shown in Table 10.3.

[32] consumers of red meat only – 97% of survey participants

[33] Average consumption of total red meat for the UK population is 66 g/d (94% of consumers). Average consumption of red meat is 85 g/d for males and 47 g/d for females (i.e. 97% of males and 91% of females are consumers of red meat).

Table 10.2: Effect of reducing total red meat consumption on mean intakes of iron and zinc

Maximum red meat intake (g/d)	Mean iron intake from all foods (mg/d) (95% CI)		Mean zinc intake from all foods (mg/d) (95% CI)	
	Men	Women	Men	Women
Current	13 (12.9-13.5)	10 (9.8-10.3)	10 (10.0-10.4)	7.5 (7.2-7.5)
180	13 (12.8-13.5)	10 (9.8-10.3)	10 (9.9-10.4)	7.4 (7.2-7.5)
120	13 (12.7-13.4)	10 (9.8-10.3)	9.9 (9.7-10.1)	7.4 (7.2-7.5)
100	13 (12.6-13.3)	10 (9.8-10.2)	9.7 (9.5-9.9)	7.4 (7.2-7.4)
90	13 (12.6-13.2)	10 (9.7-10.2)	9.5 (9.4-9.7)	7.3 (7.1-7.4)
80	13 (12.5-13.1)	10 (9.7-10.2)	9.4 (9.2-9.6)	7.2 (7.1-7.3)
70	13 (12.4-13.0)	9.9 (9.7-10.2)	9.2 (9.0-9.4)	7.1 (7.0-7.3)
60	13 (12.3-12.9)	9.9 (9.6-10.1)	8.9 (8.7-9.1)	7.0 (6.9-7.2)
50	13 (12.2-12.8)	9.8 (9.6-10.1)	8.7 (8.5-8.9)	6.9 (6.8-7.0)
0	12 (11.4-12.1)	9.2 (9.0-9.5)	7.0 (6.8-7.2)	5.6 5.5-5.7)

Table 10.3: Effect of reducing total red meat consumption on the proportion of the population with intakes of iron and zinc below the LRNI

Maximum red meat intake (g/d)	% of population exceeding threshold		IRON Estimated % with intakes below LRNI (95% CI)		ZINC Estimated % with intakes below LRNI (95% CI)	
	Men	Women	Men	Women	Men	Women
Current			0.9 (0.2-1.5)	25 (22.0-22.7)	3.7 (2.4-5.0)	3.8 (2.5-5.1)
180	5	0.3	0.9 (0.2-1.5)	25 (22.0-22.7)	3.9 (2.6-5.2)	3.9 2.6-5.1()
120	20	4.1	1.0 (0.3-1.7)	25 (22.4-28.1)	3.9 (2.6-5.2)	3.9 (2.6-5.1)
100	33	8.3	1.0 (0.3-1.7)	25 (22.6-28.3)	4.0 (2.7-5.3)	3.9 (2.6-5.17)
90	42	12	1.0 (0.3-1.7)	25 (22.6-28.3)	4.1 (2.8-5.5)	3.9 (2.6-5.1)
80	50	17	1.0 (0.3-1.7)	26 (22.7-28.4)	4.4 (3.0-5.8)	3.9 (2.6-5.1)
70	58	23	1.0 (0.3-1.7)	26 (23.3-29.1)	5.5 (3.9-7.0)	3.9 (2.6-5.1)
60	66	33	1.0 (0.3-1.7)	27 (23.7-29.5)	6.1 (4.5-7.8)	4.1 (2.8-5.4)
50	74	43	1.0 (0.3-1.7)	27 (24.3-30.1)	9.5 (7.3-11.2)	5.0 (3.6-6.4)
0	97	91	2.8 (1.7-3.9)	32 (29.3-35.4)	29 (26.3-32.5)	20 (17.3-22.5)

At current levels of total red meat consumption

617. Current mean iron intake from all foods (excluding supplements) is approximately 13 mg/d for men and 10 mg/d women. As current mean intake of iron from total red meat consumption is 1.5 mg/d for men and 0.9 mg/d for women, the contribution of red meat to total iron intake is approximately 12% and 9% for men and women respectively.

618. Current mean zinc intake from all foods (excluding supplements) is 10 mg/d for men and 7.5 mg/d women consumers. As current mean intake of zinc from total red meat consumption is 3.3 mg/d for men and 2 mg/d for women, the contribution red meat to total zinc intake is approximately 32% and 27% for men and women respectively.

619. The proportion of the population with iron intakes below the LRNI is currently 0.9% for men and 25% for women.

620. The proportion of adults with zinc intakes below the LRNI is currently 3.7% for men and 3.8% for women.

Reduction of total red meat consumption to a maximum of 180 g/d

621. Approximately 5% of men and 0.3% of women currently have intakes of total red meat exceeding 180 g/d.

622. There would be no change in total iron intake (13 mg/d for men; 10 mg/d for women). There would be no change in total zinc intake for men (10 mg/d) and a reduction from 7.5 g/d to 7.4 g/d for women.

623. There would be no change in the proportion of adults with intakes below the LRNI for iron (0.9% of men; 25% of women). The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 3.9% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 120 g/d

624. Approximately 20% of men and 4% of women currently have intakes of total red meat exceeding 120 g/d.

625. There would be no change in total iron intake (13 mg/d for men; 10 mg/d for women). Total zinc intake would be reduced from 10 mg/d to 9.9 mg/d for men and from 7.5 mg/d to 7.4 mg/d for women.

626. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and remain at 25% or women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 3.9% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 100 g/d

627. Approximately 33% of men and 8% of women currently have intakes of total red meat exceeding 100 g/d.

628. There would be no change in total iron intake (13 mg/d for men; 10 mg/d for women). Total zinc intake would be reduced from 10 mg/d to 9.7 mg/d for men and from 7.5 mg/d to 7.4 mg/d for women.

629. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and remain at 25% or women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 4.0% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 90 g/d

630. Approximately 42% of men and 12% of women currently have intakes of total red meat exceeding 90 g/d.

631. There would be no change in total iron intake (13 mg/d for men; 10 mg/d for women). Total zinc intake would be reduced from 10 mg/d to 9.5 mg/d for men and from 7.5mg/d to 7.3mg/d for women.

632. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and remain at 25% for women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 4.1% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 80 g/d

633. Approximately 50% of men and 17% of women currently have intakes of total red meat exceeding 80 g/d.

634. There would be no change in total iron intake (13 mg/d for men; 10 mg/d for women). Total zinc intake would be reduced from 10 mg/d to 9.4 mg/d for men and 7.5 mg/d to 7.2 mg/d for women.

635. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and from 25% to 26% for women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 4.4% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 70 g/d

636. Approximately 58% of men and 23% of women currently have intakes of total red meat exceeding 70 g/d.

637. There would be no change in total iron intake for men (13 mg/d) and a reduction from 10 mg/d to 9.9 mg/d for women. Total zinc intake would be reduced from 10 mg/d to 9.2 mg/d for men and from 7.5 mg/d to 7.1 mg/d for women.

638. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and from 25% to 26% for women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 5.5% for men and from 3.8% to 3.9% for women.

Reduction of total red meat consumption to a maximum of 60 g/d

639. Approximately 66% of men and 33% of women currently have intakes of total red meat exceeding 60 g/d.

640. There would be no change in total iron intake for men (13 mg/d) and a reduction from 10 mg/d to 9.9 mg/d for women. Total zinc intake would be reduced from 10 mg/d to 8.9 mg/d for men and from 7.5 mg/d to 7.0 mg/d for women.

641. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and from 25% to 27% for women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 6.1% for men and from 3.8% to 4.1% for women.

Reduction of total red meat consumption to a maximum of 50 g/d

642. Approximately 74% of men and 43% of women currently have intakes of total red meat exceeding 50 g/d.

643. There would be no change in total iron intake for men (13 mg/d) and a reduction from 10 mg/d to 9.8 mg/d for women. Total zinc intake would be reduced from 10 mg/d to 8.7 mg/d for men and from 7.5 mg/d to 6.9 mg/d for women.

644. The proportion of adults with intakes below the LRNI for iron would increase from 0.9% to 1% for men and from 25% to 27% for women. The proportion of adults with intakes below the LRNI for zinc would increase from 3.7% to 9.5% for men and from 3.8 to 5% for women.

Interpretation of results from the modelling exercise

645. The modelling exercise shows that:

646. Red meat makes a greater contribution to total zinc intake from all foods (32% for men; 27% for women) than to total iron intake (12% for men; 9% for women).

647. Compared to the current situation, reducing total red meat consumption of high consumers (100 g/d or more) down to 90 g/d (previous COMA recommendation) or 80 g/d would have a minimal effect on total iron and zinc intakes or on the proportion of adults with iron or zinc intakes below the LRNI.

648. Reducing total red meat consumption of high consumers down to 70 g/d (WCRF recommendation for red meat intake) would have a minimal effect on the proportion of adults with iron intakes below the LRNI for iron but would increase the proportion of men with zinc intakes below the LRNI to over 5%.

Limitations of the modelling exercise

649. The potential effects of reducing total red meat consumption on overall intakes of iron and zinc, and on the proportion of adults with intakes below the LRNI for iron and zinc, are likely to be overestimates of real-life effects. This is because the modelling exercise did not attempt to incorporate the effects of replacing the reduction in total red meat consumption with other foods (e.g., white meat/poultry) which also contain iron and zinc.

650. The modelling did not consider the possible effect of recommendations to reduce red meat intakes on low red meat consumers, as it was assumed that the advice would be taken up only by high red meat consumers. However it is possible that any advice

to reduce total red meat consumption could result in further reductions or complete removal of red meat intake from the diets of low consumers of red meat.

651. The modelling exercise did not take any account of adaptive increases in dietary iron absorption in response to decreases in iron and zinc intakes.

652. The modelling also did not consider the beneficial impact that reducing total red meat consumption may have on other aspects of public health. Depending on the nutrient profile of foods replacing red meat in the diet, a reduction in total red meat could also lead to reductions in intakes of salt, total energy, total fat and saturated fat. These reductions would have significant public health benefits on the UK population by contributing to a decrease in high blood pressure, obesity, and cardiovascular disease.

Summary and conclusions

653. Current total red meat consumption of those who consume red meat is approximately 70 g/d (88g/d, men; 52g/d, women).

654. Red meat makes a greater contribution to total zinc intake from all foods (32% for men; 27% for women) than to total iron intake (12% for men; 9% for women).

655. Reducing the red meat consumption of high consumers of red meat (100 g/d or more) down to 80 g/d would have little impact on the proportion of people with iron or zinc intakes below the LRNI compared to current levels. Further reduction, to 70 g/d, would have a minimal effect on the proportion of people with iron intakes below the LRNI but the proportion of men with zinc intakes below the LRNI would exceed 5%.

11. OVERALL SUMMARY AND CONCLUSIONS

656. The key issues considered in this report were: iron in the diet, the health consequences of iron deficiency and iron excess, and adequacy of iron nutrition in the UK. The effects of a recommendation to reduce red and processed meat intake on the iron and zinc status of the UK population was also considered.

657. Iron is an essential nutrient which is required for transporting oxygen around the body as haemoglobin in red blood cells and for storage and use of oxygen in muscles in the form of myoglobin. It is also a component of a number of enzymes which are essential for many metabolic and synthetic functions.

658. Iron is also potentially toxic because free iron promotes free radical reactions. In humans, the risk of toxicity is minimised by tightly regulating the amount of iron in the body and by a series of proteins which bind free iron, carry it in the circulation and deliver it to functional sites or to deposits where it is stored in a safe form.

659. The body has no means of excreting excess iron. Iron that is surfeit to immediate requirements is deposited in tissues as ferritin. Serum concentrations of ferritin reflect the levels deposited in tissue and can be used as indicators of potential excess and deficiency of iron.

660. The body's iron supply is derived mainly from recycling iron contained in red blood cells. Increased needs for iron are met initially by increased release of iron from ferritin stores. The absorption of iron from the diet (i.e. the uptake by gut mucosa and subsequent transfer into the body) is determined by the body's need for iron to replace the small amounts lost from skin, hair, gut lining, menstruation, and to supply any additional amounts needed for growth and reproduction.

661. Polymorphisms in genes involved in regulating iron metabolism can affect iron absorption. Hereditary haemochromatosis, an autosomal recessive disease caused by mutation of the gene coding for HFE protein, results in excessive absorption of dietary iron leading to the accumulation of high levels of iron in the body, which can lead to tissue and organ damage. Two mutations of this gene, C282Y and H63D have been identified. It is not clear if heterozygotes have altered iron metabolism that will affect their requirements or predispose them to excessively accumulate iron.

662. A number of haematological and biochemical indicators are used to assess iron deficiency, adequacy, or excess. No single marker of iron metabolism is considered ideal for the assessment of iron deficiency or excess as all the individual indices have limitations in terms of their sensitivity and specificity. However, in this report, haemoglobin (functional iron) and serum ferritin (iron depots) were considered to be the most useful indicators of iron deficiency, adequacy, and excess.

663. The reference ranges for markers of iron metabolism define iron sufficiency. They do not define iron deficiency or iron excess as the thresholds selected for use are not based on functional defects. For example, low serum ferritin concentrations represent low iron depots in tissues; they do not represent a functional deficiency of iron. It is not known at which level above or below the reference range for serum ferritin is associated with an increased risk of an adverse outcome. Individuals with values either above or below the reference range may still be healthy. The reference limits only indicate the possibility of iron depletion, deficiency, or excess.

664. Iron is present in foods as haem or non-haem iron compounds. Haem iron is found mainly in foods of animal origin. Non-haem iron is found in animal and plant tissues, fortified foods, and supplements. The most important determinant of dietary iron absorption is systemic iron needs: more iron is absorbed from the diet in a state of iron deficiency and less is absorbed when iron depots are replete.

665. The absorption and subsequent systemic use of iron is referred to as iron bioavailability. Haem iron is absorbed more efficiently from the diet than non-haem iron. A number of dietary components have been shown to increase (ascorbic acid; meat) or reduce (calcium, phytates; phenolic compounds) non-haem iron absorption from single test meals. However, single meal absorption studies do not take account of adaptive absorptive and homeostatic responses over time to qualitative and quantitative changes in the diet. Results from whole diet studies, over several days or weeks, suggest that single meal studies overestimate the effects of enhancers and inhibitors of iron absorption. Effects of dietary modulators of iron absorption may be diluted substantially when they are consumed with other foods as part of a whole diet.

666. Observational data have generally not shown a relationship between markers of iron status and intakes of total iron or enhancers and inhibitors of iron absorption. Long term intervention studies have also, overall, not shown a corresponding change in markers of iron status. This raises uncertainties regarding the importance of dietary advice in the UK to maximise iron absorption: for example, eating cereal sources of iron with foods rich in vitamin C or avoiding drinking tea with meals.

667. Fortification of foods with iron has been the main approach used to increase the iron supply of the UK population. Iron fortification of white and brown wheat flour, to replace iron lost during processing, is mandatory in the UK. A number of other foods, including breakfast cereals and infant formulas are fortified on a voluntary basis. Evidence suggests that foods fortified with iron make little practical contribution to improving iron status.

668. There are no data to indicate that the bioavailability of dietary iron is a significant factor in the pathogenesis of anaemia and iron deficiency in the UK population. UK diets contain a broad range of foods containing iron and various enhancers and inhibitors of iron absorption. The effects of dietary modulators of iron absorption may be more important in developing countries where populations probably have insufficient iron intakes to meet requirements as diets are more limited and contain higher levels of inhibitors and lower levels of enhancers and haem iron.

669. Causes of iron deficiency include inadequate intakes of iron, impaired absorption, and increased blood losses due to menstruation or gastrointestinal disease. In developing countries, intestinal helminthiasis is a major cause of anaemia because it can lead to significant blood loss from the intestine. Depletion of iron stores leads to mobilisation of iron depots from macrophages or hepatocytes and upregulation of iron absorption. Progressive iron deficiency leads to anaemia, i.e. a decrease in haemoglobin concentrations and in circulating number of precursor red cells and iron-dependent functions are affected. Anaemia has been reported to have adverse effects on physical work capacity, pregnancy outcomes, and cognitive, motor and behavioural development in children.

670. Studies in humans suggest that iron deficiency anaemia (haemoglobin <120g/L plus an indicator of iron deficiency) is associated with reduced aerobic capacity although there is no clear threshold of haemoglobin concentration for this effect. There is little evidence that iron deficiency in the absence of anaemia (haemoglobin>120g/L; plus an indicator of iron deficiency) has adverse effects on work capacity. There is evidence suggesting that iron deficiency may impair endurance capacity and voluntary activity in humans, however there are insufficient data to reach clear conclusions.

671. Data from observational studies have suggested that maternal haemoglobin concentrations at either the low or high end of the distribution during pregnancy are associated with increased risk of adverse birth outcomes (i.e. low birth weight, preterm birth, perinatal mortality). The physiological changes that occur during pregnancy, such as plasma volume expansion and haemodilution, make it difficult to interpret the markers of iron metabolism during this time. High haemoglobin concentrations during pregnancy are generally not caused by high intakes of dietary or supplemental iron but are the result of inadequate plasma volume expansion, which is also associated with adverse birth outcomes. Intervention studies of routine iron supplementation during pregnancy have not reported beneficial or adverse effects on pregnancy outcomes.

672. Evidence from randomised controlled trials of iron supplementation suggests that iron deficiency anaemia is associated with poor motor development in children in the first three years of life; the long-term effects are unknown. There is insufficient evidence from rigorous randomised controlled trials to determine whether iron deficiency or iron deficiency anaemia affects cognitive or language development in children 3 years or under. There is evidence to suggest iron treatment has beneficial effects on cognitive development in anaemic older children, however, it is not known whether these benefits are sustained. Based on current evidence, it is not possible to derive thresholds of iron status at which cognitive, motor and behavioural development might be at risk.

673. Iron is thought to exert adverse effects by facilitating formation of free radicals leading to oxidative damage. It may have detrimental effects on the gut through direct interaction with intestinal mucosa or on other tissues if regulation of iron metabolism is impaired. Iron may also exert adverse effects by competing with other transition metals with similar absorption and transport mechanisms (such as copper and zinc) in a number of physiological processes.

674. It has been proposed that high iron intakes may increase the risk of colorectal cancer, cardiovascular disease (CVD), infection, neurodegenerative disorders, and inflammatory conditions. In the UK, the evidence for adverse effects of iron was considered insufficient to establish a safe upper level (SUL[34]). A guidance level (GL[35]) of 17 mg/day of supplemental iron, based on gastrointestinal effects, was recommended for adults. In the USA, a UL[36] of 45 mg/day was set for adults based on gastrointestinal effects. A UL for iron has not been set in Europe as adverse

[34] The SUL represents the amount of a nutrient that can be consumed daily over a lifetime without significant risk to health and is based on adequate available evidence.
[35] The GL is based on limited data and represents an approximate indication of intakes that would not be expected to cause adverse effects.
[36] The Tolerable Upper Intake Level represents the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population.

gastrointestinal effects were not considered a suitable basis to establish a UL for iron from all sources and there were insufficient data regarding other risks.

675. Limited epidemiological data suggest that: increased dietary intakes of total or haem iron are associated with increased colorectal cancer risk however confounding by other dietary and lifestyle factors is possible; high iron depots are not associated with increased colorectal cancer risk; and heterozygosity for hereditary haemochromatosis is associated with increased colorectal cancer risk. Overall, there are insufficient data on the association between colorectal cancer risk and dietary intakes of total iron, haem iron, iron status, or heterozygosity for hereditary haemochromatosis, to reach clear conclusions.

676. Meat, particularly red meat, is the main source of haem iron. The available epidemiological evidence suggests that red and processed meat intake is *probably* associated with increased colorectal cancer risk. However, as the available evidence is based on prospective observational studies, the effects of confounding by other factors associated with increased colorectal cancer risk cannot be excluded. It is not possible to identify if there is a dose-response or threshold level of red and processed meat which may be associated with an increased colorectal cancer risk because of a number of limitations in the data.

677. The available epidemiological evidence on total iron intake or status and CVD does not suggest an association. Limited evidence suggests that high intakes of haem iron *probably* increase CVD risk; it is possible that this could be due to other components of meat (the main source of haem iron) associated with CVD risk, such as saturated fats or dietary and lifestyle factors associated with meat intake. Studies of HFE heterozygosity and CVD risk suggest that C282Y heterozygotes (but not H63D heterozygotes) may be at increased risk of CVD, however, there are insufficient data to reach clear conclusions.

678. Limited evidence suggests that iron supplementation may have a negative effect on the physical growth of iron replete infants and children but further studies are required to confirm this effect. There is insufficient evidence to suggest that high iron intake or high iron depots increase the risk of diabetes mellitus in the general population or that homozygosity or heterozygosity for hereditary haemochromatosis increases diabetes risk. The evidence for an association between iron intake and rheumatoid arthritis is limited and inconclusive. There is no evidence that dietary iron is associated with Parkinson's disease or Alzheimer's disease.

679. Evidence from animal studies suggests that iron plays a role in immunity and infection. Most human studies on iron and infection have been conducted in developing countries where there are multiple nutrient deficiencies which may also affect resistance to infection. The evidence suggests that iron supplementation does not increase the risk of non-diarrhoeal or respiratory tract infections in children but may increase diarrhoea risk. It is not clear if iron supplementation increases risk of malaria or risk of infectious diseases in areas where malaria incidence is high. There is currently insufficient evidence to draw conclusions on the relationship between iron supplementation and HIV or tuberculosis. There is no evidence to suggest that improving iron status in the UK would have any impact on infectious disease incidence or morbidity.

680. Data from the National Diet and Nutrition Survey (NDNS) series and the Low Income Diet and Nutrition Survey (LIDNS) show that the major dietary sources of iron in the UK are cereals, including bread, which account for about half of the iron intake of most of the population. Meat & meat products & vegetables also make substantial contributions.

681. Average iron intakes in the general population are near (>90%) or above the reference nutrient intake (RNI[37]) for most population groups in the UK. Intakes below 90% of the RNI were reported for children aged 1½-3½ (73-81%), girls aged 11-18y (60%) and women aged 19-49y (66-87%). Population groups with substantial proportions below the lower reference nutrient intake (LRNI[38]) were children aged 1½-3½ (12-24%), girls aged 11-18y (44-48%) and women aged 19-49y (25-40%).

682. In low income groups average daily intakes were above the RNI for all males. For females, average intakes of iron were at or above the RNI for girls aged 2-10y and women aged 65y and over; mean daily intakes were below the RNI for girls aged 11-18y (63% of RNI) and women aged 19-49y (about 60% of RNI). A high proportion of females aged 11-49y (39% of girls aged 11-18y; 50% women 19-49y) had intakes below the LRNI.

683. In the general population, substantial proportions of children aged 1½-4½y, girls aged 11-18y, women aged 19-24y and 35-49y, and free living adults 75y and over had serum ferritin concentrations below WHO thresholds indicating an increased risk of iron deficiency. In low income groups, women aged 19-49y were at greatest risk of iron deficiency.

684. In the general population, risk of iron deficiency anaemia (haemoglobin and serum ferritin concentration below WHO thresholds) was highest (5-6%) for children aged 1½-2½y, girls aged 15-18y, women aged 35-49y, men 65y and over living in institutions, and free-living adults aged 85y and over. In low income groups, a substantial proportion of women aged 19-39y were at risk of iron deficiency anaemia (9-11%).

685. Although data from the NDNS and LIDNS suggest that considerable proportions of some population groups may have iron intakes below amounts required to meet their requirements, this is not clearly consistent with the iron status data which suggests that for 95% of the general population, current intakes are adequate to maintain their iron status above internationally accepted criteria for iron deficiency anaemia. This mismatch suggests that the dietary reference values (DRVs) for iron may have been set too high. The DRVs are based on limited data and may not take full account of absorptive adaptation to increased iron needs.

686. The NDNS and LIDNS both broadly show that women aged 15-50y are at increased risk of iron deficiency anaemia which is consistent with increased iron losses in this age group due to menstrual blood loss. More women of reproductive age from low income groups are at risk of iron deficiency anaemia compared to those in the general population. The reasons for this are not clear as mean iron intakes are similar. It is possible that intakes are insufficient to compensate for menstrual losses or increased pregnancy burden or due to greater ill health in this population.

[37] The RNI represents the amount of a nutrient that is likely to meet the needs of 97.5% of the population.
[38] The LRNI represents the amount of a nutrient that is likely to meet the needs of 2.5% of the population.

687. Iron deficiency anaemia observed in some adults aged 65 years and over might be caused by decreased absorption of dietary iron due to gastric atrophy or increased blood loss due to gastrointestinal disease or medication.

688. Although data from NDNS and LIDNS suggest that iron intake and iron status in the UK may be of public health concern for toddlers, women of reproductive age, and adults aged 65y and over, this is also dependent on the confidence placed on the dietary reference values for iron intake and on iron status criteria which are not based on functional defects.

689. A modelling exercise, which was carried out to explore the potential effect of reducing red meat consumption on intakes of iron and zinc, indicates that the current average consumption of total red meat (consumers of red meat only) in the UK is approximately 70 g/d (88 g/day, men; 52 g/d, women). This is lower than the previous average total red meat intake of 90 g/d cited in the COMA report[39] in 1998 because this figure included non-meat components of composite dishes such as meat products (e.g. sausage rolls, pies) and meals containing red meat (e.g. lasagne, stew) resulting in an overestimation of red meat consumption.

690. It is estimated that 33% of men and 8% of women are currently consuming more than 100 g/d of total red meat. The modelling data suggest that reducing intakes of high consumers of red and processed meat (100 g/d or more) down to an average of 80 g/day would have a minimal impact on the proportion of individuals with average intakes below the LRNI for iron and zinc. Further reductions in the total red meat intake of high consumers to an average of 70 g/d would have little effect on iron intakes but may lead to the proportion of men with intakes of zinc below the LRNI to increase from 3.7% to 5.5%.

691. A reduction in total red meat consumption may also lead to reductions in the intakes of salt, total energy, total fat, and saturated fat; this may lead to additional public health benefits by contributing to reductions in high blood pressure, obesity, and cardiovascular disease.

[39] Department of Health. *Nutrtional Aspects of the Development of Cancer*. Report on health and social subjects 48. London: TSO, 1998.

12. RECOMMENDATIONS

692. It is important to ensure that the UK population has a safe and adequate supply of iron. Most population groups in the UK are iron replete. Groups at risk of iron deficiency[40] include toddlers, girls and women of reproductive age, and adults aged over 65 years. Health professionals need to be vigilant of poor iron status in these groups and ensure that they are provided with appropriate medical advice, including dietary advice on how to increase their iron intakes and to consider use of iron supplements if required.

693. A public health approach to increasing iron intake, i.e. a healthy balanced diet, including a variety of foods containing iron, is important in helping people achieve adequate iron status. Such an approach is more important than focusing on particular inhibitors or enhancers of the bioavailability of iron from diets or the use of iron fortified foods.

694. Lower consumption of red and processed meat would probably reduce the risk of colorectal cancer. Although the evidence is not conclusive, as a precaution, it may be advisable for intakes of red and processed meat not to increase above the current average (70 g/d) and for high consumers of red and processed meat (100 g/d or more) to reduce their intakes.

695. As previously recommended by NICE (2008), iron supplementation should not be offered routinely to all pregnant women but should be considered for women identified with haemoglobin concentrations below 110 g/L in the first trimester and 105 g/L at 28 weeks.

[40] Defined as serum ferritin concentrations below the following thresholds (WHO): children 1½-4½y, 10 & 12 µg/L; children 4-6y, 12 µg/L; children >7y and adults, 15 µg/L.

13. RESEARCH RECOMMENDATIONS

696. Consideration of a harmonised and co-ordinated approach to research on iron nutrition in the UK and elsewhere.

697. Better data are needed to identify homeostatic and functional sequelae of iron deficiency, sufficiency, and excess. This would help to determine dose-response relationships and enable characterisation of the risks associated with insufficiency and excess of iron, and identification and validation of thresholds and markers which can be used as a basis for population risk management of iron deficiency and iron excess.

698. Future prospective studies assessing the relationship between iron and chronic disease should include better measures of iron exposure and employ a standardised approach to determine iron status based on measurement of serum ferritin concentration.

699. Future prospective studies assessing the relationship between red and processed meat intake and colorectal cancer risk should: use a standardised approach to assessment of dietary intakes and classification of red and processed meat; and examine genetic markers of susceptibility.

700. Further long term prospective studies examining the relationship between haem iron intake and CVD risk, with more accurate and reliable measures of haem iron intake, and of major established risk factors for CVD including plasma lipid profile.

701. Improvement of food composition databases, with particular reference to iron content. This would improve dietary assessments of intake for studies relating to iron and chronic disease.

702. Determine if foods fortified with iron, e.g., cereals and cereal products (including white and brown wheat flour) make a worthwhile contribution to achieving reference intakes and whether intakes of fortificant iron contribute to preventing iron deficiency.

703. Further data on iron intakes and status of infants aged up to 18 months and minority ethnic groups.

704. Further studies on whether delaying clamping of the umbilical cord at birth, until it has stopped pulsating, reduces the risk of iron deficiency in mid-infancy.

REFERENCES

1. Routine iron supplementation during pregnancy. Policy statement. US Preventive Services Task Force. JAMA 1993;270:2846-8.

2. A simple genetic test identifies 90% of UK patients with haemochromatosis. The UK Haemochromatosis Consortium. Gut 1997;41:841-4.

3. Abrams B, Duncan D, Hertz-Picciotto I. A prospective study of dietary intake and acquired immune deficiency syndrome in HIV-seropositive homosexual men. J Acquir Immune Defic Syndr 1993; 6(8):949-958.

4. Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by five- to seven-month-old infants. Pediatr.Res 1997;41:384-90.

5. Adams PC, Kertesz AE, McLaren CE, Barr R, Bamford A, Chakrabarti S. Population screening for hemochromatosis: a comparison of unbound iron-binding capacity, transferrin saturation, and C282Y genotyping in 5,211 voluntary blood donors. Hepatology 2000;31:1160-4.

6. Adish AA, Esrey SA, Gyorkos TW, Jean-Baptiste J, Rojhani A. Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. Lancet 1999; 353(9154):712-716.

7. Aggett PJ, Agostoni C, Axelsson I et al. Iron metabolism and requirements in early childhood: do we know enough?: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr. Gastroenterol. Nutr 2002;34:337-45.

8. Ahluwalia N, Sun J, Krause D, Mastro A, Handte G. Immune function is impaired in iron-deficient, homebound, older women. Am.J Clin.Nutr 2004;79:516-21.

9. Akesson A, Bjellerup P, Vahter M. Evaluation of kits for measurement of the soluble transferrin receptor. Scand.J Clin.Lab Invest 1999;59:77-81.

10. Akman M, Cebeci D, Okur V, Angin H, Abali O, Akman AC. The effects of iron deficiency on infants' developmental test performance. Acta Paediatr 2004; 93(10):1391-1396.

11. Alexander D, Ball MJ, Mann J. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. Eur J Clin Nutr 1994; 48(8):538-546.

12. Algarin C, Peirano P, Garrido M, Pizarro F, Lozoff B. Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. Pediatr Res 2003; 53(2):217-223.

13. Allen L. Pregnancy and iron deficiency: unresolved issues. Nutr Rev 1997;55:91-101.

14. Allen J, Backstrom KR, Cooper JA et al. Measurement of soluble transferrin receptor in serum of healthy adults. Clin.Chem. 1998;44:35-9.

15. Altes A, Gimferrer E, Capella G, Barcelo MJ, Baiget M. Colorectal cancer and HFE gene mutations. Haematologica 1999;84:479-80.

16. Andang'o PE, Osendarp SJ, Ayah R, West CE, Mwaniki DL, De Wolf CA, Kraaijenhagen R, Kok FJ, Verhoef H. Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. Lancet 2007; 369(9575):1799-1806.

17. Andrews NC. Disorders of iron metabolism. N Engl J Med 1999; 341(26):1986-1995.

18. Aronow WS, Ahn C. Three-year follow-up shows no association of serum ferritin levels with incidence of new coronary events in 577 persons aged > or = 62 years. Am.J.Cardiol. 1996;78:678-9.

19. Arredondo M, Martinez R, Nunez MT, Ruz M, Olivares M. Inhibition of iron and copper uptake by iron, copper and zinc. Biol Res 2006; 39(1):95-102.

20. Asberg A, Hveem K, Thorstensen K et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand.J Gastroenterol. 2001;36:1108-15.

21. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation 1994;89:969-74.

22. Ascherio A, Rimm EB, Giovannucci E, Willett WC, Stampfer MJ. Blood donations and risk of coronary heart disease in men. Circulation 2001;103:52-7.

23. Assuncao MC, Santos IS, Barros AJ, Gigante DP, Victora CG. [Effect of iron fortification of flour on anemia in preschool children in Pelotas, Brazil]. Rev Saude Publica 2007; 41(4):539-548.

24. Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. Arch.Dis.Child 1986;61:849-57.

25. Bach KM, Tetens I, Alstrup Jorgensen AB, Dal Thomsen A, Milman N, Hels O, Sandstrom B, Hansen M. A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread. Eur J Nutr 2005; 44(6):334-340.

26. Bach KM, Hels O, Morberg C, Marving J, Bugel S, Tetens I. Pork meat increases iron absorption from a 5-day fully controlled diet when compared to a vegetarian diet with similar vitamin C and phytic acid content. Br J Nutr 2005; 94(1):78-83.

27. Backstrand JR, Allen LH, Black AK, de Mata M, Pelto GH. Diet and iron status of nonpregnant women in rural Central Mexico. Am J Clin Nutr 2002; 76(1):156-164.

28. Baech SB, Hansen M, Bukhave K et al. Increasing the cooking temperature of meat does not affect nonheme iron absorption from a phytate-rich meal in women. J Nutr 2003;133:94-7.

29. Bagchi K, Mohanram M, Reddy V. Humoral immune response in children with iron-deficiency anaemia. Br.Med.J. 1980;280:1249-51.

30. Bainton DF, Finch CA. The diagnosis of iron deficiency anemia. Am.J Med. 1964;37:62-70.

31. Baker E, Morgan EH. Iron transport. In: Brock JH, Halliday JW, Pippard M, Powell LW, eds. Iron metabolism in Health and Disease. London: WB Saunders 1994:63-95.

32. Balder HF, Vogel J, Jansen MC, Weijenberg MP, van den Brandt PA, Westenbrink S, van der MR, Goldbohm RA. Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. Cancer Epidemiol Biomarkers Prev 2006; 15(4):717-725.

33. Ball MJ, Bartlett MA. Dietary intake and iron status of Australian vegetarian women. Am J Clin Nutr 1999; 70(3):353-358.

34. Ballart IJ, Estevez ME, Sen L et al. Progressive dysfunction of monocytes associated with iron overload and age in patients with thalassemia major. Blood 1986;67:105-9.

35. Ballot D, Baynes RD, Bothwell TH et al. The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br.J Nutr 1987;57:331-43.

36. Baqui AH, Zaman K, Persson LA, El Arifeen S, Yunus M, Begum N, Black RE. Simultaneous weekly supplementation of iron and zinc is associated with lower morbidity due to diarrhea and acute lower respiratory infection in Bangladeshi infants. J Nutr 2003; 133(12):4150-4157.

37. Barclay SM, Aggett PJ, Lloyd DJ, Duffty P. Reduced erythrocyte superoxide dismutase activity in low birth weight infants given iron supplements. Pediatr Res 1991; 29(3):297-301.

38. Barrett JF, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. BMJ 1994;309:79-82.

39. Barton JC, Bertoli LF, Rothenberg BE. Peripheral blood erythrocyte parameters in hemochromatosis: evidence for increased erythrocyte hemoglobin content. J Lab Clin.Med. 2000;135:96-104.

40. Basta SS, Soekirman, Karyadi D, Scrimshaw NS. Iron deficiency anemia and the productivity of adult males in Indonesia. Am.J.Clin.Nutr 1979;32:916-25.

41. Baynes RD, Cook JD, Bothwell TH, Friedman BM, Meyer TE. Serum transferrin receptor in hereditary hemochromatosis and African siderosis. Am.J Hematol. 1994;45:288-92.

42. Beal VA, Meyers AJ. Iron nutriture from infancy to adolescence. Am J Public Health Nations Health 1970; 60(4):666-678.

43. Beard JL, Murray-Kolb LE, Haas JD, Lawrence F. Iron absorption prediction equations lack agreement and underestimate iron absorption. J Nutr 2007; 137(7):1741-1746.

44. Beckman LE, Van Landeghem GF, Sikstrom C et al. Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. Carcinogenesis 1999;20:1231-3.

45. Bendich A, Cohen M. Ascorbic acid safety: analysis of factors affecting iron absorption. Toxicol.Lett. 1990;51:189-201.

46. Bendich A. Calcium supplementation and iron status of females. Nutrition 2001; 17(1):46-51.

47. Benito-Garcia E, Feskanich D, Hu FB, Mandl LA, Karlson EW. Protein, iron, and meat consumption and risk for rheumatoid arthritis: a prospective cohort study. Arthritis Res Ther 2007; 9(1):R16.

48. Berger J, Dyck JL, Galan P, Aplogan A, Schneider D, Traissac P, Hercberg S. Effect of daily iron supplementation on iron status, cell-mediated immunity, and incidence of infections in 6-36 month old Togolese children. Eur J Clin Nutr 2000; 54(1):29-35.

49. Beutler E, Felitti V, Gelbart T, Ho N. The effect of HFE genotypes on measurements of iron overload in patients attending a health appraisal clinic. Ann.Intern.Med. 2000;133:329-37.

50. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.

51. Beutler E. Hemochromatosis: genetics and pathophysiology. Annu Rev Med 2006; 57:331-347.

52. Bezwoda WR, TORRANCE JD, Bothwell TH, MacPhail AP, Graham B, Mills W. Iron absorption from red and white wines. Scand J Haematol 1985; 34(2):121-127.

53. Bhaskaram C, Reddy V. Cell-mediated immunity in iron- and vitamin-deficient children. Br.Med.J. 1975;3:522.

54. Bingham SA, Day NE, Luben R, et al. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003; 361(9368):1496-1501.

55. Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. J Nutr 2002; 132(11 Suppl):3522S-3525S.

56. Bjorn-Rasmussen E, Hallberg L. Effect of animal proteins on the absorption of food iron in man. Nutr Metab 1979; 23(3):192-202.

57. Black MM, Baqui AH, Zaman K, Ake PL, El Arifeen S, Le K, McNary SW, Parveen M, Hamadani JD, Black RE. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. Am J Clin Nutr 2004; 80(4):903-910.

58. Boelaert JR, Piette J, Weinberg GA, Sappey C, Weinberg ED. Iron and oxidative stress as a mechanism for the enhanced production of human immunodeficiency virus by alveolar macrophages from otherwise healthy cigarette smokers. J Infect Dis 1996; 173(4):1045-1047.

59. Boelaert JR, Weinberg GA, Weinberg ED. Altered iron metabolism in HIV infection: mechanisms, possible consequences, and proposals for management. Infect Agents Dis 1996; 5(1):36-46.

60. Boelaert JR, Vandecasteele SJ, Appelberg R, Gordeuk VR. The effect of the host's iron status on tuberculosis. J Infect Dis 2007; 195(12):1745-1753.

61. Bothwell TH, Seftel H, Jacobs P, Torrance JD, Baumslag N. Iron overload in Bantu subjects: studies on the availability of iron in Bantu beer. Am J Clin Nutr 1964; 14:47-51.

62. Bothwell TH, MacPhail AP. Hereditary hemochromatosis: etiologic, pathologic, and clinical aspects. Semin Hematol 1998; 35(1):55-71.

63. Bradbear RA, Bain C, Siskind V et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. J.Natl.Cancer Inst. 1985;75:81-4.

64. Brink M, Weijenberg MP, de Goeij AF, Roemen GM, Lentjes MH, de Bruine AP, Goldbohm RA, van den Brandt PA. Meat consumption and K-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study. Br J Cancer 2005; 92(7):1310-1320.

65. Brissot P, Troadec MB, Bardou-Jacquet E, Le Lan C, Jouanolle AM, Deugnier Y, Loreal O. Current approach to hemochromatosis. Blood Rev 2008; 22(4):195-210.

66. British Nutrition Foundation. Iron: nutritional and physiological significance. 1995. Cornwall: T.J. Press (Padstow) Ltd.

67. Brittin HC, Nossaman CE. Iron content of food cooked in iron utensils. J Am Diet Assoc 1986; 86(7):897-901.

68. Brownlie T, Utermohlen V, Hinton PS, Haas JD. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. Am J Clin Nutr 2004; 79(3):437-443.

69. Brune M, Magnusson B, Persson H, Hallberg L. Iron losses in sweat. Am.J.Clin.Nutr. 1986;43:438-43.

70. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet 1996;348:992-6.

71. Brutsaert TD, Hernandez-Cordero S, Rivera J, Viola T, Hughes G, Haas JD. Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. Am J Clin Nutr 2003; 77(2):441-448.

72. Bryan CF, Leech SH, Ducos R et al. Thermostable erythrocyte rosette-forming lymphocytes in hereditary hemochromatosis. I. Identification in peripheral blood. J Clin.Immunol. 1984;4:134-42.

73. Bryan CF, Leech SH, Kumar P, Gaumer R, Bozelka B, Morgan J. The immune system in hereditary hemochromatosis: a quantitative and functional assessment of the cellular arm. Am J Med.Sci. 1991;301:55-61.

74. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N.Engl.J.Med. 1996;335:1799-805.

75. Burden MJ, Westerlund AJ, Armony-Sivan R, Nelson CA, Jacobson SW, Lozoff B, Angelilli ML, Jacobson JL. An event-related potential study of attention and recognition memory in infants with iron-deficiency anemia. Pediatrics 2007; 120(2):e336-e345.

76. Burt MJ, George PM, Upton JD et al. The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 1998;43:830-6.

77. Cairo G, Pietrangelo A. Iron regulatory proteins in pathobiology. Biochem.J. 2000;352 Pt 2:241-50.

78. Cantinieaux B, Hariga C, Ferster A, De Maertelaere E, Toppet M, Fondu P. Neutrophil dysfunctions in thalassaemia major: the role of cell iron overload. Eur J Haematol 1987; 39(1):28-34.

79. Cantinieaux B, Hariga C, Ferster A, Toppet M, Fondu P. Desferrioxamine improves neutrophil phagocytosis in thalassemia major. Am J Hematol 1990; 35(1):13-17.

80. Carpenter CE, Clark E. Evalation of methods used in meat iron analysis and iron content or raw and cooked meats. J Agric Food Chem 1995;43:1824-1827.

81. Cavill I, Ricketts C. Human iron kinetics. In: Iron in Biochemistry and Medicine, II, Ed.: A Jacobs and M Worwood, Academic Press, London, 1980: 573-604.

82. CDC (Centers for Disease Control). CDC criteria for anemia in children and childbearing-aged women. Morbid Mortal Weekly Rpt 1989;38:400-4.

83. Celsing F, Blomstrand E, Werner B, Pihlstedt P, Ekblom B. Effects of iron deficiency on endurance and muscle enzyme activity in man. Med.Sci.Sports Exerc. 1986;18:156-61.

84. Chambers V, Sutherland L, Palmer K, Dalton A, Rigby AS, Sokol R, Pollitt R, Tanner S, Gleeson D. Haemochromatosis-associated HFE genotypes in English blood donors: age-related frequency and biochemical expression. J Hepatol 2003; 39(6):925-931.

85. Chan AT, Tranah GJ, Giovannucci EL, Willett WC, Hunter DJ, Fuchs CS. Prospective study of N-acetyltransferase-2 genotypes, meat intake, smoking and risk of colorectal cancer. Int J Cancer 2005; 115(4):648-652.

86. Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, Rodriguez C, Sinha R, Calle EE. Meat consumption and risk of colorectal cancer. JAMA 2005; 293(2):172-182.

87. Chaparro CM, Neufeld LM, Tena AG, Eguia-Liz CR, Dewey KG. Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial. Lancet 2006; 367(9527):1997-2004.

88. Charlton RW, Bothwell TH. Definition, prevalence and prevention of iron deficiency. Clin.Haematol. 1982;11:309-25.

89. Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, Hennekens CH, Kelsey KT, Hunter DJ. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 1998; 58(15):3307-3311.

90. Chen J, Zhao X, Zhang X, Yin S, Piao J, Huo J, Yu B, Qu N, Lu Q, Wang S, Chen C. Studies on the effectiveness of NaFeEDTA-fortified soy sauce in controlling iron deficiency: a population-based intervention trial. Food Nutr Bull 2005; 26(2):177-186.

91. Choi HK. Dietary risk factors for rheumatic diseases. Curr Opin Rheumatol 2005; 17(2):141-146.

92. Choi JW, Pai SH, Im MW, Kim SK. Change in transferrin receptor concentrations with age. Clin.Chem. 1999;45:1562-3.

93. Chwang LC, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin.Nutr. 1988;47:496-501.

94. Cohen RJ, Brown KH, Canahuati J, Rivera LL, Dewey KG. Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomised intervention study in Honduras. Lancet 1994; 344(8918):288-293.

95. Colombo J. Infant cognition: predicting later intellectual functioning. Newbury Park, London: Sage Publications, 1993.

96. Conte D, Manachino D, Colli A et al. Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. Ann.Intern.Med. 1998;128:370-3.

97. Cook JD, Layrisse M, Finch CA. The measurement of iron absorption. Blood 1969; 33(3):421-429.

98. Cook JD, Monsen ER. Food iron absorption. I. Use of semisynthetic diet to study absorption of nonheme iron. Am.J.Clin.Nutr. 1975;28:1289-95.

99. Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am.J.Clin.Nutr. 1976;29:859-67.

100. Cook JD, Monsen ER. Vitamin C, the common cold, and iron absorption. Am.J Clin.Nutr 1977;30:235-41.

101. Cook JD, Morck TA, Lynch SR. The inhibitory effect of soy products on nonheme iron absorption in man. Am J Clin Nutr 1981; 34(12):2622-2629.

102. Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS. The effect of high ascorbic acid supplementation on body iron stores. Blood 1984; 64(3):721-726.

103. Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency in the US population. Blood 1986; 68(3):726-731.

104. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am.J Clin.Nutr 1991;54:717-22.

105. Cook JD, Reddy MB. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 2001; 73(1):93-98.

106. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359-64.

107. Costagliola DG, de Montalembert M, Lefrere JJ, Briand C, Rebulla P, Baruchel S, Dessi C, Fondu P, Karagiorga M, Perrimond H, . Dose of desferrioxamine and evolution of HIV-1 infection in thalassaemic patients. Br J Haematol 1994; 87(4):849-852.

108. Cowin I, Emond A, Emmett P; ALSPAC Study Group. Association between composition of the diet and haemoglobin and ferritin levels in 18-month-old children. Eur J Clin Nutr 2001;55(4):278-86.

109. Crompton DW, Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 2002; 22:35-59.

110. Cronje L, Edmondson N, Eisenach KD, Bornman L. Iron and iron chelating agents modulate Mycobacterium tuberculosis growth and monocyte-macrophage viability and effector functions. FEMS Immunol Med Microbiol 2005; 45(2):103-112.

111. Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 2003; 63(10):2358-2360.

112. Cross AJ, Gunter MJ, Wood RJ, Pietinen P, Taylor PR, Virtamo J, Albanes D, Sinha R. Iron and colorectal cancer risk in the alpha-tocopherol, beta-carotene cancer prevention study. Int J Cancer 2006; 118(12):3147-3152.

113. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med 2007; 4(12):e325.

114. Czajka-Narins DM, Haddy TB, Kallen DJ. Nutrition and social correlates in iron deficiency anemia. Am.J.Clin.Nutr 1978;31:955-60.

115. Dallman PR, Refino C, Yland MJ. Sequence of development of iron deficiency in the rat. Am.J.Clin.Nutr. 1982;35:671-7.

116. Dallman PR, Widdowson EM, Dickerson JWT. Chemical composition of body. In: Comar CL, Bronner F, eds. Mineral metabolism: and advanced treatise volume 2 part A. Orlando Academic Press 1988:1-247.

117. Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 1999;99:852-4.

118. Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber B. Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clin.Chem. 1998;44:2429-32.

119. Davey GK, Spencer EA, Appleby PN, Allen NE, Knox KH, Key TJ. EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. Public Health Nutr 2003; 6(3):259-269.

120. Davidsson L, Galan P, Kastenmayer P et al. Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. Pediatr.Res 1994;36:816-22.

121. Davies KJ, Maguire JJ, Brooks GA, Dallman PR, Packer L. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. Am J Physiol 1982;242:E418-E427.

122. Davies KJ, Donovan CM, Refino CJ, Brooks GA, Packer L, Dallman PR. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. Am J Physiol 1984;246:E535-E543.

123. Davis CD, Malecki EA, Greger JL. Interactions among dietary manganese, heme iron, and nonheme iron in women. Am.J.Clin.Nutr. 1992;56:926-32.

124. Dawson EB, Dawson R, Behrens J, DeVora MA, McGanity WJ. Iron in prenatal multivitamin/multimineral supplements. Bioavailability. J Reprod Med 1998; 43(2):133-140.

125. de Andraca I, Walter T, Castillo M, Pino P, Rivera P, and Cobo C. Iron deficiency anemia and its effects upon psychological development at pre-school age: a longitudinal study. 53-62. 1990. Nestle Foundation Annual Report.

126. De D, I, Ward DM, Nemeth E, Vaughn MB, Musci G, Ganz T, Kaplan J. The molecular basis of ferroportin-linked hemochromatosis. Proc Natl Acad Sci U S A 2005; 102(25):8955-8960.

127. Deehr MS, Dallal GE, Smith KT, Taulbee JD, Dawson-Hughes B. Effects of different calcium sources on iron absorption in postmenopausal women. Am J Clin Nutr 1990; 51(1):95-99.

128. Deinard AS, List A, Lindgren B, Hunt JV, Chang PN. Cognitive deficits in iron-deficient and iron-deficient anemic children. J.Pediatr. 1986;108:681-9.

129. Delanghe JR, Langlois MR, Boelaert JR, Van Acker J, Van Wanzeele F, van der GG, Hemmer R, Verhofstede C, De Buyzere M, De Bacquer D, Arendt V, Plum J. Haptoglobin polymorphism, iron metabolism and mortality in HIV infection. AIDS 1998; 12(9):1027-1032.

130. Delanghe JR, Langlois MR. Haptoglobin polymorphism and body iron stores. Clin Chem Lab Med 2002; 40(3):212-216.

131. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. World Health Stat.Q. 1985;38:302-16.

132. de Monye C, Karcher DS, Boelaert JR, Gordeuk VR. Bone marrow macrophage iron grade and survival of HIV-seropositive patients. AIDS 1999; 13(3):375-380.

133. Department of Health and Social Security. Nutritional Aspects of Bread and Flour. Report on Health and Social Subjects No. 23. Report by the Committee on Medical Aspects of Food Policy, pp. 1-64. 1981. London: H.M. Stationery Office.

134. Department of Health. Dietary Reference Values for Food, Energy and Nutrients in the United Kingdom. 1991. London: HSMO. Report on Health and Social Subjects, No. 41.

135. Department of Health. Nutritional Aspects of Cardiovascular Disease. 1994. London: HMSO. Report on Health and Social Subjects, No. 46.

136. Department of Health. Nutritional Aspects of the Development of Cancer. 1998. London: HMSO. Report on Health and Social Subjects, No. 48.

137. Derman DP, Bothwell TH, TORRANCE JD, Bezwoda WR, MacPhail AP, Kew MC, Sayers MH, Disler PB, Charlton RW. Iron absorption from maize (Zea mays) and sorghum (Sorghum vulgare) beer. Br J Nutr 1980; 43(2):271-279.

138. de Valk B, Marx JJ. Iron, atherosclerosis, and ischemic heart disease. Arch Intern Med 1999; 159(14):1542-1548.

139. Dewey KG, Cohen RJ, Rivera LL, Brown KH. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. Am J Clin Nutr 1998; 67(5):878-884.

140. Dewey KG, Domellof M, Cohen RJ, Landa RL, Hernell O, Lonnerdal B. Iron supplementation affects growth and morbidity of breast-fed infants: results of a randomized trial in Sweden and Honduras. J.Nutr. 2002;132:3249-55.

141. Diaz M, Rosado JL, Allen LH, Abrams S, Garcia OP. The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. Am J Clin Nutr 2003; 78(3):436-440.

142. Dinarello CA. Interleukin 1 as mediator of the acute-phase response. Surv Immunol Res 1984;3(1):29-33.

143. Disler PB, Lynch SR, Charlton RW et al. The effect of tea on iron absorption. Gut 1975;16:193-200.

144. Domellof M, Dewey KG, Lonnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. J.Nutr. 2002;132:3680-6.

145. Domellof M, Lonnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O. Sex differences in iron status during infancy. Pediatrics 2002;110:545-52.

146. Domellof M, Lonnerdal B, Abrams SA, Hernell O. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. Am.J Clin.Nutr 2002;76:198-204.

147. Dooley, J and Worwood, M. Genetic Haemochromotosis. Guidelines on Diagnosis and Therapy Compiled on behalf of the British Committee for Standards in Haemotology. 1-1-2000. Abingdon, Darwin Medical Communications.

148. Driva A, Kafatos A, Salaman M. Iron deficiency and the cognitive and psychomotor development of children: A pilot study with institutionalised children. Early Child Devel Care 1985;22:73-82.

149. Duggan MB, Steel G, Elwys G, Harbottle L, Noble C. Iron status, energy intake, and nutritional status of healthy young Asian children. Arch Dis Child 1991; 66(12):1386-1389.

150. Dunlop W, Furness C, Hill LM. Maternal haemoglobin concentration, haematocrit and renal handling of urate in pregnancies ending in the births of small-for-dates infants. Br J Obstet Gynaecol 1978; 85(12):938-940.

151. Dwyer J, Wood C, McNamara J et al. Abnormalities in the immune system of children with beta-thalassaemia major. Clin.Exp.Immunol. 1987;68:621-9.

152. Eaton SB, Konner M. Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med 1985; 312(5):283-289.

153. Edgerton VR, Bryant SL, Gillespie CA, Gardner GW. Iron deficiency anemia and physical performance and activity of rats. J Nutr 1972; 102(3):381-399.

154. Edgerton VR, Diamond LB, Olson J. Voluntary activity, cardiovascular and muscular responses to anemia in rats. J Nutr 1977; 107(9):1595-1601.

155. Edgerton VR, Gardner GW, Ohira Y, Gunawardena KA, Senewiratne B. Iron-deficiency anaemia and its effect on worker productivity and activity patterns. Br Med J 1979; 2(6204):1546-1549.

156. Edwards CQ, Griffen LM, Bulaj ZJ, Ajioka RS, Kushner JP. The iron phenotype of hemochromatosis heterozygotes. Hemochromatosis – genetics, pathophysiology, diagnosis and

treatment (ed. by JC Barton & CQ Edwards), pp. 411-418. 2000. Cambridge University Press, Cambridge.

157. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N.Engl.J Med. 1988;318:1355-62.

158. Ehrhardt P. Iron deficiency in young Bradford children from different ethnic groups. Br.Med.J.(Clin.Res.Ed) 1986;292:90-3.

159. Ekblom K, Hultdin J, Stegmayr B, Johansson I, Van Guelpen B, Hallmans G, Weinehall L, Johansson L, Wiklund PG, Marklund SL. Iron stores and HFE genotypes are not related to increased risk of ischemic stroke. A prospective nested case-referent study. Cerebrovasc Dis 2007; 24(5):405-411.

160. Ellervik C, Tybjaerg-Hansen A, Grande P, Appleyard M, Nordestgaard BG. Hereditary hemochromatosis and risk of ischemic heart disease: a prospective study and a case-control study. Circulation 2005; 112(2):185-193.

161. Emond AM, Hawkins N, Pennock C, Golding J. Haemoglobin and ferritin concentrations in infants at 8 months of age. Arch.Dis.Child 1996;74:36-9.

162. Engelmann MD, Sandstrom B, Michaelsen KF. Meat intake and iron status in late infancy: an intervention study. J Pediatr Gastroenterol Nutr 1998; 26(1):26-33.

163. English DR, MacInnis RJ, Hodge AM, Hopper JL, Haydon AM, Giles GG. Red meat, chicken, and fish consumption and risk of colorectal cancer. Cancer Epidemiol.Biomarkers Prev. 2004;13:1509-14.

164. Escalona E, Malave I, Rodriguez E, Araujo Z, Inati J, Arends A, Perdomo Y. Mitogen induced lymphoproliferative responses and lymphocyte sub-populations in patients with sickle cell disease. J Clin Lab Immunol 1987; 22(4):191-196.

165. European Food Safety Authority. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Iron. The EFSA Journal 2004; 125:1-34.

166. Expert Group on Vitamins and Minerals (EVM). Safe Upper Levels for Vitamins and Minerals. 2003.

167. Expert Scientific Working Group. Summary of a report on assessment of the iron nutritional status of the United States population. Expert Scientific Working Group. Am.J.Clin.Nutr. 1985;42:1318-30.

168. Fairweather-Tait SJ, Fox TE, Mallillin A. Balti curries and iron. BMJ 1995; 310(6991):1368.

169. Fairweather-Tait SJ, Teucher B. Iron and calcium bioavailability of fortified foods and dietary supplements. Nutr.Rev. 2002;60:360-7.

170. FAO and WHO. Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultion (Bangkok, Thailand). 2002. Rome, WHO/FAO.

171. Feder JN, Gnirke A, Thomas W et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat.Genet. 1996;13:399-408.

172. Feingold AO. Association of tuberculosis with alcoholism. South Med J 1976; 69(10):1336-1337.

173. Fidler MC, Davidsson L, Zeder C, Walczyk T, Hurrell RF. Iron absorption from ferrous fumarate in adult women is influenced by ascorbic acid but not by Na2EDTA. Br.J Nutr. 2003;90:1081-5.

174. Fidler MC, Walczyk T, Davidsson L et al. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. Br.J Nutr. 2004;91:107-12.

175. Fidler MC, Davidsson L, Zeder C, Hurrell RF. Erythorbic acid is a potent enhancer of nonheme-iron absorption. Am.J Clin.Nutr. 2004;79:99-102.

176. Finch S, Doyle, W., Lowe C, Bates, C. J, Prentice, A., Smithers, G, and Clarke P, C. National Diet and Nutrition Survey: people aged 65 years and over. Volume 1: Report of the diet and nutrition survey. 1998. London, The Stationery Office.

177. Finch CA, Deubelbeiss K, Cook JD et al. Ferrokinetics in man. Medicine (Baltimore) 1970;49:17-53.

178. Fischer WC, Kordas K, Stoltzfus RJ, Black RE. Interactive effects of iron and zinc on biochemical and functional outcomes in supplementation trials. Am J Clin Nutr 2005; 82(1):5-12.

179. Flanagan PR. Mechanisms and regulation of intestinal uptake and transfer of iron. Acta Paediatr.Scand.Suppl 1989;361:21-30.

180. Fleming RE, Bacon BR. Orchestration of iron homeostasis. N Engl J Med 2005; 352(17):1741-1744.

181. Fleming DJ, Jacques PF, Dallal GE, Tucker KL, Wilson PW, Wood RJ. Dietary determinants of iron stores in a free-living elderly population: The Framingham Heart Study. Am.J.Clin.Nutr. 1998;67:722-33.

182. Fleming DJ, Jacques PF, Massaro JM, D'Agostino RB, Sr., Wilson PW, Wood RJ. Aspirin intake and the use of serum ferritin as a measure of iron status. Am J Clin Nutr 2001; 74(2):219-226.

183. Flood A, Velie EM, Sinha R et al. Meat, fat, and their subtypes as risk factors for colorectal cancer in a prospective cohort of women. Am.J.Epidemiol. 2003;158:59-68.

184. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. J Lab Clin.Med. 1989;114:368-77.

185. Fogelholm M, Alopaeus K, Silvennoinen T, Teirila J. Factors affecting iron status in non-pregnant women from urban south Finland. Eur.J.Clin.Nutr. 1993;47:567-74.

186. Food and Agriculture Organization. Requirements of Vitamin A, Iron, Folate and B12. Report of a Joint FAO/WHO consultation. 1988. Rome, FAO. Food and Nutrition Series No 23.

187. Food Standards Agency. McCance and Widdowson's The Composition of Foods, Sixth summary edition. Cambridge: Royal Society of Chemistry, 2002.

188. Forest JC, Masse J, Moutquin JM. Maternal hematocrit and albumin as predictors of intrauterine growth retardation and preterm delivery. Clin Biochem 1996; 29(6):563-566.

189. Fox CJ, Cullen DJ, Knuiman MW et al. Effects of body iron stores and haemochromatosis genotypes on coronary heart disease outcomes in the Busselton health study. J Cardiovasc.Risk 2002;9:287-93.

190. Fracanzani AL, Conte D, Fraquelli M et al. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. Hepatology 2001;33:647-51.

191. Frazer DM, Wilkins SJ, Becker EM et al. A rapid decrease in the expression of DMT1 and Dcytb but not Ireg1 or hephaestin explains the mucosal block phenomenon of iron absorption. Gut 2003;52:340-6.

192. Freeman VE, Mulder J, van't Hof MA, Hoey HM, Gibney MJ. A longitudinal study of iron status in children at 12, 24 and 36 months. Public Health Nutr 1998;1:93-100.

193. Frey GH, Krider DW. Serum ferritin and myocardial infarct. W.V.Med.J. 1994;90:13-5.

194. Friel JK, Andrews WL, Aziz K, Kwa PG, Lepage G, L'Abbe MR. A randomized trial of two levels of iron supplementation and developmental outcome in low birth weight infants. J.Pediatr. 2001;139:254-60.

195. Friel JK, Aziz K, Andrews WL, Harding SV, Courage ML, Adams RJ. A double-masked, randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. J.Pediatr. 2003;143:582-6.

196. Friis H, Gomo E, Nyazema N, Ndhlovu P, Krarup H, Madsen PH, Michaelsen KF. Iron, haptoglobin phenotype, and HIV-1 viral load: a cross-sectional study among pregnant Zimbabwean women. J Acquir Immune Defic Syndr 2003; 33(1):74-81.

197. Galan P, Hercberg S, Soustre Y, Dop MC, Dupin H. Factors affecting iron stores in French female students. Hum Nutr Clin Nutr 1985; 39(4):279-287.

198. Galan P, Cherouvrier F, Preziosi P, Hercberg S. Effects of the increasing consumption of dairy products upon iron absorption. Eur J Clin Nutr 1991; 45(11):553-559.

199. Galan P, Noisette N, Estaquio C, Czernichow S, Mennen L, Renversez JC, Briancon S, Favier A, Hercberg S. Serum ferritin, cardiovascular risk factors and ischaemic heart diseases: a prospective analysis in the SU.VI.MAX (SUpplementation en VItamines et Mineraux AntioXydants) cohort. Public Health Nutr 2006; 9(1):70-74.

200. Galan P, Thibault H, Preziosi P, Hercberg S. Interleukin 2 production in iron-deficient children. Biol.Trace Elem.Res. 1992;32:421-6.

201. Galan P, Yoon HC, Preziosi P et al. Determining factors in the iron status of adult women in the SU.VI.MAX study. Supplementation en VItamines et Mineraux Antioxydants. Eur.J.Clin.Nutr. 1998;52:383-8.

202. Galaris D, Evangelou A. The role of oxidative stress in mechanisms of metal-induced carcinogenesis. Crit Rev Oncol Hematol 2002; 42(1):93-103.

203. Gangaidzo IT, Moyo VM, Mvundura E, Aggrey G, Murphree NL, Khumalo H, Saungweme T, Kasvosve I, Gomo ZA, Rouault T, Boelaert JR, Gordeuk VR. Association of pulmonary tuberculosis with increased dietary iron. J Infect Dis 2001; 184(7):936-939.

204. Ganz T. Hepcidin--a regulator of intestinal iron absorption and iron recycling by macrophages. Best Pract Res Clin Haematol 2005; 18(2):171-182.

205. Ganz T. Hepcidin in iron metabolism. Curr.Opin.Hematol. 2004;11:251-4.

206. Garcia OP, Diaz M, Rosado JL, Allen LH. Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. Am J Clin Nutr 2003; 78(2):267-273.

207. Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematologic levels and pregnancy outcomes. Semin.Perinatol. 1981;5:155-62.

208. Garry PJ, Hunt WC, Baumgartner RN. Effects of iron intake on iron stores in elderly men and women: longitudinal and cross-sectional results. J.Am.Coll.Nutr. 2000;19:262-9.

209. Gartside PS, Glueck CJ. The important role of modifiable dietary and behavioral characteristics in the causation and prevention of coronary heart disease hospitalization and mortality: the prospective NHANES I follow-up study. J Am.Coll.Nutr 1995;14:71-9.

210. Gear JS, Mann JI, Thorogood M, Carter R, Jelfs R. Biochemical and haematological variables in vegetarians. Br Med J 1980; 280(6229):1415.

211. Geier D, Hebert B, Potti A. Risk of primary non-hepatocellular malignancies in hereditary hemochromatosis. Anticancer Res. 2002;22:3797-9.

212. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ 2002; 325(7373):1142.

213. Gera T, Sachdev HP, Nestel P. Effect of iron supplementation on physical performance in children and adolescents: systematic review of randomized controlled trials. Indian Pediatr 2007; 44(1):15-24.

214. Gibson SA. Iron intake and iron status of preschool children: associations with breakfast cereals, vitamin C and meat. Public Health Nutr. 1999;2:521-8.

215. Gillooly M, Bothwell TH, Torrance JD et al. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br.J.Nutr. 1983;49:331-42.

216. Glassman AB, Deas DV, Berlinsky FS, Bennett CE. Lymphocyte blast transformation and peripheral lymphocyte percentages in patients with sickle cell disease. Ann Clin Lab Sci 1980; 10(1):9-12.

217. Gleerup A, Rossander-Hulthen L, Gramatkovski E, Hallberg L. Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am.J.Clin.Nutr. 1995;61:97-104.

218. Gordeuk VR, Mukkiibi J, Med M, Hasstedt SJ, Samowitz W, Edwards CQ. Iron overload in Africa. Interaction between a gene and dietary iron content. N.Engl.J Med. 1992;326:95-100.

219. Gordeuk V, Thuma P, Brittenham G, McLaren C, Parry D, Backenstose A, Biemba G, Msiska R, Holmes L, McKinley E, . Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. N Engl J Med 1992; 327(21):1473-1477.

220. Gordeuk VR, Thuma PE, Brittenham GM, Zulu S, Simwanza G, Mhangu A, Flesch G, Parry D. Iron chelation with desferrioxamine B in adults with asymptomatic Plasmodium falciparum parasitemia. Blood 1992; 79(2):308-312.

221. Graf E, Eaton JW. Dietary suppression of colonic cancer. Fiber or phytate? Cancer 1985;56:717-8.

222. Grange JM, Kardjito T, Beck JS, Ebeid O, Kohler W, Prokop O. Haptoglobin: an immunoregulatory role in tuberculosis? Tubercle 1985; 66(1):41-47.

223. Granick S. Ferritin: its properties and significance for iron metabolism. Chem Rev 1946;38:379-403.

224. Grantham-McGregor S, Schofield W, Haggard D. Maternal-child interaction in survivors of severe malnutrition who received psychosocial stimulation. Eur.J.Clin.Nutr 1989;43:45-52.

225. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J.Nutr 2001;131:649S-66S.

226. Green R, Charlton R, Seftel H et al. Body iron excretion in man: a collaborative study. Am.J.Med. 1968;45:336-53.

227. Gregory, J, Foster, K, Tyler, H, and Wiseman, M. The Dietary and Nutritional Survey of British Adults. 1990. London, HMSO.

228. Gregory, J, Collins, D. L, Davies, P. S. W, Hughes, J. M, and Clarke, P. C. National Diet and Nutrition Survey: Children Aged 1.5 to 4.5 years. Volume 1. 1995. London, HMSO.

229. Gregory, J, Lowe, S, Bates, C. J, Prentice, A., Jackson, L. V, Smithers, G, Wenlock, R, and Farron, M. National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey. 2000. London, The Stationery Office.
Ref Type: Report

230. Griffin IJ, Abrams SA. Iron and breastfeeding. Pediatr.Clin.North Am. 2001;48:401-13.

231. Grinder-Pedersen L, Bukhave K, Jensen M, Højgaard L, Hansen M. Calcium from milk or calcium-fortified foods does not inhibit nonheme-iron absorption from a whole diet consumed over a 4-d period. Am J Clin Nutr 2004;80(2):404-9.

232. Grindulis H, Scott PH, Belton NR, Wharton BA. Combined deficiency of iron and vitamin D in Asian toddlers. Arch Dis.Child 1986;61:843-8.

233. Guglielmo P, Cunsolo F, Lombardo T et al. T-subset abnormalities in thalassaemia intermedia: possible evidence for a thymus functional deficiency. Acta Haematol. 1984;72:361-7.

234. Gunn IR, Maxwell FK, Gaffney D, McMahon AD, Packard CJ. Haemochromatosis gene mutations and risk of coronary heart disease: a west of Scotland coronary prevention study (WOSCOPS) substudy. Heart 2004; 90(3):304-306.

235. Gutierrez JA, Yu J, Rivera S, Wessling-Resnick M. Functional expression cloning and characterization of SFT, a stimulator of Fe transport. J.Cell Biol. 1997;139:895-905.

236. Haas JD, Brownlie T. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr 2001; 131(2S-2):676S-688S.

237. Hallberg L. Results of surveys to assess iron status in Europe. Nutr Rev 1995; 53(11):314-322.

238. Hallberg L, Nilsson L. Constancy of individual menstrual blood loss. Acta Obstet.Gynecol.Scand. 1964;43:352-9.

239. Hallberg L, Hogdahl AM, Nilsson L, Rybo G. Menstrual blood loss--a population study. Variation at different ages and attempts to define normality. Acta Obstet.Gynecol.Scand. 1966;45:320-51.

240. Hallberg L, Hogdahl AM, Nilsson L, Rybo G. Menstrual blood loss and iron deficiency. Acta Med.Scand. 1966;180:639-50.

241. Hallberg L, Rossander L. Effect of different drinks on the absorption of non-heme iron from composite meals. Hum Nutr Appl.Nutr 1982;36:116-23.

242. Hallberg L, Rossander L. Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. Am.J Clin.Nutr 1984;39:577-83.

243. Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am.J.Clin.Nutr. 1989;49:140-4.

244. Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. Am.J Clin.Nutr 1991;53:112-9.

245. Hallberg L, Rossander-Hulten L. Iron requirements in menstruating women. Am.J.Clin.Nutr. 1991;54:1047-58.

246. Hallberg L, Rossander-Hulthen L, Brune M, Gleerup A. Inhibition of haem-iron absorption in man by calcium. Br.J Nutr. 1993;69:533-40.

247. Hallberg L, Bengtsson C, Lapidus L, Lindstedt G, Lundberg PA, Hulten L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. Br.J Haematol. 1993;85:787-98.

248. Hallberg L, Hulten L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? Am.J.Clin.Nutr. 1997;66:347-56.

249. Hallberg L. Does calcium interfere with iron absorption? Am.J.Clin.Nutr. 1998;68:3-4.

250. Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. Am.J.Clin.Nutr. 2000;71:1147-60.

251. Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. J.Neurochem. 1958;3:41-51.

252. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem.J. 1984;219:1-14.

253. Halliwell B, Gutteridge JM, Blake D. Metal ions and oxygen radical reactions in human inflammatory joint disease. Philos Trans R Soc Lond B Biol Sci 1985;311(1152):659-671.

254. Hann HW, Stahlhut MW, Blumberg BS. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. Cancer Res 1988; 48(15):4168-4170.

255. Harahap H, Jahari AB, Husaini MA, Saco-Pollitt C, Pollitt E. Effects of an energy and micronutrient supplement on iron deficiency anemia, physical activity and motor and mental development in undernourished children in Indonesia. Eur.J.Clin.Nutr. 2000;54 Suppl 2:S114-S119.

256. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. Biochim.Biophys.Acta 1996;1275:161-203.

257. Harvey LJ, Armah CN, Dainty JR, Foxall RJ, John LD, Langford NJ, Fairweather-Tait SJ. Impact of menstrual blood loss and diet on iron deficiency among women in the UK. Br J Nutr 2005; 94(4):557-564.

258. Harvey PW, Heywood PF, Nesheim MC, Galme K, Zegans M, Habicht JP, Stephenson LS, Radimer KL, Brabin B, Forsyth K, . The effect of iron therapy on malarial infection in Papua New Guinean schoolchildren. Am J Trop Med Hyg 1989; 40(1):12-18.

259. Hasanbegovic E, Sabanovic S. [Effects of iron therapy on motor and mental development of infants and small children suffering from iron deficiency anaemia]. Med Arh 2004; 58(4):227-229.

260. Haschke F, Ziegler EE, Edwards BB, Fomon SJ. Effect of iron fortification of infant formula on trace mineral absorption. J Pediatr Gastroenterol Nutr 1986; 5(5):768-773.

261. Heath AL, Skeaff CM, Williams S, Gibson RS. The role of blood loss and diet in the aetiology of mild iron deficiency in premenopausal adult New Zealand women. Public Health Nutr. 2001;4:197-206.

262. Heath AL, Roe MA, Oyston SL, Fairweather-Tait SJ. Meal-based intake assessment tool: relative validity when determining dietary intake of Fe and Zn and selected absorption modifiers in UK men. Br J Nutr 2005; 93(3):403-416.

263. Helman AD, Darnton-Hill I. Vitamin and iron status in new vegetarians. Am J Clin Nutr 1987; 45(4):785-789.

264. Henderson, L, Gregory, J, and Swan, G. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed. 2002. London, The Stationery Office.

265. Henderson, L, Gregory J, Irving, K, and Swan, G. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 2: Energy, protein, carbohydrate, fat and alcohol intake. 2003. London, The Stationery Office.

266. Henderson, L, Irving, K, Gregory, J, Bates, C. J, Prentice, A., Perks, J, Swan, G, and Farron, M. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes. 2003. London, The Stationery Office.

267. Hercberg S, Galan P. Biochemical effects of iron deprivation. Acta Paediatr.Scand.Suppl 1989;361:63-70.

268. Hernandez P, Cruz C, Santos MN, Ballester JM. Immunologic dysfunction in Sickle cell anaemia. Acta Haematol 1980; 63(3):156-161.

269. Heywood A, Oppenheimer S, Heywood P, Jolley D. Behavioral effects of iron supplementation in infants in Madang, Papua New Guinea. Am.J.Clin.Nutr 1989;50:630-7.

270. Hinton PS, Giordano C, Brownlie T, Haas JD. Iron supplementation improves endurance after training in iron-depleted, nonanemic women. J Appl.Physiol 2000;88:1103-11.

271. Hinton PS, Sinclair LM. Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. Eur J Clin Nutr 2007; 61(1):30-39.

272. Hoare, J, Henderson, L, Bates, C. J, Prentice, A., Birch, M, Swan, G, and Farron, M. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Summary report. 2004. London, TSO.

273. Hsing AW, McLaughlin JK, Olsen JH, Mellemkjar L, Wacholder S, Fraumeni JF, Jr. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. Int.J.Cancer 1995;60:160-2.

274. Hsing AW, McLaughlin JK, Chow WH, Schuman LM, Co Chien HT, Gridley G, Bjelke E, Wacholder S, Blot WJ. Risk factors for colorectal cancer in a prospective study among U.S. white men. Int J Cancer 1998; 77(4):549-553.

275. Huebers H, Josephson B, Huebers E, Csiba E, Finch C. Uptake and release of iron from human transferrin. Proc.Natl.Acad.Sci.U.S.A 1981;78:2572-6.

276. Huebers H, Csiba E, Huebers E, Finch CA. Molecular advantage of diferric transferrin in delivering iron to reticulocytes: a comparative study. Proc.Soc.Exp.Biol.Med. 1985;179:222-6.

277. Hulthen L, Lindstedt G, Lundberg PA, Hallberg L. Effect of a mild infection on serum ferritin concentration--clinical and epidemiological implications. Eur.J Clin.Nutr 1998;52:376-9.

278. Hulthen L, Lindstedt G, Lundberg P, Hallberg L. Effect of a mild infection on serum ferritin concentration - clinical and epidemiological implications. European Journal of Clinical Nutrition 2002; 52:376-379.

279. Hunt JR, Mullen LM, Lykken GI, Gallagher SK, Nielsen FH. Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women. Am.J Clin.Nutr. 1990;51:649-55.

280. Hunt JR, Gallagher SK, Johnson LK. Effect of ascorbic acid on apparent iron absorption by women with low iron stores. Am.J Clin.Nutr. 1994;59:1381-5.

281. Hunt JR, Gallagher SK, Johnson LK, Lykken GI. High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am.J.Clin.Nutr. 1995;62:621-32.

282. Hunt JR, Roughead ZK. Nonheme-iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am.J.Clin.Nutr. 1999;69:944-52.

283. Hunt JR, Zeng H. Iron absorption by heterozygous carriers of the HFE C282Y mutation associated with hemochromatosis. Am.J.Clin.Nutr. 2004;80:924-31.

284. Hunt JR, Zito CA, Erjavec J, Johnson LK. Severe or marginal iron deficiency affects spontaneous physical activity in rats. Am J Clin Nutr 1994; 59(2):413-418.

285. Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. Am J Clin Nutr 2000; 71(1):94-102.

286. Huo J, Sun J, Miao H, Yu B, Yang T, Liu Z, Lu C, Chen J, Zhang D, Ma Y, Wang A, Li Y. Therapeutic effects of NaFeEDTA-fortified soy sauce in anaemic children in China. Asia Pac J Clin Nutr 2002; 11(2):123-127.

287. Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. Am J Clin Nutr 1992; 56(3):573-578.

288. Hurrell RF. Bioavailability of iron. Eur J Clin Nutr 1997; 51 Suppl 1:S4-S8.

289. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Rev 1997; 55(6):210-222.

290. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption in humans: bovine serum albumin compared with beef muscle and egg white. Am.J.Clin.Nutr. 1988;47:102-7.

291. Hurrell RF, Reddy M, Cook JD. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. Br.J Nutr. 1999;81:289-95.

292. Idjradinata P, Watkins WE, Pollitt E. Adverse effect of iron supplementation on weight gain of iron-replete young children. Lancet 1994; 343(8908):1252-1254.

293. Idjradinata P, Pollitt E. Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 1993;341:1-4.

294. Ilich-Ernst JZ, McKenna AA, Badenhop NE, Clairmont AC, Andon MB, Nahhas RW, Goel P, Matkovic V. Iron status, menarche, and calcium supplementation in adolescent girls. Am J Clin Nutr 1998; 68(4):880-887.

295. Institute of Medicine (IOM). Iron deficiency anemia: recommended guidelines for the prevention, detection and management among U.S children and women of childbearing age. 1993. Washington, DC, National Academy Press.

296. International Nutritional Anemia Consultative Group (INAGG). Iron absorption from cereals and legumes. A report of the International Nutritional Anemia Consultative Group. New York, NY: The Nutrition Foundation, 1982:1-41.

297. Jackson HA, Carter K, Darke C et al. HFE mutations, iron deficiency and overload in 10,500 blood donors. Br.J Haematol. 2001;114:474-84.

298. James JA, Laing GJ, Logan S. Changing patterns of iron deficiency anaemia in the second year of life. BMJ 1995;311:230.

299. Jarvinen R, Knekt P, Hakulinen T, Rissanen H, Heliovaara M. Dietary fat, cholesterol and colorectal cancer in a prospective study. Br J Cancer 2001; 85(3):357-361.

300. Jehn ML, Guallar E, Clark JM, Couper D, Duncan BB, Ballantyne CM, Hoogeveen RC, Harris ZL, Pankow JS. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 2007; 165(9):1047-1054.

301. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004; 291(6):711-717.

302. Jiang R, Ma J, Ascherio A, Stampfer MJ, Willett WC, Hu FB. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. Am J Clin Nutr 2004; 79(1):70-75.

303. Johnson MA, Hove SS. Development of anemia in copper-deficient rats fed high levels of dietary iron and sucrose. J Nutr 1986; 116(7):1225-1238.

304. Johnson MB, Enns CA. Diferric transferrin regulates transferrin receptor 2 protein stability. Blood 2004; 104(13):4287-4293.

305. Kabat GC, Miller AB, Jain M, Rohan TE. A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. Br J Cancer 2007; 97(1):118-122.

306. Kalgaonkar, S., and Lonnerdal, B. (2008a). Effects of dietary factors on iron uptake from ferritin by Caco-2 cells. Journal of Nutritional Biochemistry *19*, 33-39.

307. Kalgaonkar, S., and Lonnerdal, B. (2008b). Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. J Nutr Biochem *In Press*.

308. Kalkwarf HJ, Harrast SD. Effects of calcium supplementation and lactation on iron status. Am J Clin Nutr 1998; 67(6):1244-1249.

309. Kasvosve I, Gomo ZA, Mvundura E, Moyo VM, Saungweme T, Khumalo H, Gordeuk VR, Boelaert JR, Delanghe JR, De Bacquer D, Gangaidzo IT. Haptoglobin polymorphism and mortality in patients with tuberculosis. Int J Tuberc Lung Dis 2000; 4(8):771-775.

310. Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. Nutr Cancer 1997; 28(3):276-281.

311. Kato I, Dnistrian AM, Schwartz M et al. Iron intake, body iron stores and colorectal cancer risk in women: a nested case-control study. Int.J.Cancer 1999;80:693-8.

312. Kemahli AS, Babacan E, Cavdar AO. Cell mediated immune responses in children with iron deficiency and combined iron and zinc deficiency. Nutrition Research 1988;8:129-36.

313. Kent S, Weinberg E. Hypoferremia: adaptation to disease? N.Engl.J.Med. 1989;320:672.

314. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. Circulation 1997;96:3300-7.

315. Klingshirn LA, Pate RR, Bourque SP, Davis JM, Sargent RG. Effect of iron supplementation on endurance capacity in iron-depleted female runners. Med Sci Sports Exerc 1992; 24(7):819-824.

316. Klipstein-Grobusch K, Koster JF, Grobbee DE et al. Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam Study. Am.J Clin.Nutr 1999;69:1231-6.

317. Klipstein-Grobusch K, Grobbee DE, den Breeijen JH, Boeing H, Hofman A, Witteman JC. Dietary iron and risk of myocardial infarction in the Rotterdam Study. Am.J Epidemiol. 1999;149:421-8.

318. Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int J Cancer 1999; 80(6):852-856.

319. Knuiman MW, Divitini ML, Olynyk JK, Cullen DJ, Bartholomew HC. Serum ferritin and cardiovascular disease: a 17-year follow-up study in Busselton, Western Australia. Am.J.Epidemiol. 2003;158:144-9.

320. Koerper MA, Dallman PR. Serum iron concentration and transferrin saturation in the diagnosis of iron deficiency in children: normal developmental changes. J Pediatr. 1977;91:870-4.

321. Koller O, Sagen N, Ulstein M, Vaula D. Fetal growth retardation associated with inadequate haemodilution in otherwise uncomplicated pregnancy. Acta Obstet Gynecol Scand 1979; 58(1):9-13.

322. Konijn AM, Hershko C. Ferritin synthesis in inflammation. I. Pathogenesis of impaired iron release. Br J Haematol 1977; 37(1):7-16.

323. Kordas K, Siegel EH, Olney DK, Katz J, Tielsch JM, Chwaya HM, Kariger PK, Leclerq SC, Khatry SK, Stoltzfus RJ. Maternal reports of sleep in 6-18 month-old infants from Nepal and Zanzibar: association with iron deficiency anemia and stunting. Early Hum Dev 2008; 84(6):389-398.

324. Krantman HJ, Young SR, Ank BJ, O'Donnell CM, Rachelefsky GS, Stiehm ER. Immune function in pure iron deficiency. Am.J.Dis.Child 1982;136:840-4.

325. Kupka R, Msamanga GI, Mugusi F, Petraro P, Hunter DJ, Fawzi WW. Iron status is an important cause of anemia in HIV-infected Tanzanian women but is not related to accelerated HIV disease progression. J Nutr 2007; 137(10):2317-2323.

326. Kuratko CN. Decrease of manganese superoxide dismutase activity in rats fed high levels of iron during colon carcinogenesis. Food Chem Toxicol 1998; 36(9-10):819-824.

327. Kuvibidila S, Baliga BS. Role of iron in immunity and infection. In: Calder PC, Field CJ, Gill HS, eds. Nutriton and Immune Function. Wallingford: CABI 2002:209-28.

328. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996; 42(10):1589-1600.

329. Langlois MR, Martin ME, Boelaert JR, Beaumont C, Taes YE, De Buyzere ML, Bernard DR, Neels HM, Delanghe JR. The haptoglobin 2-2 phenotype affects serum markers of iron status in healthy males. Clin Chem 2000; 46(10):1619-1625.

330. Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. Int J Cancer 2006; 119(11):2657-2664.

331. Larsson G, Milsom I, Lindstedt G, Rybo G. The influence of a low-dose combined oral contraceptive on menstrual blood loss and iron status. Contraception 1992;46:327-34.

332. Larsson SC, Rafter J, Holmberg L, Bergkvist L, Wolk A. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: The Swedish Mammography Cohort. Int.J.Cancer 2005;113:829-34.

333. Lawson MS, Thomas M, Hardiman A. Iron status of Asian children aged 2 years living in England. Arch.Dis.Child 1998;78:420-6.

334. Layrisse M, Martinez-Torres C, Leets I, Taylor P, Ramirez J. Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J.Nutr. 1984;114:217-23.

335. Layrisse M, Martinez-Torres C, Roche M. Effect of interaction of various foods on iron absorption. Am J Clin Nutr 1968; 21(10):1175-1183.

336. Layrisse M, Cook JD, Martinez C, Roche M, Kuhn IN, Walker RB, Finch CA. Food iron absorption: a comparison of vegetable and animal foods. Blood 1969; 33(3):430-443.

337. Layrisse M, Chaves JF, Mendez C, Bosch V, Tropper E, Bastardo B, Gonzalez E. Early response to the effect of iron fortification in the Venezuelan population. Am J Clin Nutr 1996; 64(6):903-907.

338. Layrisse M, Garcia-Casal MN, Mendez-Castellano H, Jimenez M, Henry O, Chavez JE, Gonzalez E. Impact of fortification of flours with iron to reduce the prevalence of anemia and iron deficiency among schoolchildren in Caracas, Venezuela: a follow-up. Food Nutr Bull 2002; 23(4):384-389.

339. Ledue TB, Craig WY. Serum concentrations of transferrin receptor in hereditary hemochromatosis. Clin.Chem. 1995;41:1053-4.

340. Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR, Jr. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. J Natl Cancer Inst 2004; 96(5):403-407.

341. Lee DH, Folsom AR, Jacobs DR, Jr. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: the Iowa Women's Health Study. Am J Clin Nutr 2005; 81(4):787-791.

342. Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of haemochromatosis amongst asymptomatic Australians. Br.J.Haematol. 1990;74:525-30.

343. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J 1986; 111(2):383-390.

344. Letsky E. The haematological system. In: Hytten F, Chamberlain G, eds. Clinical Physiology. Oxford: Blackwell Scientific Publications 1991:39-82.

345. Levitsky DA, Strupp BJ. Malnutrition and the brain: changing concepts, changing concerns. J.Nutr 1995;125:2212S-20S.

346. Li R, Chen X, Yan H, Deurenberg P, Garby L, Hautvast JG. Functional consequences of iron supplementation in iron-deficient female cotton mill workers in Beijing, China. Am J Clin Nutr 1994; 59(4):908-913.

347. Liao Y, Cooper RS, McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study. Am.J Epidemiol. 1994;139:704-12.

348. Lind T, Lonnerdal B, Persson LA, Stenlund H, Tennefors C, Hernell O. Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: a randomized intervention in infants from 6 to 12 mo of age. Am.J Clin.Nutr 2003;78:168-75.

349. Lind T, Hernell O, Lonnerdal B, Stenlund H, Domellof M, Persson LA. Dietary iron intake is positively associated with hemoglobin concentration during infancy but not during the second year of life. J Nutr 2004;134:1064-70.

350. Liu JM, Hankinson SE, Stampfer MJ, Rifai N, Willett WC, Ma J. Body iron stores and their determinants in healthy postmenopausal US women. Am J Clin Nutr 2003; 78(6):1160-1167.

351. Lonnerdal B, Hernell O. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. Acta Paediatr 1994; 83(4):367-373.

352. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. JAMA 1997;277:973-6.

353. Looker AC, Johnson CL. Prevalence of elevated serum transferrin saturation in adults in the United States. Ann.Intern.Med. 1998;129:940-5.

354. Looker AC, Loyevsky M, Gordeuk VR. Increased serum transferrin saturation is associated with lower serum transferrin receptor concentration. Clin.Chem. 1999;45:2191-9.

355. Lounis N, Truffot-Pernot C, Grosset J, Gordeuk VR, Boelaert JR. Iron and Mycobacterium tuberculosis infection. J Clin Virol 2001; 20(3):123-126.

356. Lozoff B, Jimenez E, Smith JB. Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. Arch Pediatr Adolesc Med 2006; 160(11):1108-1113.

357. Lozoff B, Brittenham GM, Viteri FE, Wolf AW, Urrutia JJ. The effects of short-term oral iron therapy on developmental deficits in iron deficient anemic infants. J Pediat 1982;100:351-7.

358. Lozoff B. Does preventing iron-deficiency anemia (IDA) improve developmental test scores? Pediat Res 1997;A39:136 (abstr).

359. Lozoff B. Explanatory mechanisms for poorer development in iron-deficient anemic infants. In: Grantham-McGregor SM, ed. Recent advances in research on the effects of health and nutrition on children's development and school achievement in the Third World: policy implications. Washington D.C: PAHO 1998.

360. Lozoff B, Brittenham G, Viteri FE, Urrutia JJ. Behavioural abnormalities in infants with iron deficiency anamia. In: Pollitt E, Leibel RL, eds. Brain Biochemistry and Behavior. New York, NY: Raven Press 1982:183-93.

361. Lozoff B, Wolf AW, Urrutia JJ, Viteri FE. Abnormal behavior and low developmental test scores in iron-deficient anemic infants. J Dev.Behav Pediatr. 1985;6:69-75.

362. Lozoff B, Klein NK, Prabucki KM. Iron-deficient anemic infants at play. J Dev.Behav Pediatr. 1986;7:152-8.

363. Lozoff B, Brittenham GM, Wolf AW et al. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics 1987;79:981-95.

364. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. N.Engl.J Med. 1991;325:687-94.

365. Lozoff B, Wolf AW, Jimenez E. Iron-deficiency anemia and infant development: effects of extended oral iron therapy. J Pediatr. 1996;129:382-9.

366. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron-deficiency anemia. Child Dev. 1998;69:24-36.

367. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics 2000;105:E51.

368. Lozoff B. Perinatal iron deficiency and the developing brain. Pediatr.Res. 2000;48:137-9.

369. Lozoff B, De A, I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics 2003;112:846-54.

370. Lu ZM, Goldenberg RL, Cliver SP, Cutter G, Blankson M. The relationship between maternal hematocrit and pregnancy outcome. Obstet Gynecol 1991; 77(2):190-194.

371. Lukaski HC, Hall CB, Siders WA. Altered metabolic response of iron-deficient women during graded, maximal exercise. Eur J Appl Physiol Occup Physiol 1991; 63(2):140-145.

372. Lyle RM, Weaver CM, Sedlock DA, Rajaram S, Martin B, Melby CL. Iron status in exercising women: the effect of oral iron therapy vs increased consumption of muscle foods. Am.J Clin.Nutr. 1992;56:1049-55.

373. Lynch SR, Dassenko SA, Cook JD, Juillerat MA, Hurrell RF. Inhibitory effect of a soybean-protein--related moiety on iron absorption in humans. Am J Clin Nutr 1994; 60(4):567-572.

374. Lynch SR, Cook JD. Interaction of vitamin C and iron. Ann.N.Y.Acad.Sci. 1980;355:32-44.

375. Lynch SR, Skikne BS, Cook JD. Food iron absorption in idiopathic hemochromatosis. Blood 1989;74:2187-93.

376. Lynn R, Harland P. A positive effect of iron supplementation on the IQs of iron deficient children. Person Individ Diff 1998;24:883-5.

377. Macdonald GA, Tarish J, Whitehall VJ et al. No evidence of increased risk of colorectal cancer in individuals heterozygous for the Cys282Tyr haemochromatosis mutation. J Gastroenterol.Hepatol. 1999;14:1188-91.

378. Macdougall LG, Anderson R, McNab GM, Katz J. The immune response in iron-deficient children: Impaired cellular defense mechanisms with altered humoral components. J.Pediatr. 1975;86:833-43.

379. Magnusson MK, Sigfusson N, Sigvaldason H, Johannesson GM, Magnusson S, Thorgeirsson G. Low iron-binding capacity as a risk factor for myocardial infarction. Circulation 1994;89:102-8.

380. Magnusson B, Bjorn-Rassmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express results to food iron absorption measurements. Scand J Haematol 1981; 27(3):201-208.

381. Mainous AG, III, Gill JM, Pearson WS. Should we screen for hemochromatosis? An examination of evidence of downstream effects on morbidity and mortality. Arch.Intern.Med. 2002;162:1769-74.

382. Majumdar I, Paul P, Talib VH, Ranga S. The effect of iron therapy on the growth of iron-replete and iron-deplete children. J Trop Pediatr 2003; 49(2):84-88.

383. Malone HE, Kevany JP, Scott JM, O'Broin SD, O'Connor G. Ascorbic acid supplementation: its effects on body iron stores and white blood cells. Ir J Med Sci 1986; 155(3):74-79.

384. Manttari M, Manninen V, Huttunen JK, Palosuo T, Ehnholm C, Heinonen OP, Frick MH. Serum ferritin and ceruloplasmin as coronary risk factors. Eur Heart J 1994; 15(12):1599-1603.

385. Marder E, Nicoll A, Polnay L, Shulman CE. Discovering anaemia at child health clinics. Arch.Dis.Child 1990;65:892-4.

386. Marniemi J, Jarvisalo J, Toikka T, Raiha I, Ahotupa M, Sourander L. Blood vitamins, mineral elements and inflammation markers as risk factors of vascular and non-vascular disease mortality in an elderly population. Int J Epidemiol 1998; 27(5):799-807.

387. Martinez-Torres C, Layrisse M. Iron absorption from veal muscle. Am.J.Clin.Nutr. 1971;24:531-40.

388. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 2007; 85(4):931-945.

389. McCord JM. Iron, free radicals, and oxidative injury. Semin Hematol 1998; 35(1):5-12.

390. McDonald SJ, Middleton P. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. Cochrane Database Syst Rev 2008;(2):CD004074.

391. Mebrahtu T, Stoltzfus RJ, Chwaya HM, Jape JK, Savioli L, Montresor A, Albonico M, Tielsch JM. Low-dose daily iron supplementation for 12 months does not increase the prevalence of malarial infection or density of parasites in young Zanzibari children. J Nutr 2004; 134(11):3037-3041.

392. Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. Lancet 1997; 350(9081):844-850.

393. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J.Med.Genet. 1997;34:275-8.

394. Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, Lonnerdal B, Stamoulakatou A, Pollitt E. Effect of iron supplementation on cognition in Greek preschoolers. Eur J Clin Nutr 2004; 58(11):1532-1542.

395. Meyers DG, Strickland D, Maloley PA, Seburg JK, Wilson JE, McManus BF. Possible association of a reduction in cardiovascular events with blood donation. Heart 1997;78:188-93.

396. Meyron-Holtz EG, Ghosh MC, Rouault TA. Mammalian tissue oxygen levels modulate iron-regulatory protein activities in vivo. Science 2004; 306(5704):2087-2090.

397. Mills AF. Surveillance for anaemia: risk factors in patterns of milk intake. Arch.Dis.Child 1990;65:428-31.

398. Mills A, Tyler HA. Food and nutrient intakes of British infants aged 6-12 months. 1992. London: HMSO.

399. Milman N, Byg KE, Ovesen L. Iron status in Danes 1994. II: Prevalence of iron deficiency and iron overload in 1319 Danish women aged 40-70 years. Influence of blood donation, alcohol intake and iron supplementation. Ann Hematol 2000; 79(11):612-621.

400. Milman N, Byg KE, Ovesen L, Kirchhoff M, Jurgensen KS. Iron status in Danish women, 1984-1994: a cohort comparison of changes in iron stores and the prevalence of iron deficiency and iron overload. Eur J Haematol 2003; 71(1):51-61.

401. Milman N, Pedersen AN, Ovesen L, Schroll M. Iron status in 358 apparently healthy 80-year-old Danish men and women: relation to food composition and dietary and supplemental iron intake. Ann Hematol 2004; 83(7):423-429.

402. Milman N, Rosdahl N, Lyhne N, Jorgensen T, Graudal N. Iron status in Danish women aged 35-65 years. Relation to menstruation and method of contraception. Acta Obstet.Gynecol.Scand. 1993;72:601-5.

403. Milsom I, Andersson K, Jonasson K, Lindstedt G, Rybo G. The influence of the Gyne-T 380S IUD on menstrual blood loss and iron status. Contraception 1995;52:175-9.

404. Minihane AM, Fairweather-Tait SJ. Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. Am.J Clin.Nutr. 1998;68:96-102.

405. Miret S, Simpson RJ, McKie AT. Physiology and molecular biology of dietary iron absorption. Annu.Rev.Nutr. 2003;23:283-301.

406. Modell B. Advances in the use of iron-chelating agents for the treatment of iron overload. Prog.Hematol. 1979;11:267-312.

407. Moffatt ME, Longstaffe S, Besant J, Dureski C. Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. J Pediatr. 1994;125:527-34.

408. Molgaard C, Michaelsen KF. Changes in body composition during growth in healthy school-age children. Appl.Radiat.Isot. 1998;49:577-9.

409. Monsen ER, Cook JD. Food iron absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. Am J Clin Nutr 1976; 29(10):1142-1148.

410. Monsen ER, Cook JD. Food iron absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. Am J Clin Nutr 1976; 29(10):1142-1148.

411. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W, Finch CA. Estimation of available dietary iron. Am J Clin Nutr 1978; 31(1):134-141.

412. Moos T, Rosengren NT, Skjorringe T, Morgan EH. Iron trafficking inside the brain. J Neurochem 2007; 103(5):1730-1740.

413. Morais MB, Fisberg M, Suzuki HU, Amancio OM, Machado NL. Effects of oral iron therapy on serum copper and serum ceruloplasmin in children. J Trop Pediatr 1994; 40(1):51-52.

414. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. Am.J Clin.Nutr. 1983;37:416-20.

415. Morley R, Abbott R, Fairweather-Tait S, MacFadyen U, Stephenson T, Lucas A. Iron fortified follow on formula from 9 to 18 months improves iron status but not development or growth: a randomised trial. Arch.Dis.Child 1999;81:247-52.

416. Morrison HI, Semenciw RM, Mao Y, Wigle DT. Serum iron and risk of fatal acute myocardial infarction. Epidemiology 1994;5:243-6.

417. Morton RE, Nysenbaum A, Price K. Iron status in the first year of life. J.Pediatr.Gastroenterol.Nutr. 1988;7:707-12.

418. Moyo VM, Gangaidzo IT, Gordeuk VR, Kiire CF, MacPhail AP. Tuberculosis and iron overload in Africa: a review. Cent Afr J Med 1997; 43(11):334-339.

419. Munn CG, Markenson AL, Kapadia A, de Sousa M. Impaired T-cell mitogen responses in some patients with thalassemia intermedia. Thymus 1981;3:119-28.

420. Munoz LM, Lonnerdal B, Keen CL, Dewey KG. Coffee consumption as a factor in iron deficiency anemia among pregnant women and their infants in Costa Rica. Am.J.Clin.Nutr. 1988;48:645-51.

421. Mura C, Raguenes O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 1999;93:2502-5.

422. Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986;1:992-5.

423. Murray MJ, Murray AB, Murray MB, Murray CJ. The adverse effect of iron repletion on the course of certain infections. Br Med J 1978; 2(6145):1113-1115.

424. Murtagh LJ, Whiley M, Wilson S, Tran H, Bassett ML. Unsaturated iron binding capacity and transferrin saturation are equally reliable in detection of HFE hemochromatosis. Am.J Gastroenterol. 2002;97:2093-9.

425. Narisawa T, Reddy BS, Weisburger JH. Effect of bile acids and dietary fat on large bowel carcinogenesis in animal models. Gastroenterol Jpn 1978; 13(3):206-212.

426. National Academy of Sciences. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. 2001. Washington, D.C., National Academy Press.

427. National Collaborating Centre for Women's and Children's Health. Antenatal care: routine care for the healthy pregnant woman. 2003. London, RCOG Press.

428. National Institute for Clinical Excellence. Antenatal care, routine care for the healthy pregnant woman. 2008. London, RCOG Press at the Royal College of Obstertricians and Gynaecologists.

429. Nelle M, Zilow EP, Bastert G, Linderkamp O. Effect of Leboyer childbirth on cardiac output, cerebral and gastrointestinal blood flow velocities in full-term neonates. Am J Perinatol 1995; 12(3):212-216.

430. Nelson M, Erens B, Bates B, Church S, Boshier T. Food Standards Agency. Low Income Diet and Nutrition Survey. Volume 2: Food consumption and nutrient intake. 2007. London: TSO.

431. Nelson M, Erens B, Bates B, Church S, Boshier T. Food Standards Agency. Low Income Diet and Nutrition Survey. Volume 3: Nutrition status, physical activity and economic, social and other factors. 2007. London: TSO.

432. Nelson RL. Dietary iron and colorectal cancer risk. Free Radic.Biol.Med. 1992;12:161-8.

433. Nelson RL, Davis FG, Persky V, Becker E. Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis. Cancer 1995;76:875-9.

434. Nemeth E, Tuttle MS, Powelson J et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306:2090-3.

435. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003; 101(7):2461-2463.

436. Nemeth E, Roetto A, Garozzo G, Ganz T, Camaschella C. Hepcidin is decreased in TFR2 hemochromatosis. Blood 2005; 105(4):1803-1806.

437. Newhouse IJ, Clement DB, Taunton JE, McKenzie DC. The effects of prelatent/latent iron deficiency on physical work capacity. Med Sci Sports Exerc 1989; 21(3):263-268.

438. Nicolas G, Bennoun M, Porteu A et al. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc.Natl.Acad.Sci.U.S.A 2002;99:4596-601.

439. Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. Int J Cancer 2002; 98(2):241-256.

440. Norat T, Bingham S, Ferrari P, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst 2005; 97(12):906-916.

441. Oba S, Shimizu N, Nagata C, Shimizu H, Kametani M, Takeyama N, Ohnuma T, Matsushita S. The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: a prospective study in Japan. Cancer Lett 2006; 244(2):260-267.

442. Ohira Y, Edgerton VR, Gardner GW, Senewiratne B, Barnard RJ, Simpson DR. Work capacity, heart rate and blood lactate responses to iron treatment. Br J Haematol 1979; 41(3):365-372.

443. Ohira Y, Koziol BJ, Edgerton VR, Brooks GA. Oxygen consumption and work capacity in iron-deficient anemic rats. J Nutr 1981; 111(1):17-25.

444. Ohlund I, Lind T, Hornell A, Hernell O. Predictors of iron status in well-nourished 4-y-old children. Am J Clin Nutr 2008; 87(4):839-845.

445. Okada S. Iron-induced tissue damage and cancer: the role of reactive oxygen species-free radicals. Pathol Int 1996; 46(5):311-332.

446. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro F. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am.J Clin.Nutr 2000;72:1191-5.

447. Olney DK, Pollitt E, Kariger PK, Khalfan SS, Ali NS, Tielsch JM, Sazawal S, Black R, Allen LH, Stoltzfus RJ. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5- to 11-mo old. J Nutr 2006; 136(9):2427-2434.

448. Olsen A, Mwaniki D, Krarup H, Friis H. Low-Dose Iron Supplementation Does Not Increase HIV-1 Load. J.Acquir.Immune.Defic.Syndr. 2004;36:637-8.

449. Oski FA, Honig AS. The effects of therapy on the developmental scores of iron-deficient infants. J Pediat 1978;92:21-5.

450. Oski FA, Honig AS, Helu B, Howanitz P. Effect of iron therapy on behaviour performance in non-anaemic iron-deficient infants. Pediat 1983;71:877-80.

451. Osler M, Milman N, Heitmann BL. Consequences of removing iron fortification of flour on iron status among Danish adults: some longitudinal observations between 1987 and 1994. Prev Med 1999; 29(1):32-36.

452. Owen GM, Lubin AH, Garry PJ. Pre-school children in the United States: who has iron deficiency? J Pediat 1971;79:563-8.

453. Pan, Y.-H., Powell, J., Bleloch, A., Gass, M., Sader, K., Trinick, J., Warley, A., Li, A., Brydson, R., and Brown, A. (2009). 3-D morphology of the human hepatic ferritin mineral core: new evidence for a subunit structure revealed by single particle analysis of HAADF STEM images. J Struct Biol *In Press*.

454. Papanikolaou G, Samuels ME, Ludwig EH et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat.Genet. 2004;36:77-82.

455. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, Andres L, MacFarlane J, Sakellaropoulos N, Politou M, Nemeth E, Thompson J, Risler JK, Zaborowska C, Babakaiff R, Radomski CC, Pape TD, Davidas O, Christakis J, Brissot P, Lockitch G, Ganz T, Hayden MR, Goldberg YP. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 2004; 36(1):77-82.

456. Patel BN, Dunn RJ, Jeong SY, Zhu Q, Julien JP, David S. Ceruloplasmin regulates iron levels in the CNS and prevents free radical injury. J Neurosci 2002; 22(15):6578-6586.

457. Pattison DJ, Symmons DP, Lunt M, Welch A, Luben R, Bingham SA, Khaw KT, Day NE, Silman AJ. Dietary risk factors for the development of inflammatory polyarthritis: evidence for a role of high level of red meat consumption. Arthritis Rheum 2004; 50(12):3804-3812.

458. Pedersen M, Stripp C, Klarlund M, Olsen SF, Tjonneland AM, Frisch M. Diet and risk of rheumatoid arthritis in a prospective cohort. J Rheumatol 2005; 32(7):1249-1252.

459. Peirano P, Algarin C, Garrido M, Pizarro F, Roncagliolo M, Lozoff B. Interaction of iron deficiency anemia and neurofunctions in cognitive development. Nestle.Nutr.Workshop Ser.Clin.Perform.Programme. 2001;19-35.

460. Pena-Rosas JP, Viteri FE. Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. Cochrane Database Syst Rev 2006; 3:CD004736.

461. Perkkio MV, Jansson LT, Henderson S, Refino C, Brooks GA, Dallman PR. Work performance in the iron-deficient rat: improved endurance with exercise training. Am J Physiol 1985;249:E306-E311.

462. Perry GS, Byers T, Yip R, Margen S. Iron nutrition does not account for the hemoglobin differences between blacks and whites. J.Nutr. 1992;122:1417-24.

463. Pietinen P, Malila N, Virtanen M, Hartman TJ, Tangrea JA, Albanes D, Virtamo J. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999; 10(5):387-396.

464. Pippard MJ, Brock JH, Halliday J.W., Powell LW. Secondary Overload in Iron Metabolism in Health and Disease. London: Saunders, 1994.

465. Pisacane A, De Vizia B, Valiante A et al. Iron status in breast-fed infants. J.Pediatr. 1995;127:429-31.

466. Pisacane A. Neonatal prevention of iron deficiency. BMJ 1996;312:136-7.

467. Plug CM, Dekker D, Bult A. Complex stability of ferrous ascorbate in aqueous solution and its significance for iron absorption. Pharm Weekbl Sci 1984; 6(6):245-248.

468. Pollitt E, Leibel RL, Greenfield DB. Iron deficiency and cognitive test performance in preschool children. Nutr Behav 1983;1:137-46.

469. Pollitt E, Soemantri AG, Yunis F, Scrimshaw NS. Cognitive effects of iron-deficiency anaemia. Lancet 1985;1:158.

470. Pollitt E, Saco-Pollitt C, Leibel RL, Viteri FE. Iron deficiency and behavioral development in infants and preschool children. Am.J Clin.Nutr 1986;43:555-65.

471. Pollitt E, Hathirat P, Kotchabhakdi NJ, Missell L, Valyasevi A. Iron deficiency and educational achievement in Thailand. Am.J Clin.Nutr 1989;50:687-96.

472. Prema K, Ramalakshmi BA, Madhavapeddi R, Babu S. Immune status of anaemic pregnant women. Br.J.Obstet.Gynaecol. 1982;89:222-5.

473. Preziosi P, Hercberg S, Galan P, Devanlay M, Cherouvrier F, Dupin H. Iron status of a healthy French population: factors determining biochemical markers. Ann.Nutr.Metab 1994;38:192-202.

474. Qi L, van Dam RM, Rexrode K, Hu FB. Heme iron from diet as a risk factor for coronary heart disease in women with type 2 diabetes. Diabetes Care 2007; 30(1):101-106.

475. Qian ZM, Shen X. Brain iron transport and neurodegeneration. Trends Mol.Med. 2001;7:103-8.

476. Raddatz D, Legler T, Lynen R, Addicks N, Ramadori G. HFE genotype and parameters of iron metabolism in German first-time blood donors - evidence for an increased transferrin saturation in C282Y heterozygotes. Z Gastroenterol 2003; 41(11):1069-1076.

477. Ramdath DD, Simeon DT, Wong MS, Grantham-McGregor SM. Iron status of schoolchildren with varying intensities of Trichuris trichiura infection. Parasitology 1995;110 (Pt 3):347-51.

478. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. J.Nutr. 2003;133:3215-21.

479. Rasmussen K. Is There a Causal Relationship between Iron Deficiency or Iron-Deficiency Anemia and Weight at Birth, Length of Gestation and Perinatal Mortality? J Nutr 2001;131:590S-601S.

480. Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis 2001;154:739-46.

481. Reddy MB, Cook JD. Effect of calcium intake on nonheme-iron absorption from a complete diet. Am.J.Clin.Nutr. 1997;65:1820-5.

482. Reddy MB, Hurrell RF, Cook JD. Meat consumption in a varied diet marginally influences nonheme iron absorption in normal individuals. J Nutr 2006; 136(3):576-581.

483. Reddy S, Sanders TA. Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores. Br J Nutr 1990; 64(2):331-338.

484. Reddy MB, Hurrell RF, Cook JD. Estimation of nonheme-iron bioavailability from meal composition. Am.J.Clin.Nutr. 2000;71:937-43.

485. Reisenberger K, Egarter C, Kapiotis S, Stemberger B, Gregor H, Husslein P. Transfer of erythropoietin across the placenta perfused in vitro. Obstet Gynecol. 1997;89:738-42.

486. Reunanen A, Takkunen H, Knekt P, Seppanen R, Aromaa A. Body iron stores, dietary iron intake and coronary heart disease mortality. J Intern.Med. 1995;238:223-30.

487. Richard SA, Zavaleta N, Caulfield LE, Black RE, Witzig RS, Shankar AH. Zinc and iron supplementation and malaria, diarrhea, and respiratory infections in children in the Peruvian Amazon. Am J Trop Med Hyg 2006; 75(1):126-132.

488. Robinson JP, Johnson VL, Rogers PA, Houlston RS, Maher ER, Bishop DT, Evans DG, Thomas HJ, Tomlinson IP, Silver AR. Evidence for an association between compound heterozygosity for germ line mutations in the hemochromatosis (HFE) gene and increased risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2005; 14(6):1460-1463.

489. Roche M, Layrisse M. The nature and causes of "hookworm anemia". Am J Trop Med Hyg 1966; 15(6):1029-1102.

490. Rockey DC, Cello JP. Evaluation of the gastrointestinal tract in patients with iron-deficiency anemia. N.Engl.J.Med. 1993;329:1691-5.

491. Roe MA, Heath AL, Oyston SL, Macrow C, Hoogewerff JA, Foxall R, Dainty JR, Majsak-Newman G, Willis G, Fairweather-Tait SJ. Iron absorption in male C282Y heterozygotes. Am J Clin Nutr 2005; 81(4):814-821.

492. Roest M, van der Schouw YT, de Valk B et al. Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. Circulation 1999;100:1268-73.

493. Roetto A, Papanikolaou G, Politou M et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat.Genet. 2003;33:21-2.

494. Rogers JT, Bridges KR, Durmowicz GP, Glass J, Auron PE, Munro HN. Translational control during the acute phase response. Ferritin synthesis in response to interleukin-1. J Biol Chem 1990; 265(24):14572-14578.

495. Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. Am.J Clin.Nutr 1998;68:683-90.

496. Rushton, D, Hoare, J, Henderson, L, Gregory J, Bates, C. J, Prentice, A., Birch, M, Swan, G, and Farron, M. The National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 4: Nutritional status (anthropometry and blood analytes), blood pressure and physical activity. 2004. London, The Stationery Office.

497. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86:803-11.

498. Salonen JT, Nyyssonen K, Salonen R. Body iron stores and the risk of coronary heart disease. N.Engl.J.Med. 1994;331:1159.

499. Salonen JT, Tuomainen TP, Salonen R, Lakka TA, Nyyssonen K. Donation of blood is associated with reduced risk of myocardial infarction. The Kuopio Ischaemic Heart Disease Risk Factor Study. Am.J.Epidemiol. 1998;148:445-51.

500. Sandstrom B, Davidsson L, Cederblad A, Lonnerdal B. Oral iron, dietary ligands and zinc absorption. J Nutr 1985; 115(3):411-414.

501. Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr 2001; 85 Suppl 2:S181-S185.

502. San Martin, C. D., Garri, C., Pizarro, F., Walter, T., Theil, E. C., and Nunez, M. T. (2008). Caco-2 intestinal epithelial cells absorb soybean ferritin by mu(2) (AP2)-dependent endocytosis. Journal of Nutrition 138, 659-666.

503. Sato Y, Nakaya N, Kuriyama S, Nishino Y, Tsubono Y, Tsuji I. Meat consumption and risk of colorectal cancer in Japan: the Miyagi Cohort Study. Eur J Cancer Prev 2006; 15(3):211-218.

504. Saudek CD, Charache S. Haemochromatosis and diabetes. Baillieres Clin.Endocrinol.Metab 1992;6:807-17.

505. Sawitsky B, Kanter R, Sawitsky A. Lymphocyte response to phytomitogens in iron deficiency. Am.J.Med.Sci. 1976;272:153-60.

506. Sayers MH, Lynch SR, Charlton RW, Bothwell TH, Walker RB, Mayet F. Iron absorption from rice meals cooked with fortified salt containing ferrous sulphate and ascorbic acid. Br.J Nutr 1974;31:367-75.

507. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006; 367(9505):133-143.

508. Schneider H, Malek A. Lack of permeability of the human placenta for erythropoietin. J Perinat Med. 1995;23:71-6.

509. Scholz BD, Gross R, Schultink W, Sastroamidjojo S. Anaemia is associated with reduced productivity of women workers even in less-physically-strenuous tasks. Br J Nutr 1997; 77(1):47-57.

510. Scientific Advisory Committee on Nutrition. A Framework for the Evaluation of Evidence. 2002.

511. Scientific Committee on Food. Nutrient and Energy Intakes for the European Community. 1993.

512. Sellers TA, Bazyk AE, Bostick RM, Kushi LH, Olson JE, Anderson KE, Lazovich D, Folsom AR. Diet and risk of colon cancer in a large prospective study of older women: an analysis stratified on family history (Iowa, United States). Cancer Causes Control 1998; 9(4):357-367.

513. Semba RD, Taha TE, Kumwenda N, Mtimavalye L, Broadhead R, Miotti PG, Chiphangwi JD. Iron status and indicators of human immunodeficiency virus disease severity among pregnant women in Malawi. Clin Infect Dis 2001; 32(10):1496-1499.

514. Sempos CT, Looker AC, Gillum RE, McGee DL, Vuong CV, Johnson CL. Serum ferritin and death from all causes and cardiovascular disease: the NHANES II Mortality Study. National Health and Nutrition Examination Study. Ann.Epidemiol. 2000;10:441-8.

515. Seshadri S, Gopaldas T. Impact of iron supplementation on cognitive functions in preschool and school-aged children: the Indian experience. Am.J Clin.Nutr 1989;50:675-84.

516. Shaheen NJ, Silverman LM, Keku T et al. Association between hemochromatosis (HFE) gene mutation carrier status and the risk of colon cancer. J Natl.Cancer Inst. 2003;95:154-9.

517. Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, Khan Y, Warley A, McCann FE, Hider RC, Frazer DM, Anderson GJ, Vulpe CD, Simpson RJ, McKie AT. Identification of an intestinal heme transporter. Cell 2005; 122(5):789-801.

518. Sherriff A, Emond A, Hawkins N, Golding J. Haemoglobin and ferritin concentrations in children aged 12 and 18 months. ALSPAC Children in Focus Study Team. Arch.Dis.Child 1999;80:153-7.

519. Siegenberg D, Baynes RD, Bothwell TH et al. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am.J.Clin.Nutr. 1991;53:537-41.

520. Siegers CP, Bumann D, Trepkau HD, Schadwinkel B, Baretton G. Role of iron in cell proliferation and tumorigenesis. Prog Clin Biol Res 1991; 369:439-444.

521. Siegers CP, Bumann D, Trepkau HD, Schadwinkel B, Baretton G. Influence of dietary iron overload on cell proliferation and intestinal tumorigenesis in mice. Cancer Lett 1992; 65(3):245-249.

522. Siimes MA, Addiego JE, Jr., Dallman PR. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. Blood 1974;43:581-90.

523. Siimes MA, Vuori E, Kuitunen P. Breast milk iron--a declining concentration during the course of lactation. Acta Paediatr.Scand. 1979;68:29-31.

524. Singh PN, Fraser GE. Dietary risk factors for colon cancer in a low-risk population. Am J Epidemiol 1998; 148(8):761-774.

525. Sipe JC, Lee P, Beutler E. Brain iron metabolism and neurodegenerative disorders. Dev Neurosci 2002; 24(2-3):188-196.

526. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990;75:1870-6.

527. Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. Ann Trop Paediatr 1989; 9(1):17-23.

528. Snetselaar L, Stumbo P, Chenard C, Ahrens L, Smith K, Zimmerman B. Adolescents eating diets rich in either lean beef or lean poultry and fish reduced fat and saturated fat intake and those eating beef maintained serum ferritin status. J Am Diet Assoc 2004; 104(3):424-428.

529. Soemantri AG, Pollitt E, Kim I. Iron deficiency anemia and educational achievement. Am.J Clin.Nutr 1985;42:1221-8.

530. Soemantri AG. Preliminary findings on iron supplementation and learning achievement of rural Indonesian children. Am.J Clin.Nutr 1989;50:698-701.

531. Soewondo S, Husaini M, Pollitt E. Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. Am.J Clin.Nutr 1989;50:667-73.

532. Sokoll LJ, Dawson-Hughes B. Calcium supplementation and plasma ferritin concentrations in premenopausal women. Am J Clin Nutr 1992; 56(6):1045-1048.

533. Sorensen M, Autrup H, Olsen A, Tjonneland A, Overvad K, Raaschou-Nielsen O. Prospective study of NAT1 and NAT2 polymorphisms, tobacco smoking and meat consumption and risk of colorectal cancer. Cancer Lett 2008; 266(2):186-193.

534. Soustre Y, Dop MC, Galan P, Hercberg S. Dietary determinants of the iron status in menstruating women. Int J Vitam Nutr Res 1986; 56(3):281-286.

535. Steer P, Alam MA, Wadsworth J, Welch A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ 1995;310:489-91.

536. Steer PJ. Maternal hemoglobin concentration and birth weight. Am.J Clin.Nutr 2000;71:1285S-7S.

537. Steinmacher J, Pohlandt F, Bode H, Sander S, Kron M, Franz AR. Randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams: neurocognitive development at 5.3 years' corrected age. Pediatrics 2007; 120(3):538-546.

538. Stevens RG, Kalkwarf DR. Iron, radiation, and cancer. Environ Health Perspect 1990; 87:291-300.

539. Stoltzfus RJ, Kvalsvig JD, Chwaya HM et al. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. BMJ 2001;323:1389-93.

540. Storey ML, Greger JL. Iron, zinc and copper interactions: chronic versus acute responses of rats. J Nutr 1987; 117(8):1434-1442.

541. Sugimura T. Nutrition and dietary carcinogens. Carcinogenesis 2000; 21(3):387-395.

542. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet 1981; 1(8233):1293-1294.

543. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet 1981;1:1293-4.

544. Sun J, Huang J, Li W, Wang L, Wang A, Huo J, Chen J, Chen C. Effects of wheat flour fortified with different iron fortificants on iron status and anemia prevalence in iron deficient anemic students in Northern China. Asia Pac J Clin Nutr 2007; 16(1):116-121.

545. Suominen P, Virtanen A, Lehtonen-Veromaa M et al. Regression-based reference limits for serum transferrin receptor in children 6 months to 16 years of age. Clin.Chem. 2001;47:935-7.

546. Svanberg B, Arvidsson B, Bjorn-Rasmussen E, Hallberg L, Rossander L, Swolin B. Dietary iron absorption in pregnancy - a longitudinal study with repeated measurements of non-haeme iron absorption from whole diet. Acta Obstet.Gynecol.Scand.Suppl 1975;43-68.

547. Swarup-Mitra S, Sinha AK. Cell mediated immunity in nutritional anaemia. Indian J.Med.Res. 1984;79:354-62.

548. Tandy S, Williams M, Leggett A, Lopez-Jimenez M, Dedes M, Ramesh B, Srai SK, Sharp P. Nramp2 expression is associated with pH-dependent iron uptake across the apical membrane of human intestinal Caco-2 cells. J Biol Chem 2000; 275(2):1023-1029.

549. Tang C, Robson AD, Dilworth MJ. The role of iron in nodulation and nitrogen fixation in *Lupinus angustifolius* L. New Phytol 1990;114:173-182.

550. Taylor A, Redworth EW, Morgan JB. Influence of diet on iron, copper, and zinc status in children under 24 months of age. Biol.Trace Elem.Res 2004;97:197-214.

551. Tetens I, Bendtsen KM, Henriksen M, Ersboll AK, Milman N. The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores. Eur J Nutr 2007; 46(8):439-445.

552. Thane CW, Walmsley CM, Bates CJ, Prentice A, Cole TJ. Risk factors for poor iron status in British toddlers: further analysis of data from the National Diet and Nutrition Survey of children aged 1.5-4.5 years. Public Health Nutr. 2000;3:433-40.

553. Thane CW, Bates CJ. Dietary intakes and nutrient status of vegetarian preschool children from a British national survey. J.Hum.Nutr Diet. 2000;13:149-62.

554. Thane CW, Bates CJ, Prentice A. Risk factors for low iron intake and poor iron status in a national sample of British young people aged 4-18 years. Public Health Nutr 2003;6:485-96.

555. Thibault H, Galan P, Selz F et al. The immune response in iron-deficient young children: effect of iron supplementation on cell-mediated immunity. Eur.J.Pediatr. 1993;152:120-4.

556. Thompson KJ, Shoham S, Connor JR. Iron and neurodegenerative disorders. Brain Res.Bull. 2001;55:155-64.

557. Tidehag P, Hallmans G, Wing K et al. A comparison of iron absorption from single meals and daily diets using radioFe (55Fe, 59Fe). Br.J Nutr. 1996;75:281-9.

558. Tielsch JM, Khatry SK, Stoltzfus RJ, Katz J, Leclerq SC, Adhikari R, Mullany LC, Shresta S, Black RE. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet 2006; 367(9505):144-152.

559. Tiemersma EW, Kampman E, Bueno de Mesquita HB et al. Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. Cancer Causes Control 2002;13:383-93.

560. Toyokuni S. Iron-induced carcinogenesis: the role of redox regulation. Free Radic Biol Med 1996; 20(4):553-566.

561. Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation : a prospective cohort study in men in eastern Finland. Circulation 1999;100:1274-9.

562. Turnbull AJ, Mitchison HC, Peaston RT et al. The prevalence of hereditary haemochromatosis in a diabetic population. QJM. 1997;90:271-5.

563. Untoro J, Gross R, Schultink W, Sediaoetama D. The association between BMI and haemoglobin and work productivity among Indonesian female factory workers. Eur J Clin Nutr 1998; 52(2):131-135.

564. Uzel C, Conrad ME. Absorption of heme iron. Semin.Hematol. 1998;35:27-34.

565. Van Aken MO, De Craen AJ, Gussekloo J et al. No increase in mortality and morbidity among carriers of the C282Y mutation of the hereditary haemochromatosis gene in the oldest old: the Leiden 85-plus study. Eur.J.Clin.Invest 2002;32:750-4.

566. van Asbeck BS, Verbrugh HA, van Oost BA, Marx JJ, Imhof HW, Verhoef J. Listeria monocytogenes meningitis and decreased phagocytosis associated with iron overload. Br.Med.J (Clin.Res Ed) 1982;284:542-4.

567. van Asbeck BS, Marx JJ, Struyvenberg A, Verhoef J. Functional defects in phagocytic cells from patients with iron overload. J Infect. 1984;8:232-40.

568. van Asperen IA, Feskens EJ, Bowles CH, Kromhout D. Body iron stores and mortality due to cancer and ischaemic heart disease: a 17-year follow-up study of elderly men and women. Int.J.Epidemiol. 1995;24:665-70.

569. van der A DL, van der HO, Roest M, van der Schouw YT, van Gils CH, Marx JJ, van Noord PA, Peeters PH. Heterozygosity for the Cys282Tyr mutation in the HFE gene and the risk of colorectal cancer (Netherlands). Cancer Causes Control 2003; 14(6):541-545.

570. van der A DL, Peeters PH, Grobbee DE, Marx JJ, van der Schouw YT. Dietary haem iron and coronary heart disease in women. Eur Heart J 2005; 26(3):257-262.

571. van der A DL, Grobbee DE, Roest M, Marx JJ, Voorbij HA, van der Schouw YT. Serum ferritin is a risk factor for stroke in postmenopausal women. Stroke 2005; 36(8):1637-1641.

572. van der A DL, Marx JJ, Grobbee DE, Kamphuis MH, Georgiou NA, Kats-Renaud JH, Breuer W, Cabantchik ZI, Roest M, Voorbij HA, van der Schouw YT. Non-transferrin-bound iron and risk of coronary heart disease in postmenopausal women. Circulation 2006; 113(16):1942-1949.

573. van der A DL, Peeters PH, Grobbee DE, Roest M, Marx JJ, Voorbij HM, van der Schouw YT. HFE mutations and risk of coronary heart disease in middle-aged women. Eur J Clin Invest 2006; 36(10):682-690.

574. van Stuijvenberg ME, Smuts CM, Wolmarans P, Lombard CJ, Dhansay MA. The efficacy of ferrous bisglycinate and electrolytic iron as fortificants in bread in iron-deficient school children. Br J Nutr 2006; 95(3):532-538.

575. van Stuijvenberg ME, Smuts CM, Lombard CJ, Dhansay MA. Fortifying brown bread with sodium iron EDTA, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren. J Nutr 2008; 138(4):782-786.

576. Verhoef H, West CE, Nzyuko SM, de Vogel S, van d, V, Wanga MA, Kuijsten A, Veenemans J, Kok FJ. Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomised controlled trial. Lancet 2002; 360(9337):908-914.

577. Vuori E. Intake of copper, iron, manganese and zinc by healthy, exclusively-breast-fed infants during the first 3 months of life. Br.J.Nutr. 1979;42:407-11.

578. Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, Carter JA. Child development: risk factors for adverse outcomes in developing countries. Lancet 2007; 369(9556):145-157.

579. Wallace DF, Pedersen P, Dixon JL et al. Novel mutation in ferroportin1 is associated with autosomal dominant hemochromatosis. Blood 2002;100:692-4.

580. Walter T, Kovalskys J, Stekel A. Effect of mild iron deficiency on infant mental development scores. J Pediatr. 1983;102:519-22.

581. Walter T, Arredondo S, Arevalo M, Stekel A. Effect of iron therapy on phagocytosis and bactericidal activity in neutrophils of iron-deficient infants. Am.J.Clin.Nutr. 1986;44:877-82.

582. Walter T, De A, I, Chadud P, Perales CG. Iron deficiency anemia: adverse effects on infant psychomotor development. Pediatrics 1989;84:7-17.

583. Walter T, Pizarro F, Abrams SA, Boy E. Bioavailability of elemental iron powder in white wheat bread. Eur.J Clin.Nutr 2004;58:555-8.

584. Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. J Clin Pathol 1973; 26(10):770-772.

585. Warrington S, Storey DM. Comparative studies on Asian and Caucasian children. 1: Growth. Eur.J.Clin.Nutr. 1988;42:61-7.

586. Waterlot Y, Cantinieaux B, Hariga-Muller C, Maertelaere-Laurent E, Vanherweghem JL, Fondu P. Impaired phagocytic activity of neutrophils in patients receiving haemodialysis: the critical role of iron overload. Br Med J (Clin Res Ed) 1985; 291(6494):501-504.

587. Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willett WC, Colditz GA. Comparison of risk factors for colon and rectal cancer. Int J Cancer 2004; 108(3):433-442.

588. Weinberg ED. Iron withholding: a defense against infection and neoplasia. Physiol Rev 1984; 64(1):65-102.

589. Wells AM, Haub MD, Fluckey J, Williams DK, Chernoff R, Campbell WW. Comparisons of vegetarian and beef-containing diets on hematological indexes and iron stores during a period of resistive training in older men. J Am Diet Assoc 2003; 103(5):594-601.

590. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 2006; 145(3):209-223.

591. Whittaker PG, Barrett JF, Lind T. The erythrocyte incorporation of absorbed non-haem iron in pregnant women. Br J Nutr 2001; 86(3):323-329.

592. Williams J, Wolff A, Daly A, MacDonald A, Aukett A, Booth IW. Iron supplemented formula milk related to reduction in psychomotor decline in infants from inner city areas: randomised study. BMJ 1999;318:693-7.

593. Wilson AK, Ball MJ. Nutrient intake and iron status of Australian male vegetarians. Eur J Clin Nutr 1999; 53(3):189-194.

594. Wingard DL, Suarez L, Barrett-Connor E. The sex differential in mortality from all causes and ischemic heart disease. Am J Epidemiol 1983; 117(2):165-172.

595. Woodson RD, Wills RE, Lenfant C. Effect of acute and established anemia on O2 transport at rest, submaximal and maximal work. J Appl Physiol 1978; 44(1):36-43.

596. World Cancer Research Fund. Food, Nutrition, Physical Activity and the Prevention of Cancer: a global perspective. 2007.

597. World Health Organisation. Iron Deficiency Anaemia. Assessment, Prevention and Control. A guide for programme managers. 2001. Geneva, WHO. WHO/NHD/01.3.

598. World Health Organisation/Centers for Disease Control and Prevention. Assessing the Iron Status of Populations. 2004. Geneva: WHO.

599. World Health Organisation/Food and Agricultural Organisation of the United Nations. Guideline on food fortification with micronutrients. 2006. Geneva: WHO.

600. World Health Organisation/UNICEF. Joint Statement: Iron supplementation of young children in regions where malaria transmission is intense and infectious disease highly prevalent. 2006.

601. Worthington-Roberts BS, Breskin MW, Monsen ER. Iron status of premenopausal women in a university community and its relationship to habitual dietary sources of protein. Am.J.Clin.Nutr. 1988;47:275-9.

602. Worwood M. Ferritin in human tissues and serum. Clin Haematol 1982; 11(2):275-307.

603. Worwood M, Ellis RD, and Bain BJ. Three serum transferrin receptor ELISAs. MDA/2000/09. 2000. Norwich, UK, The Stationery Office.

604. Worwood M. Serum ferritin. CRC Crit Rev.Clin.Lab Sci 1979;10:171-204.

605. Worwood M. Ferritin. Blood Rev. 1990;4:259-69.

606. Worwood M. Serum transferrin receptor assays and their application. Ann.Clin.Biochem. 2002;39:221-30.

607. Worwood M. HFE Mutations as risk factors in disease. Best.Pract.Res.Clin.Haematol. 2002;15:295-314.

608. Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB. Iron intake and the risk of colorectal cancer. Cancer Epidemiol.Biomarkers Prev. 1996;5:503-7.

609. Yetgin S, Altay C, Ciliv G, Laleli Y. Myeloperoxidase activity and bactericidal function of PMN in iron deficiency. Acta Haematol. 1979;61:10-4.

610. Yu GS, Steinkirchner TM, Rao GA, Larkin EC. Effect of prenatal iron deficiency on myelination in rat pups. Am.J Pathol. 1986;125:620-4.

611. Yu J, Yu ZK, Wessling-Resnick M. Expression of SFT (stimulator of Fe transport) is enhanced by iron chelation in HeLa cells and by hemochromatosis in liver. J.Biol.Chem. 1998;273:34675-8.

612. Yu J, Wessling-Resnick M. Structural and functional analysis of SFT, a stimulator of Fe Transport. J.Biol.Chem. 1998;273:21380-5.

613. Zavaleta N, Lanata C, Butron B, Peerson JM, Brown KH, Lonnerdal B. Effect of acute maternal infection on quantity and composition of breast milk. Am J Clin Nutr 1995; 62(3):559-563.

614. Zhao X, Lu Q, Wang S, Yin S. [Efficiency of NaFeEDTA fortified soy sauce on anemia prevention]. Wei Sheng Yan Jiu 2004; 33(2):202-204.

615. Zhou LM, Yang WW, Hua JZ, Deng CQ, Tao X, Stoltzfus RJ. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am.J Epidemiol. 1998;148:998-1006.

616. Zhu YI, Haas JD. Response of serum transferrin receptor to iron supplementation in iron-depleted, nonanemic women. Am J Clin.Nutr 1998;67:271-5.

617. Zhu YI, Haas JD. Altered metabolic response of iron-depleted nonanemic women during a 15-km time trial. J Appl.Physiol 1998;84:1768-75.

618. Zhu YI, Haas JD. Iron depletion without anemia and physical performance in young women. Am J Clin Nutr 1997; 66(2):334-341.

619. Zimmermann MB, Winichagoon P, Gowachirapant S, Hess SY, Harrington M, Chavasit V, Lynch SR, Hurrell RF. Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized, double-blind, controlled trial in Thai women. Am J Clin Nutr 2005; 82(6):1276-1282.

620. Zimmermann MB, Zeder C, Chaouki N, Saad A, Torresani T, Hurrell RF. Dual fortification of salt with iodine and microencapsulated iron: a randomized, double-blind, controlled trial in Moroccan schoolchildren. Am.J Clin.Nutr 2003;77:425-32.

Annex 1

Membership of SACN & Iron Working Group

ANNEX 1

MEMBERSHIP OF SCIENTIFIC ADVISORY COMMITTEE ON NUTRITION: IRON WORKING GROUP

Chairman

Professor Peter Aggett	Formerly Head of School, Lancashire School of Health and Postgraduate Medicine, Emeritus Professor of Child Health and Nutrition, University of Central Lancashire

Vice Chairman

Dr Ann Prentice	Director, MRC Human Nutrition Research, Cambridge

Members

Professor Philip Calder	Professor in Nutrition Immunology, Developmental Origins of Health & Disease, School of Medicine, University of Southampton
Professor Sue Fairweather-Tait	Personal Chair in School of Medicine, Health, Policy and Practive, University of East Anglia
Mrs Christine Gratus	Honorary Senior Research Fellow, University of Birmingham, School of Primary Care Clinical Sciences. Former advertising and marketing research director.
Professor Joe Lunec	Head of Cranfield Health, Cranfield University
Professor Timothy Key	Professor in Epidemiology and Deputy Director of Cancer Epidemiology Unit, University of Oxford
Professor Sally Grantham-McGregor	Centre for International Child Health, Institute of Child Health
Professor Kim Fleischer Michaelsen	Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark
Professor Martin Pippard	Dean of the Medical School, University of Dundee
Professor Mark Worwood	Emeritus Professor at Cardiff University and honorary Clinical Scientist in the Cardiff and Vale NHS Trust

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Dr Alison Tedstone
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Ms Rachel Elsom (2002 – February 2005)

Department of Health
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ANNEX 1

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Professor Timothy Key	Professor in Epidemiology and Deputy Director of Cancer Epidemiology Unit, University of Oxford
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Mrs Stella Walsh	Postgraduate Programme Leader, Leeds Metropolitan University
Dr Anthony Williams	Reader in Child Nutrition and Consultant in Neonatal Paediatrics, St George's Hospital, University of London

Annex 2

Studies considered in relation to iron in the diet

Table 5.1: Prospective studies of the association between dietary factors influencing iron status

Study/year/country	Sample (number/age/SF & Hb concentrations)	Dietary Assessment Method	Biochemical markers of iron status	Total dietary iron	Non-haem iron	Dietary Enhancers Of Iron Absorption		Dietary Inhibitors Of Iron Absorption			Other Factors Considered In Analysis	Comments
						Haem iron/meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Munoz et al (1988) Coffee consumption as a factor in iron deficiency anaemia among pregnant women and their infants in Costa Rica COSTA RICA	Women (n=48) Age: 17-30y Divided into coffee drinkers(≥450ml/d) (n=22) and non-drinkers (0ml/d) (n=26) SF (µg/L) (mean) Coffee drinkers: 16 Non-drinkers: 14 Hb (g/L) Not provided Follow-up: 4 months	Three 24h recalls during last trimester & FFQ One 24h recall post partum All ♀ received prenatal iron supplements	SF Hb	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	- ↓	n/a n/a	Intakes of energy, protein, ascorbic acid, iron, red meat, supps. Age, parity, income, education, weight gain	23% of coffee consumers had Hb levels below 110g/L compared to 0% for non-drinkers
Garry et al (2000) Effects of iron intake on iron stores in elderly men & women: Longitudinal & cross-sectional results USA	Men & women (n=125) SF (µg/L) (mean) ♀: 95.6/♂: 113 Follow up: 10y	3-day food records (mean values for 3 separate years) (supps included)	SF	-	-	-	n/a	n/a	n/a	n/a	Sex, age, BMI, energy intake, intakes of dietary & supplemental iron, inflammation	Significant positive association with supplemental iron intake

ANNEX 2

Study/year/country	Sample (number/age/SF & Hb concentrations)	Dietary Assessment Method	Biochemical markers of iron status	Total dietary iron	Non-haem iron	Dietary Enhancers Of Iron Absorption		Dietary Inhibitors Of Iron Absorption			Other Factors Considered In Analysis	Comments
						Haem iron/meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Backstrand et al (2002) Diet & iron status of nonpregnant women in rural central Mexico MEXICO	Women (n=125) Age:16-44y SF (µg/L) (mean) Non-breast feeding: 16.4 Breastfeeding: 21.9 Hb (mg/L) (mean) Non-breast feeding: 133 Breastfeeding: 130 Follow-up: 1 year	Diet history (>10 days dietary data collected over 1y)	SF Hb Hct	- - -	↑ - -	- - -	↑ ↑ ↑	- - -	n/a n/a n/a	- - -	Age, breastfeeding status, BMI, time since birth of last child, season, haem & non-haem iron	SF – significant positive association with breast-feeding, age, more days since birth of last child
Liu et al (2003) Body iron stores and their determinants in healthy postmenopausal US women (NURSES HEALTH STUDY) USA	Women (n=620) Age: 44-69y (mean: 61.6y) Median SF: 73.8 µg/L Follow-up: 10y	Average of 3 FFQs over 10 years (80 items initially; expanded to 116) (supps incl)	SF	-	-	↑	-	-	-	-	Energy intake, age, PA, BMI, aspirin use, PMH use, GI ulcer, haem iron, non-haem iron, iron supps, alcohol, phytate, calcium, ascorbic aid, coffee	Significant positive association with age, BMI, supplemental iron intake & alcohol. Significant negative association with PMH use, aspirin use, & GI ulcer .

ANNEX 2

Study/year/country	Sample (number/age/SF & Hb concentrations)	Dietary Assessment Method	Biochemical markers of iron status	Total dietary iron	Non-haem iron	Dietary Enhancers Of Iron Absorption		Dietary Inhibitors Of Iron Absorption			Other Factors Considered In Analysis	Comments
						Haem iron/meat	Ascorbic acid	Calcium	Poly-phenols	Phytate		
Öhlund et al (2008) *Predictors of iron status in well-nourished 4y old children* SWEDEN	Infants (n=127) Age: 6-12m SF (mean) (µg/L) Girls: 55.6 Boys35.04 Hb (mean) g/L Girls: 114.9 Boys: 115 Follow-up: 12 months	5-d food records	SF Hb MCV	- -	n/a n/a	↑ (boys) -	- -	- -	n/a n/a	n/a n/a	Growth, Hb conc of parents, intakes of iron, meat products, ascorbic acid, calcium, milk, milk-based fortified cereals (MFCs), milk-based cereal drinks (MCD), porridge	Iron status improved from 6m to 4 years. Hb concentration was significantly associated with previous Hb concentration at 6m, 12m & 18m Mothers' Hb concentration correlated with chld's Hb concentration over time

↑ significant positive association; ↓ significant negative association; - no association; n/a not applicable as this factor was not considered in study
SF, serum ferritin,;Hb, haemoglobin; MCV, mean corpuscular volume; TS, transferrin saturation; EP, erythocyte protporphyrin
♀, female; ♂, male

Table 5.2: Intervention studies

Study/year/country	Study population	Duration of study	Treatment groups	Serum ferritin (µg/L) Initial/Final	Hb (g/L) Initial/Final	Comments
MEAT						
Lyle et al (1992) *Iron status in exercising women: the effect of oral therapy vs increased consumption of muscle foods* USA	Women (n=60)	12 weeks	50mg ferrous sulphate, low food-iron diet & exercise 10mg ferrous sulphate, low food-iron diet (total 18mg iron) & exercise placebo, free-choice diet & exercise High food iron diet (18mg) mainly from haem iron & exercise Free-choice diet & no exercise	27/27.5 (NS) 48.9/34.7 (NS) 40/23.9 (p<0.05 23.7/29.2 (NS) 22.2/20.4 (p<0.05)	126/124 (NS) 129/125 (p<0.05) 120/115 (p<0.05) 116/124 (p<0.05) 121/121 (NS)	At baseline, group differences in SF concentration At 12 wks SF concentrations of the 50mg ferrous sulphate group & the high food-iron group were signficantly higher than the other group At 12 wks Hb concentration of high food-iron group was signficantly higher than the other group
Hunt et al (1995) *High- vs low-meat diets: effects of zinc absorption, iron status, & calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women* USA	Postmenopausal women (n=14) Age: 51-70y (mean: 62.9y)	7 wks	High meat (289g) Low meat (38.5g) Low meat (38.5g) with mineral supplements (748 mg K, 594mg P, 3.3 mg Fe, 55mg Mg, 5.5mg Zn)	-/74 -/82 -/82	n/a	Baseline measures of iron indices not provided. Significant decrease in SF concentration with high meat diet (p=0.01)
Engelmann et al (1998) *Meat intake and iron status in late infancy: an intervention study* DENMARK	Infants (n=41) Age: 8m	2 months	High meat diet (27g/d) Low meat diet (10g/d)			Significant difference (p=0.008) in change in Hb concentration No significant differences in SF concentration
Wells et al (2003) *Comparisons of vegetarian & beef containing diets on hematological indexes and iron stores during period of resistive training in older men* USA	Men (n=21) 59-78y Undergoing resistance training	12 weeks (plus 2 wks baseline period when all participants consumed veg diet	Beef (0.6g protein/kg body wt/d) Texturized vegetable protein (0.6g protein/kg body wt/d)	132/131 (p<0.01) 95/72 (p<0.01)	140/151 (p<0.01) 143/145 (p<0.01)	SF – significant decrease in both groups over time but not between groups Hb – significant increase for both groups with time & between groups

Study/year/country	Study population	Duration of study	Treatment groups	Serum ferritin (µg/L) Initial/Final	Hb (g/L) Initial/Final	Comments
Snetselaar et al (2004) *Adolescents eating diets rich in either lean beef or lean poultry & fish reduced fat & saturated fat intake & those eating beef maintained serum ferritin status* USA	Adolescents (n=86) (age not given)	3 months	Beef 5x/wk & poultry/fish ≤2x/wk Poultry/fish 5x/wk & beef ≤2x/wk	31.2/38.7 (NS) 32.5/26.7 (NS) Significant diff between gps in change in SF after 3m (p<0.01)	n/a	Median beef consumption of beef group increased beef consumption by 26g. Median poultry/fish consumption increased by <10g. after 3 months
Tetens et al (2007) *The impact of a meat- versus a vegetable-based diet on iron status in women of childbearing age with small iron stores* SWEDEN	Women (n=57) 19-39y	30 weeks	Meat-based diet - usual diet + 150g meat/day Vegetable based diet - allowed to consume maximum of 250g meat & 125g fish per week	16.3/16.5 (NS) 17.3/11.2 (p<0.001)	126/125 (NS) 124/121 (P=0.003)	Signficant difference between dietary groups in SF and Hb concentration
ASCORBIC ACID						
Cook et al (1984) *The effect of high ascorbic acid supplementation on body iron stores* USA	Men and women (n=17) 20-30y	16 weeks + 20 more months (n=9)	2000 mg/d ascorbic acid (2x500mg with each of two meals)	46.3/43.9 (NS)	n/a	No significant effect on serum ferritin concentration when ascorbic acid supplementation was continued for additional 20 months with 5 iron replete and 4 iron deficient participants.
Malone et al, 1986 *Ascorbic acid supplementation: its effects on body iron stores and white blood cells* IRELAND	Men & women (n=58) 17-21y	8 weeks	300 mg/day ascorbic acid Placebo	27/31.3 (NS) 23/23 (NS)	n/a	Increase in the intervention group was significant at 10% level. Increase not significant when compared with change in serum ferritin in the control group over same period.
Hunt et al, 1990 *Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women* USA	Women (n=11) 22-36y	5.5 weeks	Diet containing 13.7 mg/2000 kcal + 1500 mg/d ascorbic acid Diet containing 13.7 mg/2000 kcal + placebo	Not provided	Not provided	Hb concentration was significantly higher in the group supplemented with ascorbic acid (p<0.05) Ascorbic acid had no effect on SF concentration

Study/year/country	Study population	Duration of study	Treatment groups	Serum ferritin (µg/L) Initial/Final	Hb (g/L) Initial/Final	Comments
Hunt et al, 1994 *Effect of ascorbic acid on iron absorption by women with low iron stores* USA	Women (n=25) 20-45y	10 weeks Crossover design	Diet of low iron bioavailability + 1500 mg/d ascorbic acid 5 wks placebo 5 wks Typical western diet + 1500 mg/d ascorbic acid 5 wks Placebo 5 wks	-/12.9 (NS) -/11.4 (NS) -/11.9 (NS) -/10.7 (NS)	-/132 (NS) -/131 (NS) -/132 (NS) -/130 (NS)	Combined data suggested slightly higher ferritin concentration with ascorbic acid $p<0.06$
Garcia et al, 2003 *Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico* MEXICO	Women (n=36) Mean age: 28y	8 months	50mg ascorbic acid as limeade Placebo group: lime-flavoured beverage	6.4/9.0 (NS) 6.2/8.7 (NS)	137/140 (NS) 139/137 (NS)	No significant differences in sf OR Hb concentrations between the groups after 8 months
CALCIUM						
Sokoll and Dawson-Hughes, 1992 *Calcium supplementation and plasma ferritin concentrations in premenopausal women* USA	Women (n=109) 18-52y	12 weeks	1000 mg/d calcium Control group did not receive placebo	34.9/-2.2% decrease (NS) 47.2/2.6% increase (NS)	135/1% increase (NS) 136/0.6% increase (NS)	No significant differences in SF and Hb concentrations between groups at the end of the intervention period
Ilich-Ernst et al, 1998 *Iron status, menarche, and calcium supplementation in adolescent girls* USA	Girls (n=354) Pre-menarcheal 8-13y	4 years	1000 mg/d calcium Placebo	29.1/30.6 (NS) 29.3/29.5 (NS)	N/A	No significant differences between groups at the end of the intervention period
Kallwarf & Harrast, 1998 *Effects of calcium supplementation and lactation on iron status* USA	Lactating (n=95) and non-lactating (n=92) women	6 months	<u>*Lactating women*</u> 1000 mg/d calcium Placebo <u>*Non-lactating women*</u> 1000 mg/d calcium Placebo	Initial & final SF concentrations not provided by these groupings. After 6 months, no significant differences in SF concentration between calcium supplemented & placebo groups.	 135/133 (NS) 133/130 (NS) 132/129 (NS) 132/130 (NS)	At baseline SF concentrations significantly higher in lactating than non-lactating women.

Study/year/country	Study population	Duration of study	Treatment groups	Serum ferritin (µg/L) Initial/Final	Hb (g/L) Initial/Final	Comments
Minihane and Fairweather-Tait, 1998 *Effect of calcium supplementation on daily nonhaem-iron absorption and long-term iron status* UK	Men & women (n=24) Mean age 43y	6 months	1200 mg/d Control group did not receive placebo	47/50 (NS) 40/38 (NS)	139/136 (NS) 143/139 (NS)	
PHYTATE						
Lind et al, 2003 *Effect of weaning cereals with different phytate contents on haemoglobin, iron stores, and serum zinc: a randomized intervention in infants from 6 to 12 mo of age* SWEDEN	Infants (n=267) 6 months	6 months	Commercial milk-based cereal drink and porridge (containing 124 µmol/d at 6-8 m & 189 µmol/d at 9-11 m) Phytate reduced milk based cereal drink & phytate reduced porridge (containing 48 µmol/d at 6-8 m & 36 µmol/d at 9-11 m) Milk based infant formula & porridge with usual phytate content (containing 26 µmol/d at 6-8 m & 62 µmol/d at 9-11 m)	48.5/25.3 (p<0.05) 40.9/21.3 (p<0.05) 44.1/25.2 (p<0.05)	116/119 (p<0.05) 115/120 (p<0.05) 115/117 (NS)	At 12 m of age, Hb significantly lower in infant formula group compared to phytate reduced group (p=0.015) SF concentration did not differ between groups
Bach Kristensen et al, 2005 *A decrease in iron status in young healthy women after long-term daily consumption of the recommended intake of fibre-rich wheat bread* DENMARK	Women (n=41) 19-37y	4 months	300g fibre rich bread with reduced phyate (molar ratio of phytic acid:iron 8.5:1) 300g fibre-rich bread (molar ratio of phytic acid:iron 6.7:1)	44/34 (p<0.001) 45/32 (p<0.001)	127/130 (NS) 127/130 (NS)	No significant difference in SF concentration between groups after 4 months

Table 5.3: Mean dietary iron intake, haemoglobin concentration and serum concentration ferritin in meat-eaters and vegetarians

Study	Country	Participants (n)		Dietary iron (mg/d)		Haemoglobin (g/L)		Ferritin (µg/L)	
		Meat	Veg	Meat	Veg	Meat	Veg	Meat	Veg
WOMEN PREMENOPAUSAL									
Reddy & Sanders, 1990	UK	22 cauc	18 caucasian 21 Indian	12.1	13.8 12.7	136	136 126*	20	11* 8*
Alexander et al, 1994	New Zealand	36	36	13.5	15.5	-	-	34	14*
Donovan & Gibson, 1995	Canada	29	79	11.3	11.2	138	138	20	18
Ball & Bartlett, 1999	Australia	24	50	9.9	10.7	134	130	46	25*
Harvey et al, 2005	UK	30 (red meat) 30 (poultry/ fish)	30	10.9 12.8	14.5	134 137	135	7 (median) 18† (median)	11 (median)
WOMEN, ALL AGES									
Harman & Parnell, 1998	New Zealand	12	12	12.8	14.7	129	124	60	50
Haddad et al, 1999	US	10	15 vegans	15.3	17.6	133	132	22	27
MEN									
Alexander et al, 1994	New Zealand	14	14	17.4	20.2	-	-	105	37*
Harman & Parnell, 1998	New Zealand	11	12	16.2	15.5	142	151	148	80
Haddad et al, 1999	US	10	10	15.0	26.4	156	154	141	72*
Wilson & Ball, 1999	Australia	25	39	15.8	20.4	173	140*	121	64*
MEN AND WOMEN									
Gear et al, 1980	UK	264	91	-	-	143	139*	-	-
Helman & Darnton-Hill, 1987	Australia	37	93	-	-	-	-	70	45*
Hua et al, 2001	USA	30	30	-	-	-	-	72	35*
Li et al, 2000	Australia	60 (moderate meat)	43	16.8	20.5	149	142*	111	48*

* significantly different from omnivores
† significantly different from red meat eater

Annex 3

Studies considered in relation to iron and cognitive function

Table 6.1: Short term treatment trials in children ≤ 3 years with iron deficiency anaemia or iron deficiency

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Drop out	Findings	Remarks
Oski & Honig, 1978 USA	IDA (n=24) IDA treated IDA (n=12) IDA untreated (n=12) (IDA=Hb<105 g/L, MCV<74 serum Fe<15µg/L, TS<13%)	9–26m	DBRCT Treatment = IM Fe Placebo = IM saline Fe dose = enough to raise Hb to 120g/L Duration: 5–8 d	Intercurrent illness or chronic disease	BSID IBR		Baseline: No significant differences in MDI or PDI between groups. Treatment: Change in MDI or PDI scores not significantly different between groups; Fe treated group significantly increased in MDI; No significant change in PDI of either group; Treated group improved more than controls in reactivity ($p < 0.05$), gross and fine motor ratings ($p < 0.01$); Attention not significantly different.	Small groups
Lozoff et al. 1982a GUATEMALA	Total (n= 68) IDA treated (n=15) IDA placebo (n=13) NA treated (n=19) NA placebo (n=21) (IDA group = Hb≤105 g/L plus 2 of 3: SF≤12µg/L, transferrin ≤10% , EP> mg/L of packed cells) (Non-anaemic group= Hb≥ 120g/L)	6-24 months	DBRCT Both IDA and non-IDA randomly assigned to Fe (5 mg/kg ferrous ascorbate) or placebo; Duration: 1 week	Hb ≤60g/L, acute or chronic illness, birth complications, prematurity, congenital anomalies, retardation, malnutrition, birth weight <5lb	IBR	7 non-IDA children	Baseline: significant differences between groups; IDA group more withdrawn, fearful, tense, unreactive to usual stimuli compared with non-IDA group. Treatment: No significant treatment effect. IDA group improved on all above measures with significant change in responsiveness and tension; Non-IDA group were unchanged in 3 scales and deteriorated in 3 others. Only post-treatment difference was IDA group remained more fearful (p=0.06)	Small groups
Lozoff et al. 1982b GUATEMALA	As above	As above	As above	As above	BSID		Baseline: IDA significantly lower MDI and PDI than NA Treatment: No significant treatment effect on MDI. All groups improved in MDI.	

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Drop out	Findings	Remarks
Oski et al, 1983 USA	NA (n=38) (Hb>110 g/L) divided into 4 groups: a) Fe replete (n=10) b) Fe depleted (n=10) (SF<12µg/L) c)Fe deficient (n=10) (b+EP>0.3 mg/L) d) Fe deficient (n=8) (c+MCV<70 fL)	9–12 months	No randomisation All subjects received IM Fe Duration: 1 wk	Prematurity, neonatal distress, congenital anomalies, chronic illness	BSID MDI; IBR		Baseline: MDI of group d less than group b (p=0.01) but a & b not different from c & d. Less involved (p<0.5), more solemn (p<0.054), attention, goal directed, responsivity, irritability not different. Treatment: Mean increase in MDI of groups a & b significantly less than groups c & d combined (p<0.01); Goal directness c+ d improved more than a+ b (p< 0.05); All groups had normal SF levels	Small groups; not randomised; no placebo group; some rating change differences not given.
Walter et al., 1983 CHILE (Subsample of Walter et al 1989)	Total (n=37) Divided into 3 groups: IDA (n=10) NA ID (n=15) Fe replete (n=12) (IDA = Hb<109 g/L and at least 2 abnormal Fe measures or a response in Hb or MCV to Fe treatment) (Fe deficient = Hb ≥110 g/L but one or more abnormal Fe measures) (Fe replete = Hb≥110 g/L, MCV≥0 70 fL, TS≥ 10%, SF≥10 µg/L)	15 months	Original cohort randomised at 3 months to Fe fortified or unfortified formula; At 15 months Fe status evaluated & all received 3-4 mg/kg/d ferrous sulphate; Duration = 11 days	BW < 2.5kg, neonatal complications, chronic or congenital disorders, inadequate growth or development	BSID IBR.		Baseline: IDA group had significantly lower MDI than Fe replete group (p<0.0025); non-anaemic ID group not different. No significant group differences in PDI; IDA infants more unhappy than Fe replete group (p< 0.05) on IBR. Treatment: Improvement in MDI larger in IDA group than Fe replete group (p<0.05); Post test, Fe replete group still had higher scores than IDA group (p< 0.05); NA ID group improved but not significantly different from Fe replete group; No significant PDI differences; IDA improved in cooperativeness and attention on the IBR (p<0.05), but treatment effect not reported.	Small groups; not randomised; other behaviour ratings not reported; .

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Drop out	Findings	Remarks
Driva et al, 1985 GREECE	Total (n=48) NA (n=8) (Hb>110g/L) IDA (n=40) (Hb<109 g/L): Group A, IDA Fe treated after first test (n=20) Group B, IDA Fe treated after 2nd test (n=20) No other Fe status cut-offs	3–25 months	RCT IDA tested 3 times, 10 days apart; randomly assigned to treatment after 1st or 2nd test NA treated after 1st test and tested twice Treatment = IM Fe 50 mg; no placebo Duration = 10 days	None given	BSID	0	Baseline: no significant difference in MDI. Treatment: no significant treatment effect on PDI; Group A significantly improved between 1st and 2nd but not between 2nd and 3rd test; Group B significantly improve between 2nd and 3rd test, not between 1st and 2nd NA - no significant increase Treatment effect not reported	
Lozoff et al, 1987 COSTA RICA	Total (n= 191) Five groups: a) IDA (n= 52) (Hb<105 g/L; 2 abnormal Fe status measures) b) Intermediate Hb & Fe deficient (n=45) (Hb=106–119g/L; 2 abnormal Fe status measures) c) Non-anaemic Fe deficient (n=21) (Hb≥120g/L; 2 abnormal iron status measures) d) Non-anaemic Fe deficient (n=38) (Hb≥120g/L; SF<12µg/L) e) Fe replete (n=35) (Hb≥120g/L; normal measures of iron status	12–23 months	DBRCT Groups a & b randomised to IM Fe, oral Fe, or placebo; Groups c, d, e randomised to oral Fe or placebo; Treatment = 10mg/kg/day oral Fe or placebo; Dose of IM Fe = increase Hb level to 125g/L Duration: 1 week	LBW, multiple pregnancy, perinatal complications, congenital anomalies, iron therapy after 6m, IM Fe treatment at any age, acute or chronic ill health, retardation, abnormal Hb, or missing iron data	BSID	7%	Baseline: Hb<100 g/L significantly lower MDI than rest combined (p=0.0002); Hb<105g/L significantly lower PDI than rest combined (p = 0.0001); Treatment: Significant increase in MDI for all groups (p<0.001); no significant treatment effect on PDI. Only Fe treated IDA group increased Hb by 10g/L.	Study 1: short duration; Study 2: differences remained after controlling for covariates; not randomised

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Drop out	Findings	Remarks
Walter et al, 1989 CHILE	Total (n=196) IDA (n=39) Non-Fe deficient (n=127) Fe replete (n=30) (IDA = Hb<110g/L + 2 or more abnormal Fe measures) (NA ID = Hb >110g/L but not Fe replete) (Fe replete = Hb≥110 g/L, MCV ≥70 fL, TIBC<10%, SF≥10µg/L+ <10g/L increase in Hb after Fe treatment)	12 months	DBRCT All groups randomized to placebo or 15 mg of Fe^{2+} Duration = 10 days	Same as above	BSID IBR.	0	Baseline: IDA group had lower MDI than Fe replete and NA ID group (p<0.01); IDA group scored lower on PDI than Fe replete group (p< 0.01) and NA ID group (p<0.0001); PDI and MDI showed sigmoid curve relationship with Hb levels with intermediate point (Hb=105-109g/L) significantly different (p< 0.05) from both extremes (i.e. < 105 or >110g/L). Treatment: Study 1 - no significant treatment effect after 10 days;	

Also see Walter et al 1989 and Lozoff et al 1987 in Table 6.2
Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomized controlled trial; EP, erythrocyte protoporphyrin, Fe, iron; Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron-deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin;TS, transferrin saturation; TIBC, total iron binding capacity.

Table 6.2: Longer term treatment trials in children ≤ 3 years with IDA or iron deficiency

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dro pout	Findings	Remarks
Walter et al, 1989 CHILE	Total (n=196) IDA (n=39) Non-Fe deficient (n=127) Fe replete (n=30) (IDA = Hb<110g/L + 2 or more abnormal Fe measures) (NA ID = Hb >110g/L but not Fe replete) (Fe replete = Hb≥110 g/L, MCV ≥70 fL, TIBC<10%, SF≥10µg/L+ <10g/L increase in Hb after Fe treatment)	12 months	Study 1: DBRCT All groups randomized to placebo or 15 mg of Fe^{2+}until 15 months of age Duration = 3 months	Same as above	BSID IBR.	0	Baseline: IDA group had lower MDI than Fe replete and NA ID group (p<0.01); IDA group scored lower on PDI than Fe replete group (p< 0.01) and NA ID group (p<0.0001); PDI and MDI showed sigmoid curve relationship with Hb levels with intermediate point (Hb=105-109g/L) significantly different (p< 0.05) from both extremes (i.e. < 105 or >110g/L). Treatment: No significant improvement in any group after 3 months.	Study 1, short duration; small groups. Study 2, not randomised
Lozoff et al, 1987 COSTA RICA	Total (n= 191 Five groups: a) IDA (n= 52) (Hb<105 g/L & 2 other abnormal Fe status measures) b) Intermediate Hb & Fe deficient (n=45) (Hb=106–119g/L & 2 abnormal Fe status measures) c) Non-anaemic Fe deficient (n=21) (Hb≥120g/L & 2 abnormal iron status measures) d) Non-anaemic Fe deficient (n=38) (Hb≥120g/L and ferritin <12µg/L) e) Fe replete (n=35) (Hb≥120g/L & normal measures of iron status	12–23 months	Groups a & b randomised to IM Fe, oral Fe, or placebo; Groups c, d, e randomised to oral Fe or placebo; Treatment = 10mg/kg/day oral Fe or placebo; Dose of IM Fe = increase Hb level to 125g/L After 1 week, IM treated groups (a & b) and Fe replete group (e) given placebo; other groups (c & d) treated with oral Fe. No randomisation Treatment = 6mg/kg/day oral Fe or placebo Duration: 12 weeks	LBW, multiple pregnancy, perinatal complications, congenital anomalies, iron therapy after 6m, IM Fe treatment at any age, acute or chronic ill health, retardation, abnormal Hb, or missing iron data	BSID	7%	Baseline: Hb<100 g/L significantly lower MDI than rest combined (p=0.0002); Hb<105g/L significantly lower PDI than rest combined (p = 0.0001); Treatment: Study 1 - Significant increase in MDI for all groups (p<0.001); no significant treatment effect on PDI. Only Fe treated IDA group increased Hb by 10g/L. Study 2 - No significant difference in change of scores between NA & IDA groups; but IDA infants (<100 g/L) whose anaemia and Fe lack were corrected caught up to infants with initial Hb>100g/L who became Fe replete) but those remaining Fe deficient continued to have lower MDI. IDA (<100g/L) who became Fe replete increased PDI significantly to catch up with NA Fe sufficient; those remaining Fe deficient still had significantly lower PDIs. Increase in Hb of at least 10 g/L in 93% of Fe treated infants, 64% no longer anaemic but still Fe deficient	Study 1: short duration; Study 2: differences remained after controlling for covariates; not randomised

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dro pout	Findings	Remarks
Lozoff et al, 1998 COSTA RICA	As above	12-23 months	As study 2 above	As above	Free play; IBR; behaviour ratings & quality of maternal participation HOME	? Non e	Baseline: IDA infants lower mental test scores but no differences in motor test scores. Also more wary and hesitant, maintained closer contact with caregiver, showed less pleasure & delight, tired easily, less playful & less attentive. Treatment: no significant treatment effect; formerly IDA continued to spend time near caregivers, attempted less tasks, more likely to be crying, irritable, asleep; less likely to play interactively. Post treatment, increase in Hb as above.	
Lozoff et al, 1996 COSTA RICA	IDA (n=34) NA (n=54) NA=Hb>125g/L IDA=Hb ≤100g/L+ 2 of 3 measures - SF ≤ 12µg/L, TS ≤10% free erythrocyte protopophyrin > 1 mg/L	12-24 months	IDA all treated; Non-anaemic randomised to treatment or placebo; Treatment = 6 mg/kg/day; Duration: 6 months	BW < 2500g, birth complications, multiple pregnancy, acute or chronic health problems	BSID IBR		Baseline: IDA group significantly lower MDI than NA group; No group difference in PDI; IDA more fearful ($p<0.03$) and unhappy ($p<0.01$). Treatment: No significant treatment effect. IDA group disadvantaged in maternal education & home stimulation & less breast feeding; When controlled for all covariates, IDA not significantly different from NA group in MDI (limited power); No difference in IBR post treatment but treatment effect not reported.	No randomisation
Harahap et al, 2000 INDONESIA	All children stunted or wasted. IDA from treatment groups 1 & 2 (n=18); NA from treatment group 3 (n=18). IDA = Hb<110g/L, TS>16% or change in Hb>10 g/L	IDA 12m (n=10) 18m (n=8) NA: 12m (n=9) 18m (n=9)	3 treatments: 1) Condensed milk+ micronutrient (12 mg Fe) 2) Skimmed milk & micronutrient (12 mg Fe) 3) Skimmed milk & placebo IDA groups 1 & 2 compared with NA group 3 matched for age & sex. Duration = 6 months	Chronic disease	BSID Response to novelty, object concept, motor milestones, activity at home, behaviour at home		Baseline: PDI & motor activity significantly different. Motor milestones, MDI, object concept, novelty recognition, all not significant Treatment: IDA group improved significantly more than NA group in motor development. MDI, object concept, novelty recognition and milestones, all not significant.	Small groups, some children on ceiling of motor milestone scale. No randomised placebo anaemic group.

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Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dro pout	Findings	Remarks
Hasanbegovic et al 2004 Sarajevu, Bolnicka	IDA = 90; A) 45 with Hb < 95, B) 45 with 95-110g/L NA = 30 (Hb>110g/L)	6-24 months	IDA all treated Duration: 3 months		BSID IDA given pre and post tests NA tested at baseline only		Baseline: IDA significantly lower in MDI and PDI than NA. Also A)lower than B) Treatment: MDI, PDI improved in B) but not in A).	No randomisation No post test in NA
Akman et al, 2004 Turkey	1) 37 IDA= Hb<110 g/L 2) 40 non-anaemic iron deficient (NAID) =Hb ≥ 110g/L, SF ≤12µg/L, MCV ≥ 70fl 3) 31 NA iron replete (control) = Hb≥110g/L,	6-30 months	NAID only randomised to ferrous sulphate 6mg/kg or no treatment All IDA treated All control no treatment Duration= 3 months	BW < 2500g, no birth complications, multiple pregnancy, acute or chronic health problems	BSID Denver Screening test		Baseline: IDA & NAID significantly lower scores in MDI than controls, and IDA significantly lower PDI than controls. IDA & NAID had significantly lower scores in Denver than control. Treatment: initial 3 groups not different at end. Change significantly different across the 3 groups. No intent to treat analysis with randomised groups	Only NAID randomised No placebo

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomized controlled trial; EP, erythrocyte protoporphyrin, Fe, iron; Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron-deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin;TS, transferrin saturation; TIBC, total iron binding capacity.

Table 6.3: Longer term randomised treatment trials in children ≤ 3 years with IDA or iron deficiency

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Aukett et al, 1986 UK	Total (n=110) Treated (n=54) Placebo (n=56) All with Hb=80–110 g/L (no other Fe cut off)	17–19 months	DBRCT Treatment = 24mg Fe+10 mg vitamin C/day Placebo = 10 mg vitamin C/day Duration = 2 months	Hb < 80g/L, lead poisoning, chronic health problems	Denver screening test Anthropometry	13	No significant treatment effect on psychomotor skills; No difference between those with Hb increase > 20 g/L and those with less; Expected rate of development was achieved by 31% of iron treated and 12% of the placebo group ($p < 0.05$); Hb increased by mean of 22 g/L in group receiving Fe and 0.3 g/L in placebo group	Denver not sensitive; rate of development was a post-hoc analysis
Idjradinata & Pollitt, 1993 INDONESIA	Total (n=126) IDA Fe treated (n=25) IDA placebo (n=25) NA ID, Fe treated (n=14) NA ID, placebo (n=15) NA Fe replete, Fe treated (n=24) NA Fe replete placebo (n=23) IDA = Hb ≤105g/L, TS≤ 10%, SF≤12µg/L Fe depleted=Hb≥120g/L, TS≤10% , SF≤12µg/L Fe replete=Hb≥120g/L, TS>10%, SF>12µg/L	12–18 months	DBCRT Stratified by Fe status group then randomised to treatment or placebo Treatment = Fe sulphate, 3mg/kg/day Duration: 4 months	BW<2.5 kg, multiple pregnancy, congenital anomalies, perinatal complications, hemoglobinopathy, weight & height<2SD of reference standards, , acute or chronic illness, Hb between 10.5-120g/L.	BSID	? 7 lost	Baseline: IDA groups scored significantly less in MDI than non-anaemic ID & Fe replete groups and significantly less in PDI than ID & Fe replete groups; latter two groups not significantly different. Treatment: significant treatment effect in IDA groups in MDI and PDI. No longer any differences between treated IDA & non-anaemic ID & Fe replete groups. Fe replete & ID groups had no significant treatment effect. Significant treatment effects on Hb concentration in IDA and ID group.	Previous delay reversed. Non-anaemic ID group small.

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Stoltzfus et al, 2001 ZANZIBAR	685 randomised, 538 completed study 417 aged 12- 48mths had language assessment 293 aged 12-36mths had motor assessment 97% , Hb<110g/L 85% , malarial parasitaemia	12-48 months	DBCRT Received Fe supplement (10mg/day) or placebo and anthelmintic treatment (500mg mebendazole) or placebo. (Children with Hb<70g/L treated with 60 mg/day of Fe for 30 days in addition to randomly allocated iron). Duration = 12 months		Assessed by parental interview. Language milestones assessed in children 12-48 months & motor milestones assessed in those aged 12-36 months	5% loss from language test 9% loss from motor test	Baseline: After adjustment for age, scores on motor & language scales significantly associated with Hb concentration. Treatment: significant Fe treatment effect on motor scores only in children with baseline Hb<90g/L. Significant Fe treatment effect on language scores across Hb range No significant anthelmintic treatment effect on motor and language milestones No treatment effect on Hb concentration but significant effect on ferritin	motor improvement related to initial Hb concentration but language improvement was not .

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomized controlled trial; EP, erythrocyte protoporphyrin, Hb, haemoglobin; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron-deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin;TS, transferrin saturation; TIBC, total iron binding capacity.

Table 6.4: Longer term randomised trials with children ≤ 3 years with mixed iron status.

Study/year/ country	Sample	Age	Study Design and Treatment	Exclusions	Outcome measures	Drop out	Findings	Remarks
Black et al, 2004 BANGLADESH	Total (n=221) subsample from larger study 5 groups: 1) 20mg Fe + 1mg riboflavin (n=49) 2) 20mg Zn + 1mg riboflavin (n=49) 3) 20mg Fe + 20 mg Zn + 1 mg riboflavin (n=43) 4) Micronutrient mix (MM) (with 16 vitamins & minerals incl 20mg Fe, 20mg Zn, 1mg riboflavin) (n=35) 5) 1 mg riboflavin (n=45) All with Hb ≥90g/L; approx 68% , Hb<110g/L	6 months	DBRCT Treatments given weekly All received 30mg vitamin A at beginning of study. Duration: 6 months	Severely malnourished, neurologic disorders, physical disability, chronic illness Hb <90g/L	BSID HOME scale Behaviour ratings: 3 factors Orientation-engagement Emotional regulation Motor quality	Initially 43 refused 28 absent + 125 dropped out (36%)	Baseline: Hb concentration not associated with any developmental or behavioural measures. Treatment: Significant group x time interaction for PDI and Orientation PDI scores decreased significantly less in the Fe+Zn and the MM groups compared to riboflavin group. Fe group and Zn group ns MDI scores not affected by any treatment. Orientation decreased significantly less in the Fe and Fe +Zn groups than in the riboflavin group No treatment effect on Hb concentration.	Small groups; controlled for number of covariates Only iron effect was in orientation
Lind et al, 2004 INDONESIA	Total (n=680) Each group (n=170) 4 treatment groups: 1) Fe (10mg/d) 2 Zn (10mg/d) 3) Fe (10mg/d)+ Zn (10mg/d) 4) placebo All with Hb>90g/L 41% anaemic	6 months	DBRCT Each dose of all treatments also contained 30mg ascorbic acid. Duration: 6 months	Chronic illness, twins, metabolic or neurologic disorder	BSID Behaviour ratings	25 (4%)	Baseline: No significant differences between groups. Treatment: Significant interaction between Fe & Zn treatment for PDI. Significant iron effect on PDI (p=0.042). no other group significant No effect of Fe+Zn combined on PDI. No treatment effect on MDI or behaviour	Controlled for number of covariates
Olney et al, 2006 Zanzibar	n=404 103 = Fe 12.5 mg + folate (FeFA) 87= Zinc 10mg 101= FeFA + zinc 114= placebo 65% anaemic	5-11 months	DBRCT Duration until walked or up to 12 months		Age of walking By interview every 2 weeks	12%	Treatment: Fe (+/- zinc) had a significant effect on age of walking. Improvement greatest in children with initial IDA zinc not significant	

Abbreviations: BSID, Bayley Scales of Infant Development; DBRCT, double-blind randomized controlled trial; EP, erythrocyte protoporphyrin, Hb, haemoglobin; FE, iron; HOME, Home Observation Measurement of Environment; IBR, Infant Behaviour Record; ID, iron deficient; IDA, iron-deficiency anaemia; IM, intramuscular; LBW, low birth weight; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PDI, psychomotor development index; RCT, randomised controlled trial; SF, serum ferritin;TS, transferrin saturation; TIBC, total iron binding capacity; Zn, zinc.

Table 6.5: Preventive trials with non-anaemic children ≤ 3 years

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Moffatt et al, 1994 CANADA	n=283 blood not assayed on enrolment	< 2 months	DBRCT Randomised to: Fortified formula (12.8 mg/L Fe) or low iron formula (1.1 mg/L Fe) until 15months of age. Duration = 13 months	Perinatal complications; congenital anomalies; BW <2.5kg; prematurity	BSID; IBR	225, 204, 186 and 186 infants tested at 6, 9, 12 and 15m respective ly	PDI - significant treatment effect at 9 and 12m but not at 15m. MDI - no effect. Hb significantly higher in fortified group at each test. Percentage < 110 g/L in fortified and unfortified was 8.1 and 28.0 at 6m, 8.1 and 18.6 at 9m, 2.3 and 12.4 at 12m and 2.6 and 10.4 at 15m respectively.	Large loss Difference between groups in iron status small
Williams et al. 1999 UK	n=100 Infants who had started on unmodified cows milk at 6m 14.5% anaemic	5.7-8.6 months	RCT Randomized to: Fe fortified formula (1.2mg Fe/100 m1) or usual cows' milk (0.05 mg Fe/100 ml) and given money to buy cows' milk Duration = until 18 months of age Observed to 24 months old.	Preterm, Hb <90 g/L, hemoglobinopat hy, chronic ill health	Griffiths Scale	15%	Significant treatment effect on development. Mean developmental quotients fell in both groups but in fortified group they fell significantly less by 24m ($p<0.05$). Difference not significant at end of treatment at 18m. Drop in all subscale scores was less in fortified group but only significant in personal social subscale ($p<0.05$). At baseline no significant difference between groups in Hb concentration. Percentage with Hb < 110 g/L at 12m was 2% in fortified and 33% in cow's milk group; at 18m , fortified 0% and cow's milk 24%.	Subjects not blind to treatment; other constituents of formula may have caused the effect.
Morley et al, 1999 UK	n = 493 from 3 centres. Only one centre had Hb estimations	9 months	DBRCT Stratified by 3 areas and Asian/other, then randomised to: Cows' milk (0.05 mg Fe/L); Low Fe formula (0.9 mg Fe/L). High Fe formula (1.2 mg Fe/L) Duration = 9 months	Prematurity, BW < 2.5kg; multiple pregnancy; previous iron supplement or blood transfusion; delayed development.	Pretest Sherard's screen; Post test BSID	13%	No significant treatment effect on MDI or PDI. Similar ferritin levels in cows' milk and low Fe formula groups and both significantly lower than high Fe formula group ($p< 0.01$). Hb data missing from most at beginning and end of study, therefore % anaemic unknown.	Doubtful statistical power.; large loss of Hb data.

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Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Dropout	Findings	Remarks
Lozoff et al, 2003 CHILE	n=1657 Fe supplemented (n=1123) No added Fe (n=534)	6 months	6 groups varying in entrance criteria & supplementation procedures by time of enrolment 1991-94: Infants on ≥250ml/d cow milk/formula assigned to high iron formula (12mg/L) (n=430) or low iron formula (2.3 mg/L) (n=405) 1994-96: Infants on ≥250ml/d cow milk/formula assigned to high iron formula (n=176) or unmodified cow milk and multivitamins with no iron (n=404) Infants on ≤250ml/d cow milk/formula assigned to multivitamins with Fe (n=112) and multivitamins without Fe (n=130) Duration = 6 months	Premature; BW<3.0 kg low Hb, acute or chronic illness, congenital anomalies, IDA, iron therapy	BSID; Fagan Test	?	No significant differences in mental or motor Bayley scores at 12m. Fagan test - significant effect of Fe supplementation on looking time with non-supplemented infants looking longer. Significant effect of supplementation on age of crawling/creeping – non-supplemented infants crawled later. IDA in Fe supplemented group = 3.1% IDA in non-supplemented group 22.6%	Results should be interpreted with caution owing to possible confounding effects of cow's milk and breastfeeding with treatment.
Friel et al, 2003 CANADA	Total (n=77) Successfully breast feeding, healthy with Hb > 120g/L SF>19µg/L	1 month	DBCRT Received either Fe (7.5 mg/day) or placebo until 6 months of age Developmental outcomes assessed between 12-18 months of age. (80% were 12 months) Duration = 5 months	Gestation<37 wks; BW<2.5kg; multiple pregnancy; major illness; major congenital anomaly	BSID Visual acuity	40%	Baseline: No significant difference between groups for Hb, MCV and plasma ferritin concentration. Developmental scores not assessed. Treatment: Fe treated group higher PDI ($p<0.05$) and visual acuity ($p=0.07$) than placebo group. No treatment effect on MDI. Fe treatment significantly improved MCV at 3.5m and 6m of age, and Hb at 6 months of age; no significant differences between groups in Hb, MCV, or SF at 12 months of age.	Small sample size; high dropout rate; By 6 m majority of children in both groups received formula containing Fe. Small difference in iron status

Abbreviations: BSID, Bayley Scales of Infant Development; BW, birth weight; DBRCT, double-blind randomized controlled trial; Hb, haemoglobin; IBR, Infant Behaviour Record; IDA, iron-deficiency anaemia; MCV, mean corpuscular volume; MDI, mental development index; PDI, psychomotor development index; RCT, randomised controlled trial.

Table 6.6: Therapeutic treatment trials in children > 3 years

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Pollitt et al, 1983 USA	Fe depleted (n=15) NA (n=15) Fe depleted - change in TS-1 SD to+1.5 SD, mean Hb = 112 g/L; NA = Hb>110 g/L, TS>20%	3–6 y	Both groups treated; Treatment = 4–5mg/kg Fe/d Duration = 4–6 months	Physical handicap	Discrimination -learning tasks; Oddity learning; Short term memory; Stanford Binet IQ	Baseline: IDA group took more trials to reach criterion in 1st part but not in reversal learning, IQ, short-term memory and oddity learning not significantly different. Treatment: No significant difference between groups in discrimination. Mean Hb increase in Fe depleted = 13 g/L	No randomisation; small groups
Soemantri et al, 1985 CENTRAL JAVA	IDA (n=78) Fe replete (n=41) IDA = Hb ≤110 g/L and TS ≤15% Fe replete = Hb≥ 120 g/L+ TS ≥ 20%. Mean Hb: IDA = 97 g/L Fe replete = 132 g/L	11y	DBRCT Treatment: 10 mg/kg ferrous sulphate /day Placebo = tapioca & saccharin; Duration = 3 months .	<80th percentile of weight & height; <85th percentile for MUAC, parasite egg after deworming, malaria, hematological diseases, severe illness, physical handicap, IQ<75	Baseline: Raven Progressive Matrices (IQ), Pre- and post-treatment: abbreviated standard achievement test, Bourden-Wisconsin test for concentration.	Baseline: IDA and NA not significantly different in IQ and concentration; NA group significantly higher school achievement than IDA group; Treatment: Fe treated IDA group improved significantly more in concentration and in school achievement than placebo IDA group; No significant difference between Fe treated and placebo NA groups; Post-treatment score of NA group still significantly better than Fe treated IDA group Change in Hb Fe treated IDA = 26.7 g/L; Placebo IDA =-11.7 g/L; Fe treated NA = 7.6 g/L; Placebo NA = 6.7 g/L	Clear treatment effect; ? Control for school and grade level ; extremely low post test Hb in IDA placebo group.
Pollitt et al, 1985 EGYPT	IDA (n=28) Fe replete (n=40) IDA = Hb≤115g/L+ TS ≤ 25%, or SF ≤20 µg/L; Fe replete = Hb>130g/L+ TS >25% or SF>12µg/L	9.5 y	DBRCT Treatment: 50mg ferrous sulphate/day Placebo: not stated Duration: 3–4 months	?	Matching familiar figure test	Baseline: NA children had significantly greater efficiency than IDA children. Treatment: Efficiency of Fe treated IDA group significantly greater ($p<0.05$) than placebo treated IDA group. No significant effect on Fe replete children.	Limited details (letter); clear treatment effect not reported; small groups.

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Pollitt et al, 1986 GUATEMALA	IDA (n=25) NA (n=25) IDA: At baseline, Hb≤100 g/L & EP>1 mg/L. Post treatment, Hb>110 g/L & EP≤175 or EP<100+Hb> 100g/L or ΔHb>20 g/L. NA: At baseline, Hb>110 g/L & EP≤1.5 mg/L. Post treatment, Hb>110 g/L & EP≤1.5 mg/L	3-6y	Not randomised. All infants treated with Fe. Treatment: 3 mg/kg ferrous sulphate day /day Duration 11–12 weeks	Birth weight < 2.5kg, chronic illness, severe malnutrition, hematological disorder	Discriminant learning; short term memory; oddity learning tasks(measure attention, memory and conceptual learning)	Baseline: IDA group had more trials to criterion in the discrimination task compared with NA group ($p<0.05$); no significant difference between groups in memory and oddity tasks. Treatment: Discrimination test - IDA group improved significantly; no longer significant difference between groups. Memory test - no differences. Oddity test - NA group improved more than IDA (not significant) and had significantly better scores post treatment. Mean Hb increase in IDA = 29 g/L.	No randomization
Deinard et al, 1986 USA	IDA (n=25) Fe deficient NA (n=45) NA Fe replete matched to IDA group for sex, age mother's education, and race (n=?). IDA = Hb≤110 g/L, PCV ≤ 33%, EP≥ 35, MCV< 74 fl, SF<20 µg/L; NA Fe deficient = Hb≥ 110g/L, PCV≥34%, EP = ≥35, MCV ≥ 75, SF<20; NA Fe replete = Hb≥110g/L, PCV≥34%, EP<35, MCV ≥ 75, SF>20 µg/L.	18–60 months	Double blind intervention All IDA treated; NA Fe deficient: alternately assigned to Fe (n=22) or placebo (n=23) NA Fe replete: placebo; Treatment = 6 mg/kg elemental Fe /day Duration = 6 months	Gestational age ≥38 wk, BW≥2.5kg, head circumference, height and weight within± 2 SD of NCHS, chronic illness, developmental retardation	BSID MDI for infants 18–24m Stanford Binet IQ for children > 2 y; Behaviour rating.	Baseline: No significant difference between IDA and Fe replete group; Fe treated but not placebo Fe deficient group significantly lower than Fe replete group; IDA significantly less responsive to the environment and more unhappy, Treatment: Fe replete group's score significantly improved at 3 and 6 months & significantly higher than IDA at 3 but not 6 months. IDA and Fe deficient groups showed no significant improvement. NA group significantly more responsive to examiner than IDA (3 and 6 months), and more responsive to environment (baseline, 3 and 6 months) and emotional tone (baseline and 3 months). IDA Fe treated and both Fe deficient groups showed complete hematological correction.	Wide age range; No analysis of group differences in change of scores.

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Soewondo et al, 1989 INDONESIA	IDA (n=49) Fe depleted (n=57) Fe replete (n=70) IDA = Hb< 10 g/L + two of SF<12 µg/L, TS<16%, EP>1.77 µmol/L; Fe depleted = Hb ≥110g/L + two of above; Fe replete = Hb≥110g/L, + two of SF≥12µg/L, TS ≥16%, EP≤ 1.77	IDA = 54 months NA = 58 months	DBRCT All groups received Fe or placebo. Treatment = 50mg Fe/day in syrup or placebo; Duration = 8 weeks	?	Peabody Picture Vocabulary test (PPVT). Two discrimination learning tasks Four oddity tasks	Baseline: No difference between Fe deficient and NA in any test. No differences between IDA and Fe replete in PPVT or discrimination learning tasks; Fe replete faster than IDA on 2 oddity tasks ($p< 0.05$). Treatment: PPVT no treatment effect; discrimination learning tasks remained too difficult; discrimination learning: treated IDA group earned significantly faster than treated Fe replete group ($p< 0.05$); oddity task: no effect in trials 1 & 2; in trials 3 & 4 interaction between Fe treatment and group; Fe treated IDA group had significantly higher scores than Fe treated Fe replete group & Fe replete placebo group had higher scores than the IDA placebo group. Hb change in Fe treated IDA = 9 g/L; placebo IDA = 1 g/L	Too many on floor of discrimination learning tasks, using subgroup who were on the test of doubtful validity
Pollitt et al, 1989 THAILAND	IDA (n=101) Fe depleted (n=47) Fe replete (n=1210) IDA = Hb<120g/L+ two of: SF<10µg/L, TS<16%, EP>700µg/L; Fe depleted = Hb 120g/L+ same as above; Fe replete = Hb≥120g/L+ two of: SF>9 µg/L, TS>15%, EP<701 µg/L	9–11 y	DBRCT All dewormed on enrolment & after 3 months; Randomised to Fe or placebo before Fe status known, then divided into groups by iron status; Treatment: 50 mg ferrous sulphate/d for 2 weeks, then 100 mg/d for 14 weeks Duration = 16 weeks	Thalassemia, cyanotic heart disease	Raven Progressive Matrices; Thai language and maths test; controlled for school and grade.	Baseline: IDA group scored significantly lower on IQ than NA control groups; IDA and Fe deficient group scored lower on Thai language than NA controls; maths scores not significantly different. Treatment: no treatment effect; Fe status groups still significantly different in language and IQ after controlling for anthropometry and SES. Hb: IDA placebo and Fe treated groups increased in Hb by 14 & 20g/L respectively; Fe depleted placebo and Fe treated groups increased by 1& 5 g/L respectively; both Fe replete placebo and Fe treated decreased by 2 g/L.	Hb of IDA placebo group improved, therefore validity threatened ? due to deworming

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Seshadri & Gopaldas, 1989 (study 1) INDIA	n=94 IDA= Hb<110 g/L No other measure	5–8 years	Before Hb level known, children stratified by age, then every third child randomly assigned to control group and the next two to iron treatment group. Treatment = 20mg Fe+ 0.1mg folic acid/day; Placebo not stated; Duration = 60 days;	Severe malnutrition (weight-for-age < 60% of NCHS standards).	Indian adaptation of WISC	Baseline: Anaemic children had significantly lower WISC scores than NA children only in 7 to 8-y-olds; Treatment: Fe treated group improved significantly in verbal, performance, and global IQ for all ages; no change in controls; In Fe treated group both IDA and NA children improved in IQ; Improvement in global IQ of IDA children significantly higher than for NA children for the 7–8-y-olds only. Hb increased significantly in Fe treated group	Analysis of treatment effect not reported by randomized groups; age gps too small to interpret separately; no placebo; folic acid may have independent benefits.
Seshadri & Gopaldas, 1989 (study 2) INDIA	n=28 (14 pairs of boys matched for IDA, height, weight, Hb, IQ, per capita income, and mother's educational level) IDA = Hb < 105 g/L	5–6 years	DBRCT Each pair randomized to iron or placebo Both groups dewormed. Treatment = 40 mg Fe+ 0.2mg folic acid/day Placebo = sugar. Duration = 60 days	Weight-for-age < 61% of NCHS standard; Draw-a-man IQ of <70 and >110.	Draw a man IQ; WISC	Baseline: No significant differences. Treatment: Both groups improved significantly in WISC; Fe treated group significantly better than controls in verbal and performance tasks. Mean change in Hb =+24 g/L in treated group and-8 g/L in controls.	Significance of group differences not reported; folic acid may have independent benefits; may have been over matched; small groups.

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Seshadri & Gopaldas, 1989 (study 3) INDIA	n= 48 (16 groups of three, each matched for age, Hb level, and scores in cognitive function tests.) IDA = Hb<105g/L; NA = Hb>115g/L	8–15 years	DBRCT Each matched triplet randomised into 3 groups; Treatment (a) 30 mg Fe/d; Treatment (b) 40 mg Fe/d; Placebo = brown sugar; Duration = 60 days		Visual-recall; Digit-span; Maze (visual motor coordination); Clerical task.	Baseline: No differences because of matching. Treatment: Both Fe treatment groups significantly improved in all cognitive tests except for the maze test in the 30 mg group, no change in the placebo group (?significant difference); Compared with placebo, the 30mg group had significantly higher scores in the clerical-task and visual-recall tests, and the 40mg group had significantly higher scores in digit-span, mazes, clerical-task and visual-recall. IDA placebo boys showed no significant improvements; the 40mg and 30mg iron treated IDA group significantly improved in several tests.	No analysis reported of differences between groups in change of scores; may have over matched; small groups
Seshadri and Gopaldas, 1989 (study 4) INDIA	n=130 65 pairs matched for age, and Hb.	8–15 years	DBRCT Matched pairs randomised to: Treatment = 60 mg Fe/d. Placebo = sugar tablets. Duration = 60 days	Family income > Rs500	Visual-recall; Digit-span; Maze; Clerical task.	Baseline: No differences in Hb or cognitive test scores in treated or placebo group or between IDA and NA groups. Treatment: Fe treated IDA children significantly better than placebo IDA children in overall scores and in clerical tasks, and mazes; Fe treated NA group improved significantly only in mazes.	Analysis not presented by randomised group; adding test scores of doubtful validity.
Bruner et al, 1996 USA.	NA Fe deficient girls (n=81); Treated (n=40) Placebo (n=41 IDA = Hb<120g/L for white & 115g/L for black girls Fe deficient = normal Hb+ ferritin< 12µg/L	13–18 years	DBRCT Treatment ≡ 260 mg Fe/d Duration = 8 wk;	Boys	Brief Test of Attention (BTA); Symbol Digits Modalities Test (SDMT); Visual Search and Attention (VSAT); Hopkins Verbal Learning Test (HVLT).	Baseline: No differences in haematologic & cognitive measures. Treatment: No significant effect on BTA, SDMT or VSAT. HVLT - iron treated group improved significantly more in score of 3 free recall items than the placebo group ($p< 0.02$). No significant difference in delayed recall or recognition parts of test. Hb and serum ferritin higher for Fe treated group.	Benefits limited to free recall; ferritin only other measure of iron status.

ANNEX 3

Study/year/ country	Sample	Age	Study Design & Treatment	Exclusions	Outcome measures	Findings	Remarks
Lynn and Harland, 1998 ENGLAND	Treated (n=208) Placebo (n=205) 2.9% Hb < 120g/L, 16.9% SF>12 µg/L.	12–16 years	Divided into 2 groups matched for age, sex and IQ; method of assignment not stated. Treatment = 17 mg Fe + 17 mg ascorbic acid Duration = 16 weeks		Ravens Progressive Matrixes (IQ)	Baseline: Significant correlation between Hb and IQ ($p< 0.01$); ferritin and IQ not significant. Treatment: No significant difference between the groups. Sub group with ferritin <12 µg/L improved significantly more with treatment than placebo ($p= 0.02$); subgroups with ferritin 12–20 µg/L not different; treated subgroups with ferritin >20µg/L improved significantly more than placebo ($p< 0.05$).	Moderate and high ferritin groups combined showed no treatment effects
Metallinos-Katsaras et al, 2004 GREECE	IDA (n=21) Fe replete (n=28) IDA=Hb<112g/L & TS<16%, SF<12 µg/L or Hb increase>10g/L after Fe supplementation Fe replete = Hb>120g/L & either TS>20% or SF>12 µg/L	3-4 years	DBCRT Treatment: 15mg/d Fe & multivitamins Placebo (multivitamins only) Duration: 2 months	BW<2.5kg; IQ≤1SD below age-adjusted mean; blood lead levels≥ 20µg/dL; head, weight & head circumference≤ 10th percentile of US NCHS	Simple reaction time (SRT) test; continuous performance task (CPT); oddity learning (OL) tasks	Baseline: No significant differences between IDA and Fe replete children in SRT & CPT scores, mean trials to criterion, or in proportion reaching criterion in any of the OL tasks. IDA group had significantly higher proportion correct on 1st OL task than Fe replete group. Treatment: SRT – no treatment effect in IDA or Fe replete children; CPT – Fe treated IDA children made significantly fewer errors of commission ($p<0.05$) & showed higher accuracy ($p<0.05$) and significantly more efficient ($p<0.05$) than Fe replete children given placebo. OL – no treatment effect.	Small subgroups

Abbreviations: BSID, Bayley Scale of Infant Development; BW, Birth weight ; DBRCT, double-blind randomised controlled trial; EP, erythrocyte protophyrin; Hb, haemoglobin; IDA, iron-deficiency anaemia; IQ, intelligence quotient; MUAC, mid-upper arm circumference; NCHS, National Center for Health Statistics; MCV, mean corpuscular volume; MDI, mental development index; NA, non-anaemic; PCV, packed cell volume; SF, serum ferritin; SES, socioeconomic status; TS, transferrin; WISC, Wechsler Intelligence Scale for Children.

Annex 4

Studies considered in relation to iron and colorectal cancer and cardiovascular disease

PROSPECTIVE STUDIES OF IRON AND COLORECTAL CANCER

Table 7.1: Total dietary iron

Study/year/country	**Age baseline (y)**	**Mean Follow-up**	**Cases**	**Non-cases**	**Cancer site**	**Comparison** (median intake or quantile range)	**Adjustments**	**RR (95% CI)**
Wurzelmann et al, 1996 USA	25-74	15	52 M+F	8,740 M+F	Proximal colon	Top fourth vs bottom fourth *(intakes in quartiles not specified)*	Age, sex	1.44 (1.23-1.69) *p trend not given*
As above	As above	As above	57 M+F	As above	Distal colon	As above	As above	1.03 (0.80-1.32) *p trend not given*
Kato et al, 1999 USA	34-65	4.7	105 F	523 F	Colorectum	Top fourth vs bottom fourth *(intakes in quartiles not specified)*	Age, beer intake, physical activity, family history CRC	1.17 (0.6-2.3) *p trend=0.44*
Balder et al, 2006 NETHERLANDS	55-69	9.3	869 M	2,156 M	Colorectum	Top fifth (17mg/d) vs bottom fifth (9.5mg/d)	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, veg	1.34 (0.93-1.93) *p trend=0.12*
Balder et al, 2006 NETHERLANDS	As above	As above	666 F	2,215 F	As above	Top fifth (15mg/d) vs bottom fifth (8.5mg/d)	As above	1.08 (0.72-1.62) *p trend=0.90*
Cross et al, 2006 FINLAND	50-69	14.2	130 M	260 M (smokers)	Colorectum	Top fourth (25mg/d) vs bottom fourth (12.2mg/d)	Age, education, BMI, smoking, physical activity, energy intake, alcohol, aspirin use	0.4 (0.1-1.1) *p trend=0.06*
Kabat et al, 2007 CANADA	40-59	16.4	617 F		Colorectum	Top fifth (≥14.99mg/d) vs bottom fifth (<11.90mg/d)	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.07 (0.8-1.43) *p trend=0.94*

Table 7.2: Haem iron

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Cancer site	Comparison (median intake or quantile range)	Adjustments	RR (95% CI)
Lee et al, 2004* USA	55-69	15	438 F	33,967	Proximal colon	Top fifth (≥2.05mg/d) vs bottom fifth (≤0.76mg/d)	Age, energy, BMI, physical activity, smoking, alcohol, HRT, diabetes, intake of: sat fat, calcium, vit E, folate, fibre, multivitamins	1.41 (0.90-2.21) *p trend=0.24*
Lee et al, 2004** USA	As above	As above	303 F	As above	Distal colon	As above	As above	0.65 (0.38-1.11) *p trend=0.09*
Larsson et al, 2005*** SWEDEN	40-75	14.8	547 F	60,886 F	Colon	Top fifth (≥2.06mg/d) vs bottom fifth (<0.67 mg/d)	Age, BMI, education; intakes of energy, sat fat, folate, calcium, fibre, zinc	1.31 (0.98-1.75) *p trend=0.03*
Balder et al, 2006 NETHERLANDS	55-69	9.3	869 M	2,156 M	Colorectum	Top fifth (1.85mg/d) vs bottom fifth (0.60mg/d)	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, veg.	1.32 (0.96-1.80) *p trend=0.08*
Balder et al, 2006 NETHERLANDS	As above	As above	666 F	2,215 F	As above	Top fifth (1.54mg/d) vs bottom fifth (0.47mg/d)	As above	1.20 (0.86-1.69) *p trend=0.24*
Kabat et al, 2007 CANADA	40-59	16.4	617 F	48,049	Colorectum	Top fifth (>2.95mg/d) vs bottom fifth (<1.58mg/d)	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.06 (0.8-1.42) *p trend=0.99*

*Neither haem iron or zinc intake associated with risk of proximal colon cancer; however when haem & zinc were mutually adjusted both positive association of haem iron and inverse association of zinc intake were statistically significantly associated with proximal colon cancer (RR, 2.18 [1.24-3.86], ptrend=0.01. Strength of associations of both haem iron intake and Zn intake became stronger with increasing levels of consumption.
**After mutual adjustment for haem iron and Zn only inverse trend for Zn was statistically significant. Association not affected by alcohol consumption.
***For women consuming 20g or more per week alcohol, multivariate RR =- 2.29 (1.25-4.21) p trend=0.007

Table 7.3: Serum ferritin

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Cancer site	Comparison (median intake or quantile range)	Adjustments	RR (95% CI)
Kato et al, 1999 USA	34-65	4.7	105 F	523 F	Colorectum	Top fourth vs bottom fourth *(intakes in quartiles not specified)*	Age, beer intake, physical activity, family history CRC	0.40 (0.2-0.8) *p trend<0.01*
Cross et al, 2006 FINLAND	50-69	14.2	130	260	Colorectum	Top fourth (312 µg/L) v bottom fourth (59 µg/L)	Age, education, BMI, smoking, physical activity, energy intake, alcohol, aspirin use	0.4 (0.2-0.9) *p trend=0.09*

Table 7.4: Prospective studies of C282Y heterozygosity and colorectal cancer

Study/year	Country	Cases	Non-cases	OR (95% CI)
Nelson et al, 1995	USA	47 M	26 M	1.28 (1.07-1.53)
As above	USA	45 F	36 F	1.08 (0.87-1.34)
Altes et al, 1999	Spain	116 M+F	108 M+F	0.86 (0.25-2.94)
Beckman et al, 1999	Sweden	173 M +F	294 M + F	1.02 (0.57-1.82)
Macdonald et al, 1999	Australia	229 M+F	228 M+F	0.90 (0.48-1.69)
Shaheen et al, 2003	USA	475 M+F	833 M+F	1.27 (0.83-1.95)
Van der A et al, 2003	Netherlands	240 F	635 F	1.20 (0.6-2.2)
Robinson et al, 2005	UK	327 M+F	322 M+F	1.01 (0.73-1.40)

Table 7.5: Prospective studies of red meat and colorectal cancer published after 1996

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR (95% CI)
Kato et al, 1997 USA	34-65	7.1	100 F	14,627 F	Colorectal cancer	Top fourth vs bottom fourth red meat *(intake in each quartile not provided)* *(classification of red meat not defined)*	Energy intake, age, place at enrolment, education	1.23 (0.68-2.22) *p trend=0.55*
Chen et al, 1998 USA	40-84	13	212 M	221 M	Colorectal cancer	Top third (>1 serving/day) vs bottom third (≤0.5 serving/day) *(beef, pork, lamb, as main dish, mixed sish, or sandwich, hot dogs)*	Age, smoking, BMI, physical activity, alcohol	1.17 (0.68-2.02) *p trend=0.59*
Hsing et al, 1998 USA	≥35y	20	145 M	17,488 M	Colorectal cancer	Top fifth (≥60 times/month) vs bottom fifth (<15 times/month) red meat *(beef, bacon, fresh pork, smoked ham)*	Age, smoking, alcohol, energy intake.	1.9 (0.9-4.3) *p trend=0.10*
Sellers et al, 1998 USA	55-69	10	241 F	34,975 F	Colon cancer	Top third (<7 servings/wk) vs bottom third (≤3 servings/wk) red meat *(includes liver, hamburger, beef stew, beef, venison)*	Age, energy intake, history of rectal colon polyps	1.3 (0.8-2.0) *p trend=0.30*
Singh & Fraser, 1998 USA	≥25y	6	157 M+F	31,894 M+F	Colon cancer	Top third (≥1 time/wk) vs bottom third (never) *(beef, pork)*	Age, sex, BMI, physical activity, family history, smoking, alcohol, aspirin use	1.41 (0.9-2.21) *p trend=0.46*
Pietinen et al, 1999 FINLAND	50-69	8	185 M (smokers)	26,926 M (smokers)	Colorectal cancer	Top fourth (99g/d) vs bottom fourth (35g/d) red meat (beef, pork, lamb)	Age, supplement group, smoking years, BMI, alcohol, education, physical activity, calcium intake	0.8 (0.5-1.2) *p trend=0.74*
Järvinen et al, 2001 FINLAND	Not specified	27-32 (mean not provided)	109 M+F	9850 M+F	Colorectal cancer	Top fourth (>206g/d, M; 134g/d F) vs bottom fourth (<94g/d, M; <61g/d, F) red meat *Classification of red meat not defined*	Age, sex, BMI, occupation, smoking, geographical area, energy intake, consumption of veg, fruit & cereals	1.50 (0.77-2.94) *p trend not given*
Tiemersma et al, 2002 NETHERLANDS	20-59	9	102 M+F	537 M+F	Colorectal cancer	≥5 vs ≤3 times/wk red meat *(fresh beef & pork)*	Age, sex, height, energy intake, alcohol	1.6 (0.9-2.9) *p trend=0.10*

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Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR (95% CI)
Flood et al, 2003 USA	61.9	8.5	487 F	45,009 F	Colorectal cancer	Top fifth (52.2g/1000 kcal) vs bottom fifth (6.1g/1000kcal) red meat *(bacon, beef, hamburger, ham/other lunch meat, hot dogs, liver, pork, sausage, & meat components of beef stew, chilli, salad, spaghetti, veg soup)*	Energy intake, age	1.10 (0.83-1.45) *p trend=0.39*
English et al, 2004[1] AUSTRALIA	27-75	9	451 M+F	36,661 M+F	Colorectal cancer	Top fourth (>126g/d) vs bottom fourth (<57g/d) red meat *(veal or beef schnitzel, roast beef or veal, beef steak, rissoles (meat balls), meatloaf; mixed dishes with beef, roast lamb or lamb chops, mixed dishes with lamb, roast pork or pork chops, & rabbit or other game)*	Age, sex, energy, fat, cereal products, BMI, physical activity	1.4 (1.0-1.9) *p trend=0.20*
Wei et al, 2004 USA	40-75 M 30-55 F	14 M 20 F	1139 M+F	132,887 M+F	Colon cancer	Top fifth (≥5 times/wk) vs bottom fifth (0 times/wk) red meat *(beef, pork, lamb as main dish)*	Age, sex, BMI, physical activity, folate, calcium, alcohol, family history, height, smoking	1.43 (1.00-2.05) *p trend=0.25*
As above USA	As above	As above	339 M+F	132,887 M+F	Rectal cancer	As above	As above	0.90 (0.47-1.75) *p trend=0.55*
Chan et al, 2005 USA	30-55	10	183 F	443 F	Colorectal cancer	>0.5 vs ≤0.5 servings/day red meat *(beef, pork, lamb as main dish)*	Age, BMI, family history, post-menopausal hormone use, previous endoscopy, multivit use, aspirin use, smoking	1.21 (0.85-1.72) *p trend not given*
Chao et al, 2005 USA	50-74	8-9	1197 M+F	14,6943 M+F	Colon cancer	Top fifth (M>800g/wk; F>560g/wk) vs bottom fifth (M≤180g/wk; F≤90g/wk) red meat *(bacon, sausage, hamburgers, cheeseburgers, meatloaf, or casserole with ground beef; beef [steaks, roasts etc, incl. sandwiches], beef stew or pot pie with veg; liver, pork [incl. chops, roast], hot dogs, ham, bologna, salami, lunch meat)*	Age, sex, BMI, energy, fruits, vegetables, high fibre grain foods, education, smoking, physical activity, multivitamin use, aspirin use, alcohol, HRT (women)	1.15 (0.90-1.46) *p trend=0.04*
As above USA	As above	As above	470 M+F	As above	Rectal cancer	As above	As above	1.71 (1.15-2.52) *p trend=0.007*

[1] Significantly increased risk in 2nd quartile (57g/d), RR=1.4 1.1-1.9) and 3rd quartile (86g/d), RR=1.5 (1.1-2.1)

ANNEX 4

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR (95% CI)
Larsson 2005 SWEDEN	40-75	13.9	733 F	60,700 F	Colorectal cancer	Top fourth (≥94g/d) vs bottom fourth (<50 g/d) red meat *(whole beef, chopped meat, minced meat, bacon, hot dogs, ham or other lunch meat, blood pudding, kidney, liver, liver pate)*	Age, BMI, energy, alcohol saturated fat, calcium, folate, fruits, vegetables, whole grain foods, education	1.32 (1.03-1.68) *p trend=0.03*
Norat et al, 2005 EUROPE (10 COUNTRIES)	35-70	4.5	1329 M+F	476,711 M+F	Colorectal cancer	Top fifth (≥80g/d) vs bottom fifth (<10 g/d) red meat *(All fresh, minced, and frozen beef, veal, pork, lamb)*	Age, sex, BMI, energy, fat, height, weight, physical activity, smoking, fibre, alcohol	1.17 (0.92-1.49) *p trend=0.08*
Balder et al, 2006 NETHERLANDS	55-69	9.3	869 M	2,156 M	Colorectal cancer	Top fifth (158g/d) vs bottom fifth (56g/d) total fresh meat *(meat that had not undergone preservation; includes beef, pork, minced meat, chicken, liver, & other meat, i.e. horse & lamb)*	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, veg.	0.82 (0.62-1.08) *p trend=0.15*
As above	As above	As above	666 F	2,215 F	As above	Top fifth (146g/d) vs bottom fifth (56g/d) total fresh meat	As above	1.10 (0.80-1.51) *p trend=0.57*
Oba et al, 2006 JAPAN	≥35	7	111 M	13,783 M	Colon	Top third (56.6g/d) vs bottom third (18.7g/d) red meat *(beef, pork)*	Age, height, BMI, smoking, alcohol, physical activity	1.03 (0.64-1.66) *p trend=0.86*
As above JAPAN	As above	As above	102 F	16,225 F	As above	Top third (42.3g/d) vs bottom third (10.7g/d) red meat *(Meat classification as above)*	As above	0.79 (0.49-1.28) *p trend=0.20*
Sato et al, 2006 JAPAN	40-64	11	474 M+F	41,361 M+F	Colorectal cancer	Top fourth (70.4g/d) vs bottom fourth (40.3g/d) total meat *(beef, pork, ham, sausage, chicken, liver)*	Sex, age, smoking, alcohol, BMI, education, family history, physical activity, intakes of fat, calcium & dietary fibre	1.10 (0.80-1.51) *p trend=0.38*
Cross et al, 2007 USA	50-71	6.8	5107 M+F	440,640 M+F	Colorectal cancer	Top fifth (62.7g/1000kcal) vs bottom fifth (9.8g/1000kcal) red meat *(All types beef, pork, lamb; including bacon, ham, hamburger, hot dogs, liver, pork, sausage, steak. Also included meats added to complex food mixtures such as pizza, chilli, lasagne, & stew)*	Age, sex, education, family history, race, BMI, smoking, physical activity, total energy intake, alcohol, fruit & veg intake	1.24 (1.12-1.36) *p trend<0.001*

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)/meat classification	Adjustments	RR (95% CI)
Kabat et al, 2007 CANADA	40-59	16.4	617 M+F	48,049 M+F	Colorectal cancer	Top fifth (>40.3g/d) v bottom fifth (<14.25g/d) red meat *(beef, pork, ham, bacon, pork-based luncheon meats, veal)*	Age, BMI, menopausal status, HRT, smoking, alcohol, education, physical activity; intakes of energy, fat, fibre, folic acid.	1.12 (0.86-1.46) *p trend=0.66*
Sørensen et al, 2008 DENMARK	50-64	6-10	379 M+F	769 M+F	Colorectal cancer	Per 25g/day red meat *(beef, veal, pork, lamb, offal)*	Intake of poultry, fish, alcohol and fibre; BMI, HRT, smoking; mutually adjusted for fried & processed red meat	1.03 (0.97-1.09) *p trend not given*

Table 7.6: Prospective studies of processed meat and colorectal cancer published after 1996

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR (95% CI)
Kato et al, 1997 USA	34-65	7.1	100 F	14,627	Colorectal cancer	Top fourth vs bottom fourth *(intake in each quartile not provided)* *(ham, sausages)*	Energy intake, age, place at enrolment, education	1.09 (0.59-2.02) *p trend=0.74*
Pietinen et al, 1999 FINLAND	50-69	8	185 M (smokers)	26,926 M (smokers)	Colorectal cancer	Top fourth (122g/d) vs bottom fourth (26g/d) (*Classification of processed meat not defined*)	Age, supplement group, smoking years, BMI, alcohol, education, physical activity, calcium intake	1.2 (0.7-1.8) *p trend=0.78*
Sellers et al, 1998 USA	55-69	10	241 F	34,975 F	Colon cancer	Top third (<1.5 servings/wk) vs bottom third (≤0.5 servings/wk) nitrate meat *(includes bacon, hot dogs, processed meats)*	Age, energy intake, history of rectal colon polyps	1.00 (0.7-1.4) *p trend=0.90*
Knekt et al, 1999 FINLAND	Not specified	18-24y Mean not provided	73 M+F	9912 M+F	Colorectal cancer	Top fourth vs bottom fourth cured meat and sausages *(intake in each quartile not defined)* *(classification of cured meat not defined)*	Age, sex, municipality, smoking	1.84 (0.98-3.47) *p trend not given*
Flood et al, 2003 USA	61.9	8.5	487 F	45,009 F	Colorectal cancer	Top fifth (22.2g/1000 kcal) vs bottom fifth (0.02g/1000 kcal) processed meat *(bacon, ham/lunch meat, hot dogs, sausages)*	Energy, age	1.00 (0.76-1.31) *p trend=0.22*
English et al, 2004 AUSTRALIA	27-75	9	451 M+F	36,661 M+F	Colorectal cancer	Top fourth (>29g/d) vs bottom fourth (<9g/d) processed meat *(salami, sausages, bacon, ham, corned beef, luncheon meats)*	Age, sex, energy, fat, cereal products, BMI, physical activity	1.5 (1.1-2.0) *p trend=0.01*
Wei et al, 2004 USA	40-75y M 30-55y F	14 M 20 F	1139 M+F	132,887 M+F	Colon cancer	Top fifth (≥5 times/wk) vs bottom fifth (0 times/wk) processed meat *(Classification of processed meat not defined)*	Age, sex, BMI, physical activity, folate, calcium, alcohol, family history, height, smoking	1.33 (1.04-1.70) *p trend=0.008*
As above	As above	As above	339 M+F	132,887 M+F	Rectal cancer	As above	As above	0.90 (0.52-1.57) *p trend=0.93*
Chao et al, 2005 USA	50-74	8-9	11,977M+F	147,413 M+F	Colon cancer	Top fifth (M>240g/wk; F>120g/wk) vs bottom fifth (0g/wk) *(bacon, sausage, hot dogs, ham, bologna, salami, lunch meat)*	Age, sex, BMI, energy, fruits, veg, high fibre grain foods, education, smoking, physical activity, multivitamin use, aspirin use, alcohol, HRT (women)	1.13 (0.91-1.41) *p trend=0.02*

Study/year/country	Age baseline (y)	Mean Follow-up	Cases	Non-cases	Outcome	Comparison (median intake or quantile range)	Adjustments	RR (95% CI)
As above	As above	As above	470 M+F	146,943 M+F	Rectal cancer	As above	As above	1.26 (0.86-1.83) *p trend=0.18*
Larsson 2005 SWEDEN	40-75	13.9	733 F	60,700 F	Colorectal cancer	Top fourth (≥32g/d) vs bottom fourth (<12 g/d) processed meat *(bacon, hot dogs, ham, or other lunch meat, blood pudding)*	Age, BMI, energy, alcohol saturated fat, calcium, folate, fruits, vegetables, whole grain foods, education	1.07 (0.85-1.33) *p trend=0.23*
Norat et al, 2005 EUROPE (10 COUNTRIES)	35-70	4.5	1329 M+F	476,711 M+F	Colorectal cancer	Top fifth (≥80g/d) vs bottom fifth (<10g/d) processed meat *(mostly pork & beef preserved by methods other than freezing, e.g. salting, smoking, marinating, air drying, heating: ham, bacon, sausages, blood sausages, meat cuts, liver pate, salami, bologna, tinned meat, luncheon meat, corned beef, and others)*	Age, sex, BMI, energy, fat, height, weight, physical activity, smoking, fibre, alcohol	1.42 (1.09-1.86) *p trend=0.02*
Balder et al, 2006 NETHERLANDS	55-69	9.3	869 M	2,156 M	Colorectal cancer	Top fourth (≥20g/d) vs bottom fourth (0g/d) *(meat items that had undergone some form of preservation, i.e. smoking, fermentation, and/or treatment with nitrate and/or nitrite salt [curing])*	Age, BMI, family history, smoking, physical activity; intakes of energy, alcohol, veg.	1.18 (0.84-1.64) *p trend=0.25*
As above	As above	As above	666 F	2,215 F	As above	As above	As above	1.05 (0.74-1.48) *p trend=0.62*
Oba et al, 2006 JAPAN	≥35	7	111 M	13,783 M	Colon cancer	Top third (20.3g/d) vs bottom third (3.9g/d) processed meat *(ham, sausage, bacon, roasted pork)*	Age, height, BMI, smoking, alcohol, physical activity	1.98 (1.24-3.16) *p trend<0.01*
As above	As above	As above	102 F	16,225 F	As above	Top third (16.3g/d) vs bottom third (3.0g/d) processed meat *(meat classification as above)*	As above	0.85 (0.50-1.43) *p trend=0.62*
Cross et al, 2007 USA	50-71	6.8	5107 M+F	440,640 M+F		Top fifth (22.6g/1000kcal) vs bottom fifth (1.6g/1000kcal) *(bacon, red meat sausage, cold cuts [red & white meat], ham, hot-dogs. Also included meat added to complex food mixtures, e.g. pizza, chilli, lasagne, stew)*	Age, sex, education, family history, race, BMI, smoking, physical activity, total energy intake, alcohol, fruit & veg intake	1.20 (1.09-1.32) *p trend<0.001*
Sørensen et al, 2008-07-03 DENMARK	50-64	6-10	379 M+F	769 M+F	Colorectal cancer	Per 25g/d *(bacon, smoked ham, salami, frankfurter, Cumberland sausage, cold cuts, liver pate*	Intake of poultry, fish, alcohol and fibre; BMI, HRT, smoking; mutually adjusted for fried & processed red meat	0.99 (0.84-1.16) *p trend not given*

Prospective studies of iron and cardiovascular disease

Table 7.7: Total dietary iron

Study/year/country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Salonen et al, 1992 FINLAND	42-60	3	51 M	1880 M	MI	1mg/d increment	Age, BMI, smoking, HDL & LDL cholesterol, family history, blood pressure, diabetes, maximal oxygen uptake, diabetes, number of other risk factors (no other dietary factors)	Yes	1.05 (1.01-1.09)
Liao et al, 1994 USA	25-74	13	633 M	1,194 M	IHD	Top fourth vs bottom fourth (5mg/d increment)	Age, BP, serum cholesterol, education, smoking	Yes	0.74 (0.55-0.99) *p<0.05* (0.97 (0.89-1.06))
As above	As above	As above	518 F	1,892 F	IHD	Top fourth vs bottom fourth (5mg/d increment)	As above	Yes	0.84 (0.62-1.15) *p<0.05* (0.91 (0.80-1.03))
Ascherio et al, 1994 USA	40-75	4	386 M	44,089 M	Coronary disease	Top fifth (37mg/d) vs bottom fifth (11mg/d)	Age, energy, BMI, smoking, alcohol intake, hypertension, diabetes, hypercholesterolemia, family history, profession; quintiles of intake of: vit E, total iron, haem iron, sat fat & cholesterol	Yes	0.73 (0.51-1.06) *p trend = 0.03*
Morrison et al, 1994 CANADA	35-79	15-17	? M+F	9920 M+F	MI	Not reported	Age, smoking, hypertension, serum cholesterol, diabetes	*Yes*	No association (RR not reported)
Gartside & Glueck, 1995 USA	25-74	10	492 M+F	7,759 M+F	CHD	Top third (≥13.1 mg/d) vs bottom third (<8.4 mg/d)	Sex, physical activity, weight, alcohol, riboflavin intake, serum Mg	*Yes*	0.83 (0.66-1.03) p=0.097
Reunanen et al, 1995 FINLAND	45-64	13.8	984 M+F	11,204 M+F	CHD deaths	Top fifth vs bottom fifth	Age, serum cholesterol, hypertension, diabetes, obesity	Yes	No association (RR not reported)

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Study/year/country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Klipstein-Grobusch et al, 1999 NETHERLANDS	≥55	3-7	124 M+F	4,678 M+F	MI	Top third (14.3 mg/d) vs bottom third (9.3 mg/d)	Age, sex, BMI, smoking, household income, education, alcohol; intakes of: ß-carotene, vit C & E, total fat, fat, sat fat, cholesterol; antioxidant vit supps	Yes	1.11 (0.67-1.87) *p trend = 0.787*
van der A et al, 2005 NETHERLANDS	49-70	4.3	252 F	15,884 F	CHD	Top fourth (>11.43mg/d) vs bottom fourth (<9.56mg/d) per mg/day	Age, energy intake, BMI, smoking, physical activity, hypertension, hypercholesterolemia, sat fat, carbohydrate, fibre, alcohol, ß-carotene, vit E, vit C	Yes	0.98 (0.61-1.58) *p trend = 0.878* 0.92 (0.79-1.06)
Qi et al, 2007 USA	30-55	20	550 F (with type 2 diabetes)	5,611 F (with type 2 diabetes)	CHD	Top fifth vs bottom fifth (intake in quintiles not reported)	Age, BMI, smoking, smoking, alcohol, physical activity, diabetes; hypertension, hypercholesterolemia, HRT, CHD history, fibre, glycaemic load, polyunsat/sat fat ratio, trans fat, multivits, vit C	Yes	1.32 (0.95-1.84) *p trend = 0.04*

Table 7.8: Dietary haem iron

Study/Year/Country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Ascherio et al, 1994 USA FFQ	40-75	4	386 M	44,089 M	MI	Top fifth (2.1mg/d) vs bottom fifth (0.7mg/d)	Age, energy, BMI, smoking, alcohol intake, hypertension, diabetes, hypercholesterolemia, family history, profession; quintiles of intake of: vit E, total iron, haem iron, sat fat & cholesterol	Yes	1.48 (1.01-2.16) *p=0.03*
Klipstein-Grobusch et al, 1999 NETHERLANDS (semi-quantitative FFQ)	≥55	3-7	124 M+F	4,678 M+F	MI	Top third (1.36 mg/d) vs bottom third (0.48 mg/d)	Age, sex, BMI, smoking, household income, education, alcohol; intakes of: ß-carotene, vit C & E, total fat, sat fat, cholesterol; antioxidant vit supps	Yes	1.86 (1.14-3.09) *p trend = 0.01*
Lee et al, 2005 USA (FFQ)	55-69	15	1767 F	32,725 F	CVD deaths	Top fourth (2.43mg*) vs bottom fourth (0.57mg/day)	Age, energy intake, BMI, WHR, physical activity, smoking, alcohol, HRT, BP, sat fat, trans fat, polyunsat fat, folate, ß-carotene, vits E & C, non-haem iron, zinc	Yes	0.94 (0.71-1.26)** *p trend = 0.82*
van der A et al, 2005 NETHERLANDS (FFQ/supp intake included)	49-70	4.3	252 F	15,884 F	CHD	Top fourth (>2.27mg/d) vs bottom fourth (<1.28mg/d) per mg/day	Age, energy intake, BMI, smoking, physical activity, hypertension, hypercholesterolemia, sat fat, carbohydrate, fibre, alcohol, ß-carotene, vit E, vit C	Yes	1.65 (1.07-2.53) *p trend = 0.019* 1.15 (0.95-1.40)
Qi et al, 2007 USA (FFQs)	30-55	20	550 F (with type 2 diabetes)	5,611 F (with type 2 diabetes)	CHD	Top fifth (2.83 mg/d) vs bottom fifth (1.70mg/d)	Age, BMI, smoking, smoking, alcohol, physical activity, diabetes; hypertension, hypercholesterolemia, HRT, CHD history, fibre, glycaemic load, polyunsat/sat fat ratio, trans fat, multivits, vit C	Yes	1.43 (1.01-2.01) *p trend = 0.01*

upper half of top quartile

*** alcohol consumption 0-9 g/d; RR for alcohol consumption ≥10g/day = 2.47 (1.10-5.55), p trend=0.04*

Table 7.9: Serum ferritin

Study/year/country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Frey & Krider, 1994 USA	42-60	5.2	32 M	266 M	MI	Difference in mean SF concentrations between cases & controls	Age	No	No association (RR not reported)
Salonen et al, 1994 FINLAND	42-60	5	83 M	1848 M	MI	≥ 200 µg/L vs < 200 µg/L	Age, markers of chronic inflammatory disease, CVD, pulmonary function; socioeconomic status, diabetes, family history of CVD, smoking, blood leucocyte count	Yes	2.0 (1.2-3.1) *p=0.004*
Magnusson et al, 1994 ICELAND	25-74	8.5	81 M+F	1,955 M+F	MI	1 µg/L increment	Age, BP, HDL cholesterol, total cholesterol, smoking	No	0.999 (0.998-1.001) *p=0.23*
Manttari et al, 1994 FINLAND	40-55	5	134 M (with lipid abnormalities)	268 M (with lipid abnormalities)	CHD	≥85 µg/L vs ≤42 µg/L	Age, BP, cholesterol, HDL-cholesterol, smoking	No	0.78 (0.39-1.54) *p trend=0.5*
Aronow & Ahn, 1996 USA	62-100	3	235 M+F	342 M+F	CHD	M: >282 µg/L vs <282 µg/L F: >219 µg/L vs <219 µg/L	Age, sex, prior CHD	No	1.0 (0.998-1.001) *p=0.61*
Kiechl et al, 1997 ITALY	40-79	5	401 M+F	425 M+F	Artherosclerosis	1 SD increment (approx 166 µg/L)	Age, sex, baseline vascular status, alcohol	No	1.50 (CI not reported) *p=0.0002*
Marniemi et al, 1998 FINLAND	≥65	13	142 M+F	119 M+F	CVD deaths	Top third vs bottom third *(amount in each tertile not reported)*	Age, sex, smoking, alcohol, BMI, CHD, hypertension, diabetes, serum cholesterol, HDL cholesterol, triglycerides	No	0.65 (0.42-1.01) *p not reported*
Klipstein-Grobusch, 1999 NETHERLANDS	≥55	4	60 M+F	112 M+F	MI	>171 µg/L vs <77 µg/L	Age, sex, BMI, smoking, income, alcohol	Yes	1.28 (0.98-1.67) *p trend=0.066*
Sempos et al, 2000 USA	45-74	12-16	254 M	404 M	CVD deaths	≥ 200 µg/L vs < 50 µg/L	Age, anaemia, BP, hypertension, serum total cholesterol, smoking, diabetes, chronic conditions	Yes	0.7 (0.4-1.3) *p trend=0.22*
As above	As above	As above	168 F	550 F	As above	≥ 200 µg/L vs < 50 µg/L	As above	Yes	0.9 (0.4-2.1) *p trend = 0.92*
Fox et al, 2002 AUSTRALIA	20-79	3-4	235 M+F	1,796 M+F	CVD	> 300 µg/L vs ≤ 300 µg/L	Age, sex, BMI, BP, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb	Yes	1.02 (0.69-1.50) *p not reported*

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Study/year/country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Comparison	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Knuiman et al, 2003 AUSTRALIA	40-89	17	217 M+F	450 M+F	CHD	Top third (M, >233 µg/L; F, >122 µg/L) vs bottom third (M, ≤126 µg/L; F, ≤49 µg/L)	Age, sex, BMI, BP, diabetes, total cholesterol, HDL cholesterol, smoking, Hb,	Yes	0.96 (0.60-1.5) *p not reported*
van der A et al, 2005 NETHERLANDS	49-70	4.3	63 F	1134 F	Stroke	195 µg/L vs 51.8 µg/L <200 vs ≥200 µg/L	Age, BMI, alcohol, CRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL & HDL cholesterol	Yes	1.45 (0.87-2.42) *p trend=0.158* 1.77 (1.03-3.05)
van der A et al, 2006 NETHERLANDS	49-70	4.3	185 F	1134 f	CHD	137 µg/L VS <75.7 µg/L <200 vs ≥200 µg/L	Age, BMI, alcohol, CRP, smoking, hypertension, hypercholesterolemia, diabetes, glucose, LDL & HDL cholesterol	yES	0.55 (0.23-1.31) *p trend=0.142* 0.82 (0.35-1.95)
Galan et al, 2006 FRANCE	35-60	7.5	148 M	3,075 M	IHD	>160 µg/L vs <30 µg/L	Age, smoking, BMI, total cholesterol, serum triglycerides	Yes	1.31 (0.52-3.27) *p not reported*
As above	As above	As above	39 F	6,655 F	As above	As above	As above + menopausal status	Yes	2.18 (0.64-7.43) *p not reported*
Ekblom et al, 2007 SWEDEN	25-74	?	126 M+F	304 M+F	Ischemic stroke	Top fourth vs bottom fourth *(SF concentration in quartiles not reported)*	BMI, hypertension, smoking, diabetes, cholesterol, CRP, HFE C282Y & H63D	Yes	0.80 (0.46-1.40) *p=0.250*
As above	As above	As above	27 M+F	As above	Hemorrhagic stroke	As above	As above	As above	1.07 (0.17-6.94) *p=0.576*

Prospective studies of heterozygosity for hereditary haemochromatosis and CVD

Table 7.10: C282Y heterozygosity and CVD

Study/year/country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Tuomainen et al, 1999 FINLAND	42-60		68 M	1082 M	MI	13 risk factors incl. age, WHR, HDL & VLDL cholesterol, socioeconomic status, BP, smoking	Yes	2.21 (1.05-4.67) *p=0.04*
Roest et al, 1999 NETHERLANDS	51-69	17	531 F	551 F	CVD deaths	Age, smoking, hypertension, obesity	No	1.6 (1.1-2.4) *p=0.028*
Rasmussen et al, 2001 USA	45-64	2.9	243 F	535 F	CHD	No adjustments	Yes	1.6 (0.88-2.91) *p value not reported*
Fox et al, 2002 AUSTRALIA	20-79	3-4	235 M+F	1796 M+F	CVD	Age, sex, BMI, BP, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb	Yes	0.96 (0.65-1.42) *p value not reported*
Gunn et al, 2004 UK	45-64	4.9	482 M	1104 M		BMI, BP, LDL & HDL cholesterol white cell count, fibrinogen, CRP	No	0.87 (0.63-1.19) *p value not reported*
Ellervik et al, 2005 DENMARK	20-80	24	1035 M+F	8080 M+F	IHD	Sex, smoking, cholesterol, triglycerides, lipoprotein (a), diabetes, hypertension, BMI, fibrinogen, HRT, menopausal status	Yes	(0.9-1.4) *p value not reported*
van der A et al, 2006 NETHERLANDS	49-70	4.3	211 F	1526 F	CHD	Age, BMI, smoking, hypertension, alcohol, diabetes, cholesterol, CRP	Yes	1.25 (0.74-2.09) *p value not reported*
Ekblom et al, 2007 SWEDEN	25-74	?	231 M+F	550 M+F	Ischemic stroke	Age, sex	Yes	0.74 (0.41-1.32) *p=0.332*
As above	As above		41 M+F	As above	Hemorrhagic stroke	As above	Yes	1.34 (0.46-3.94) *p=0.352*

Table 7.11: H63D heterozygosity and CVD

Study/Year/Country	Age baseline (y)	Mean Follow-up (y)	Cases	Non-cases	Outcome	Adjustments	Exclusion of chronic disease at baseline	RR (95% CI)
Fox et al, 2002 AUSTRALIA	20-79	3-4	235 M+F	1796 M+F	CVD	Age, sex, BMI, BP, diabetes, total cholesterol, HDL cholesterol, smoking, alcohol, Hb	Yes	0.98 (0.72-1.34) *p value not reported*
Ellervik et al, 2005 DENMARK	20-80	24	1035 M+F	8080 M+F	IHD	Sex, smoking, cholesterol, triglycerides, lipoprotein (a), diabetes, hypertension, BMI, fibrinogen, HRT, menopausal status	Yes	1.2 (1.0-1.4) *p values not reported*
van der A et al, 2006 NETHERLANDS	49-70	4.3	211 F	1526 F	CHD	Age, BMI, smoking, hypertension, alcohol, diabetes, cholesterol, CRP	Yes	0.73 (0.43-1.24) *p value not reported*
Ekblom et al, 2007 SWEDEN	25-74	?	231 M+F	550 M+F	Ischemic stroke	Age, sex	Yes	0.74 (0.49-1.12) *p=0.363*
As above	As above	?	41 M+F	As above	Hemorrhagic stroke	As above	Yes	1.51 (0.69-3.27) *p=0.577*

Annex 5

Consideration of possible mechanisms to explain the association between meat and colorectal cancer risk

ANNEX 5

Consideration of possible mechanisms to explain the association between colorectal cancer risk and red and processed meat intake[2]

Background

1. Epidemiology data suggest that consumption of red and/or processed meat is associated with increased risk of colorectal cancer (CRC); the data do not suggest that consumption of white meat or fish is associated with an increased CRC risk. The definition of processed meat varies between studies, but is defined by the World Cancer Research Fund (WCRF) as meat that has been preserved by curing, smoking, salting or the addition of chemical preservatives.

2. This paper considers whether the difference in risk between red, processed, and white meat can be explained by the presence of cooked food mutagens, haem iron, or preservatives. It also considers whether for modelling purposes (see Annex 7) red meat can be separated from processed meat based on the presence of preservatives in processed meat. More detailed background briefings considering the evidence for the association of cancer with food mutagens, haem iron, and preservatives are attached as Appendices. As many of the proposed mechanisms involve the formation of nitrosamines and N-nitroso compounds, background data on these are also considered.

<u>Differences in the risks of CRC associated with red, white and processed meat consumption on the basis of cooked food mutagens, haem iron or preservatives</u>

Cooked food mutagens – Heterocyclic amines

3. Heterocyclic amines (HAs) are formed from creatine and therefore are predominantly found in the muscle parts of meat and fish. The most common HA ingested is PhiP[3] which is more common in chicken than other meats. Other heterocyclic amines can also be formed in all meat types. Their formation is influenced by cooking temperature, cooking time and cooking method, and some mutagens are more likely to occur in certain types of meat or as a result of particular procedures, eg grilled steak, or pan fried burgers. Where various meat types and products have been analysed, foods treated with preservatives such as ham or sausages tend to have lower levels of HAs, possibly due to the cooking methods used.

4. In the modelling exercise (see Annex 7), processed meat is defined as meat that has been treated with preservatives excluding salt and including some cured and smoked meat cuts. However it is possible that mechanically processed (eg minced) meat or burgers could form more mutagens due to the greater surface area being exposed directly to heat, but would be classed as unprocessed in this instance since no chemical preservatives have been used. This could affect the interpretation and comparison of the epidemiological data.

5. Although HAs are genotoxins and known animal carcinogens, the data suggesting a role in human CRC is unconvincing.

Cooked food mutagens- polycyclic aromatic hydrocarbons

6. The other main class of cooked food mutagens are polycyclic aromatic hydrocarbons (PAHs) and these are formed by incomplete pyrolysis of organic compounds. It is possible that people are more likely to char red meat and therefore ingest these mutagens, compared to other meats (including processed meats in general or meats treated with preservative) but the data are not there to support or refute this possibility. PAHs are widespread in foods and meat is not the major contributor to total dietary exposure. Regular consumers of barbecued foods may have higher intakes of PAHs but it is uncertain whether this would be due to the preferential consumption of a particular meat type. The role of PAH intake in the association of red meat consumption and cancer is unconvincing.

Haem iron

[2] Prepared by Chemical Risk Assessment Unit of Food Standards Agency, January 2009
[3] 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

7. The haemoglobin content of red meat is significantly higher than that of white meat or fish. However, the majority of red meats treated with preservatives are made from pork and would have a lower haem level than other red meats.

Preservatives

8. There are no differences in the preservatives permitted for use in red and white meat. However, meat treated with preservatives is most likely to be red (bacon, ham, burgers etc). The epidemiology data suggest that processed meat (as defined by WCRF) is associated with a higher risk of CRC than red meat. Although it is sometimes claimed that this is due to the use of nitrates and nitrites as preservatives, and the possible formation of nitrosamine, the data do not support this. It should be noted that while preservatives are permitted in processed meats, not all processed meats will contain them.

Conclusion

9. None of the above appears to explain the epidemiological findings that red meat is associated with a higher risk of colorectal cancer than white meat or fish.

Can unprocessed red meats and meats treated with preservatives be separated on the basis of risks of cooked food mutagens?

10. There are no data comparing red meats and meats treated with preservatives directly; however it is possible some foods considered to be processed (e.g. mixed products such as sausage) are less likely to form mutagens. It is possible that one of the meat categories might be preferentially cooked in ways that form particular mutagens, but no data have been identified that support this.

11. Processed meat is usually defined as meat that has been treated with preservatives. However it is possible that mechanically processed (e.g. minced) meat or burgers could form more mutagens due to the greater surface area being exposed to heat, but would be classed as unprocessed. This could affect the interpretation and comparison of the epidemiological data.

Conclusion

12. The available data support different risks due to the presence of cooked food mutagens in red or processed meats. However, the data are limited, and a direct comparison has not been done.

Are there different risks associated with unprocessed red meats and meats treated with preservatives due to the effect of haem iron?

13. It has been proposed that the presence of haem iron enhances nitrosation. There is evidence that supports and contradicts this suggestion. However, intake of haem iron is likely to be higher from unprocessed meats than meats treated with preservatives, since these tend to be pork rather than beef based and thus contain lower levels of haem. Thus this does not explain the larger increase in CRC associated with processed meats (as defined by WCRF), unless there is some other interaction occurring, e.g. directly with nitrate preservatives. This latter possibility has not been investigated.

Conclusion

14. The available data do not support different risks associated with red meat or meats treated with preservatives due to haem iron. However, the data are limited, and a direct comparison has not been done.

Are the different risks of colorectal cancer associated with unprocessed red meats and meats treated with preservatives due to the effect of preservative use?

15. The preservatives permitted for use in meat are listed in appendix 1. The main preservatives used are ascorbic acid, benzoates and sorbates (which have limited use), nitrates and nitrites which are used in cured meats such as bacon and ham, though not all cured meats will contain these preservatives. Some of the preservatives permitted for use in meats are also permitted for use in other food products. There are

many sources of dietary nitrate, with green leafy vegetables being a particularly rich one. Nitrate and nitrite are also produced endogenously in the body as a result of intermediary metabolism.

16. Nitrosamines can be formed from nitrate via nitrite production. Following dietary exposure to nitrate, the levels of nitrosamines formed are low, and vary between individuals, depending on nutritional and disease status. Nitrosamines have been measured directly in bacon and other cured, smoked or salted meats as well as malted beverages. Ascorbate is used in bacon to reduce nitrosamine formation. It has been suggested that the increased risk of stomach cancer associated with consumption of smoked preserved fish may be due to nitrosamine formation, but other factors such as PAHs and salt levels may also be involved. Evidence linking nitrosamines to CRC is much weaker. There is no clear evidence linking specific food types preserved with nitrates or nitrites, such as bacon or ham, with cancer. Nitrates and nitrites used in food as preservatives are no more likely to form nitrosamines than other sources of dietary nitrate and noted that more nitrate is ingested from sources such as vegetables than from processed meats. Similarly, nitrites are produced through endogenous conversion of nitrate at a much greater level than arises from the consumption of dietary nitrite.

Conclusion

17. None of the available data support an increased risk of colorectal cancer associated with processed meats due to preservative use.

Overall conclusion

18. Other issues may be relevant to this assessment. These could include differences in potency of particular mutagens, or a combination of differing effects resulting in the findings seen in the epidemiology studies, but not in the individual mechanistic studies.

19. Current understanding of cooked food mutagens, preservatives or haem iron do not provide a basis for separating red meat and meat treated with preservatives for modelling purposes.

ANNEX 5

Appendix 1

Cooked food mutagens and cancer

1. Genotoxic carcinogens are formed during the heating of meat. All meats and meat products show some level of mutagenic activity following cooking. There are two main classes of mutagens in cooked meat: heterocyclic amines and polycyclic aromatic hydrocarbons.

Heterocyclic amines:

2. Heterocyclic amines (HAs) are referred to as 'thermic mutagens', which form at temperatures below 300°C, or 'pyrolytic mutagens', which form at higher temperatures (Alajeos *et al*, 2008). More than 20 HAs have been isolated; the principal HAs are 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).

Formation of HAs

3. HAs are formed from natural constituents in foods via the Maillard reaction during heating (Skog, 1993). Creatine, sugars, and amino acids (phenylalanine, threonine, alanine) are all precursors in their formation. Mutagenic activity is largely found in the crust of cooked meat and fish, with negligible levels being reported in the inner parts.

4. The factors that influence the formation of mutagenic activity are cooking temperature, cooking time, and cooking method (Skog, 1993). Cooking temperature appears to be the most important factor: the mutagenic activity of cooked meats increases with increasing cooking temperature as well as with increased cooking time. Cooking methods influence mutagenic activity in terms of the temperature achieved. Cooking at temperatures of 200°C and above, such as contact frying, deep-fat frying, barbecuing, and broiling, give much higher levels of mutagenic activity than other cooking methods such as oven roasting, stewing, boiling and microwave cooking. Meats cooked at fast-food restaurants have been shown to contain lower levels of mutagens due to the short cooking times that do not allow HAs to form (Knize *et al*, 1995). The degree of "doneness" (closely related to cooking time and surface browning) is important to the formation of HAs. In addition, the greater the external charring of the meat (determined by cooking method), the higher the concentration of HAs.

5. The formation of HAs is also influenced by the amounts of the different precursors: fat, amino acid, glucose, and creatine. Mutagenic activity is primarily found in the crust of cooked meat and fish, as well as in the pan residue. The formation of a crust is the result of the steady transportation of water and dissolved compounds to the surface by capillary flow, so that the precursors of HAs are present at or near the surface of the meat. Generally, levels of HAs are higher in cooked meats than in fish, and in pure meat than in mixed meat products, such as sausage.

6. The definition of processed meat is unclear. In principle, mincing of meat is a process, and if this results in a larger surface area for direct contact with heat during cooking (e.g. in burgers compared to steak), the HA content could be higher. However there is no basis for assuming a difference in the HA content of, for example, burgers containing preservatives and those that do not. For the purposes of the modelling in the SACN report (see Annex 7), processed meat is considered to be meat that has been treated with preservatives, excluding salt but including some cured meats. Meat which, for example, has been mechanically processed into burgers but that has not been treated with preservatives would be classed as unprocessed.

7. The mutagens IQ, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP have all been found in cooked fish, chicken, pork and beef. PhIP is the most abundant mutagen in beef, chicken, pork, mutton, and fish, followed by MeIQx. The mutagen PhIP seems to form more easily in chicken than in beef, pork or fish; whereas MeIQx formation is lower in cooked chicken than in cooked beef and pork. In a population-based study quantifying HAs in a range of meats (beef steak, beef hamburger, pork loin, pork sausages, chicken breast, and lamb steak) cooked by a variety of methods (pan-frying, griddling, coating-frying, and roasting), pan-fried chicken breast was the main source of HAs and PhIP was estimated to be the most consumed HA (Busquets *et al.*,

2009). Using data from cooking method questionnaires and food frequency questionnaires, Wu *et al.* (2006) examined the associations between HA and meat intake. PhIP was by far the predominant HA, found primarily in broiled and grilled chicken and grilled steak. MeIQx and DiMeIQx were found at much lower levels than PhIP in all meats, with the highest levels found in pan-fried hamburger (MeIQx) and grilled chicken (DiMeIQx) (Wu *et al*., 2006). These findings are in agreement with those of Martinez *et al.* (2007) who found PhIP to predominate in grilled chicken and grilled steak. MeIQx was found predominantly in grilled and pan-fried hamburgers followed by grilled steak; and DiMeIQx was found predominantly in grilled hamburgers and grilled and pan-fried pork chops, followed by grilled chicken (Martinez *et al*., 2007).

8. The levels of HAs in meat products such as sausages and ham made from pork and probably treated with preservatives (though this is not stated) are lower than found in pan fried beef or chicken. Levels in hot dogs or ham slices were low or undetectable, HA levels in sausage links or patties increased with doneness (Sinha *et al*, 1998).

9. The content of each IQ compound has been estimated to be less than 20 ng/g in fried beef and fish (summarised Skog, 1995). PhIP is present in fried, ground beef at 48.5 ng/g and 73 ng/g in barbecued salmon. PhIP levels in pork products are lower being 4.8 ng/g and 30.3 ng/g in very well done pan fried and oven-broiled bacon respectively. PhiP levels in cooked chicken have been reported to be approximately 20 ng/g, reaching 70 ng/g in pan-fried, skinless, chicken breasts (Skog and Solyakov, 2002).

Dietary exposure to HAs

10. Assessing exposure to HAs is complex because of the high degree of variability from different cooking methods and preferences. Estimated potential intakes of total and individual HAs have been summarised by Alaejos *et al* (2008). The limited data and high level of uncertainty in these estimates is noted.

HA	Intake ng/day	Geographical location
Phip	5-300	Japan
	72	Sweden
	158.3	US
MeIQx	300-390	Japan
	72	Sweden
	52.1	US
DiMeIQx	16	Sweden
	3.5	US
Total HAs	8.53	Sweden
	330	Switzerland
	455	US

Adapted from Alajeos *et al*, 2008.

Metabolism and mutagenicity of heterocyclic amines

11. HAs are genotoxic in bacterial systems and mammalian cells following metabolic activation (Alaejos *et al*, 2008). The major pathway for metabolic activation starts with the N-hydroxylation of the exocyclic amino group, mainly catalysed by cytochrome P450 1A2 (CYP1A2), these metabolites may directly react with DNA, but this step is generally followed by sulfation or acetylation by means of sulfotransferase 1A1 or N-acetyltransferases.

12. The carcinogenic potency of HAs is dependent on the balance of their metabolic activation and detoxication by carbon oxidation, glucuronidation and sulfation at sites other than at the hydroxylamine (Gooderham *et al*, 2001) Variability in these enzymes between species and between humans will therefore influence the carcinogenic potency. Non mutagenic HAs may enhance the mutagenicity of the mutagenic ones.

Carcinogenicity in animals

13. HAs have been demonstrated to be carcinogenic in rats, mice and monkeys (reviewed Skog, 1993). Amino acid pyrolysates have been shown to induce primarily tumours of the liver and blood vessels in mice and tumours of the liver and intestines of rats. The thermic HAs (IQ, MeIQ or MeIQx) (see table below) also produced mainly liver tumours but tumours of the lung, forestomach, lymphoid tissues and haemopoietic system were observed in mice and tumours of the intestines, skin, Zymbal gland, mammary gland, clitoral gland and skin were reported in rats. PhIP has been reported to produce lymphomas in mice and intestinal, colon and mammary gland tumours in rats. IQ has also been shown to produce liver tumours in monkeys.

Compound	**Species**	**Conc in diet (%)**	**Target organs**
IQ	Mice	0.03	Liver, forestomach, lung
	Rats	0.03	Liver, small and large intestines, Zymbal gland, clitoral gland, skin.
MeIQ	Mice	0.04,0.01	Liver, forestomach
	Rats	0.04, 0.01	Large intestine, Zymbal gland, skin, oral cavity, mammary gland,.
MeIQx	Mice	0.06	Liver, lung, haemopoietic system
	Rats	0.04	Liver, Zymbal gland, clitoral gland, skin.
PhiP	Mice	0.04	Lymphoid tissues
	Rats	0.01-0.04	Intestines
	Rats	0.04	Colon, mammary gland

(adapted from Skog, 1993)

14. Thus with the exception of liver tumours, a slightly different pattern of tumour occurrence is seen in different laboratory animals, suggesting that the tumours observed in humans could also differ. In general it cannot be assumed that there is concordance between tumour sites in animal studies and in humans. The dietary concentrations of HAs used in feeding studies are in the range 0.06% to 0.1% (600 to 1000 mg/kg diet) which is significantly higher than estimated human intakes. Using standard default values, this is equivalent to doses of 30 to 50 mg/kw bw/day in older rats, compared to 7.5 ng/kg bw/day (assuming an intake of 450 ng total HAs by an average 60 kg adult; a dose over 4 million times higher).

Studies in humans

15. As noted above, HAs have to be activated by various enzymes, including cytochrome P450 (CYP) 1A2 and N-acetyltransferase-2 (NAT-2) before they can form DNA adducts. Enzyme activities vary between individuals, suggesting that some individuals could be more susceptible to colorectal cancer, due to enhanced enzyme activation.

16. A study on HAs found that in cancer patients given trace amounts of HAs prior to surgery, more PhiP than MeIQx was bound to DNA in the colon (Garner, 2004), suggesting that PhiP might be more important in the carcinogenic process than MeIQx. There was a 100 fold difference in binding between individuals, but there was no correlation between age, sex, site or severity of the cancer and the amount of HA binding to DNA. In a large study of colo-rectal cancer patients and controls, there was no difference in CYP1A2 and NAT-2 activity between cases and controls as assessed by measurement of urinary caffeine metabolism. CYP1B1 can also mediate the formation of N-hydroxy species and an association was found between a specific CYP1B1 genotype and higher levels of DNA adducts. However as CYP1B1 expression was not associated with adduct formation, the significance of this observation with regard to increased individual risk was uncertain (Garner, 2004).

17. On the basis of small trials, it had previously been suggested that “fast” NAT2 acetylators showed a greater association between HA intake and risk of CRC than did slow acetylators. However, Barrett *et al* (2003) found no difference in the number of fast acetylators in CRC patients compared to matched controls, suggesting that “fast” acetylators were not at increased risk of CRC even when exposed to high levels of HAs from well cooked meat. In addition, high HA exposure from well cooked meat (as assessed by self reported cooking preferences) was not associated with an increased risk of CRC.

18. No difference was detected in the mutation frequency of the *hprt* gene in the DNA of white blood cells of 10 vegetarians compared to 14 meat eaters as assessed by comparison of cloning efficiency into selective and non selective media (Gooderham and Boobis, 2004). However, it was noted that this finding could be due to small study size, young age of the volunteers or use of the wrong marker for DNA damage. In this study, NAT-2 and CYP1A2, enzymes involved in HA activation, were more active in healthy volunteers compared to CRC patients, again indicating that the role of HAs in the risk of CRC is uncertain.

Risk characterisation

19. HAs are known to be genotoxic *in vitro* and are able to produce tumours in laboratory animals. However, in humans it is not possible to link the increased risk associated with red meat consumption to HA intake, since the evidence of carcinogenicity of HAs in humans is not convincing.

20. Comparison of the estimated human dietary exposure with the doses producing tumours in animal studies indicates that human exposure is at least 4 million times less than used experimentally (30 mg/kg bw/day compared to 7.5 ng/kg bw/day) see above.

Polycyclic aromatic hydrocarbons:

Formation of polycyclic aromatic hydrocarbons

21. Polycyclic aromatic hydrocarbons (PAHs) are formed by the incomplete combustion (pyrolysis) of organic compounds. They are formed whenever fossil fuels or vegetation is burned and can be present in a wide variety of cooked and processed foods, including meats, cereal products, oils and fats, fruits, vegetables, and sugars, as a result of environmental contamination or formation during drying or cooking. The two highest contributors to dietary PAH exposure are cereals & cereal products and seafood & seafood products. PaHs are found in smoked meat products and grilled or barbecued meats but these contribute only a small amount to total dietary exposure to PAHs. Benzo(*a*)pyrene (BaP) is the most widely studied and measured PAH.

22. Like HAs, PAH formation in food is dependent on the method of cooking, particularly, the distance of the food from the heat source. When meat is in direct contact with a flame, pyrolysis of the fats in the meat generates PAHs that become deposited on the meat. Even if the meat is not in direct contact with a flame, PAHs can be formed when fat/meat juices drip onto a hot fire/flame. This then yields flames containing PAHs which are carried back to the meat and adhere to its surface. Increased fat content increases PAH formation. As with HAs, the content of PAHs could be higher in meat with a larger surface area in direct contact with the heat.

23. The cooking methods that give rise to the highest amounts of PAHs are barbecuing and grilling, especially when the meat is charred. Charred meat of any type will contain PAHs. PAH levels increase with increasing cooking temperature. Using questionnaires to determine meat intake and preparation methods, Martinez *et al.* (2007) estimated BaP intake from meat and meat sources to be 30.89 ng/day with the largest contributions coming from grilled steak (20.64 ng/day or 67% of intake), followed by grilled chicken (7.47 ng/day or 24% of intake). Pan fried bacon and sausage were estimated to provide 0.06 and 0.01 ng/day BaP respectively

24. No direct comparison has been done, but as with HAs, there is no basis for assuming a difference in the PAH content of burgers or other meat products containing preservatives and those that do not, since cooking procedures appear to be the most important determinant of PAH content.

25. In a study by White and colleagues (EFSA, 2008) only 3/77 retail samples contained detectable levels of BaP. In experiments to mimic home cooking practices, little evidence of BaP production was apparent. However, barbequing beef burgers with charcoal plus woodchips gave the highest levels of PAH increasing with closeness to the heat source. In contrast, for sausages cooked over briquettes and beef burgers, beef and salmon cooked over charcoal PAHs decreased closer to the heat source. Increased cooking time led to a moderate increase in PAHs in some foods but in beef burgers levels appeared to fall.

26. PAHs are also formed during the curing and processing of meats that use smoking as a preservation method: smoke containing PAHs is deposited on the surface of meats.

Dietary exposure to PAHs

27. Mean dietary exposure to BaP in EU countries for which data are available is 235 ng/day (3.9 ng/kg bw for a 60kg adult) (EFSA, 2008). For a group of 8 defined PAHs, dietary exposure is 1729 ng/day (28.8 ng/kg bw for a 60kg adult) and 3078 ng/day (51.3 ng/kg bw) for mean and high level consumers respectively. For individual food groups, mean exposure is as below:

Category	**Consumption median g/day**	**BaP (ng/day)**	**PAH8 (ng/day)**
Cereals & cereal products	257	67	393
Sugar & sugar products (inc chocolate)	43	5	39
Fats (vegetable and animal)	38	26	239
Vegetables, nuts and pulses	194	50	378
Fruits	153	5	87
Coffee, tea, cocoa (expressed as liquid)	601	21	156
Alcoholic beverages	413	4	74
Meat & meat products	**132**	**42**	**279**
Seafood & seafood products	27	36	421
Fish & fishery products	41	21	210
Cheese	42	6	30

28. It is apparent that the cereals, seafood, fish and vegetable groups contribute more to dietary PAH exposure than do meat and meat products. However, regular consumers of home barbecued food could be exposed to higher levels of PAHs.

Carcinogenicity of PAHs in animals and humans

29. PAHs are carcinogenic by the oral, dermal and inhalation routes of exposure (SCF, 2002; EFSA 2008) and the tumours are generally related to these routes, so that gastric tumours occur after oral administration, and skin tumours after dermal application. However, studies of BaP and coal tar mixes have also been reported to produce tumours of the liver, lung, mammary gland, kidney and auditory canal.

30. Studies with PAH-containing coal tar mixes suggest that different PAHs produce different tumour profiles. Few individual PAHs other than BaP have been tested by the oral route, however, dibenz[*a,h*]anthracene and benz[*a*]anthracene produced tumours of the gastrointestinal tract, lungs and liver in mice (SCF, 2002).

31. Based on evidence from occupational exposure and studies in laboratory animals, many PAHs including BaP are classified as known or probable human carcinogens (IARC, 2006). However, there are few human studies of associations between cancer and dietary exposure to PAHs, the majority of studies relate to occupational and environmental exposure (EFSA, 2008).

32. In a small case-control study, consumption of wine from bottles impregnated with tar increased the risk of stomach cancer, but this was only statistically significant for men consuming more than 2L of wine per week, and the study is subject to a number of limitations that mean the results cannot be attributed to PAH (Lopez-Abente *et al*, 2001). In a second case control study, an increased risk of colorectal adenomas was associated with benzo[*a*]pyrene intake from meat but more strongly with total intake from the diet (Sinha *et al,* 1998; Sinha *et al*, 2001; Sinha *et al*, 2005). In this US based study, the BaP intake of 146 cases and 228 controls was assessed by means of a food frequency questionnaire, with additional questions on meat cooking preferences. Foodstuffs linked to the database were also analysed. In the controls, median BaP intake was 5 ng/day from meat and 73 ng/day from all food sources compared to 17 ng/day from meat and 76 ng/day from all food sources in the cases. Consumption of charcoal grilled beef has been reported to increase the number of DNA adducts in the peripheral blood mononucleocytes of human volunteers (Fontana *et al,* 1999).

33. In an case-control study designed to investigate the proposed association between HAs and CRC (Sachse *et al*, 2002), polymorphisms were observed in genes for the enzymes CYP1A1*2B and GSTM1*2/*2 (Glutathione-S-transferase M1, a phase 2 detoxifying enzyme, thought to be important in PAH metabolism) showed that inheritance of the CYP1A1*2B and the GSTM1*2/*2 "null" allele was associated with an increased risk of disease. This suggests that a genotype conferring increased activation and decreased detoxification respectively could increase susceptibility to the carcinogenic effects of PAHs though this would apply to PAHs from all sources, not just meat.

PAH metabolism

34. The majority of PAHs require metabolic activation for their toxic, mutagenic and carcinogenic action (reviewed EFSA, 2008). In most cases, a CYP450 catalysed epoxide formation is the initial step followed by formation of highly reactive electrophillic metabolites capable of binding to cellular macromolecues, including nucleic acids. Three main metabolic pathways have been described. The most important of these is the bay region dihydrodiol epoxide pathway, responsible for the mutagenicity of many PAHs and is catalysed by CYPs and epoxide hydrolase. A one-electron oxidation pathway leading to reactive PAH radicals and the formation of unstable DNA adducts and an ortho-quinone pathway also leading to reactive electrophillic PAH metabolites have been described and stable and unstable DNA adducts. At present it is not possible to predict which pathway will be of most importance.

35. As noted above, PAHs such as BaP are activated by the bay region dihydrodiol epoxide route. The enzymes involved include CYP1A1, the most effective catalyst for most reactions, whilst CYP1A2 and CYP1B1 appear to be involved in BaP metabolism. The PAH derived diols can then undergo phase 2 metabolism catalysed by sulfotransferases and uridine-diphosphate-glucuronyltransferases. Individual PAHs may undergo more than one pathway and other metabolic pathways than the three noted above may be involved in metabolic activation.

36. Many of the enzymes involved in the metabolism of PAHs have been shown to be polymorphic, suggesting that some individuals will have differing sensitivities to PAH exposure. Some genetic polymorphisms have been associated with an increased risk of cancer, but the role of these has not been completely elucidated because compensatory or alternative pathways may exist. It seems likely that polymorphisms may be more important at lower levels of PAH exposure.

Risk characterisation

37. It is not possible to link the increased risk associated with red meat consumption to PAH intake, since there are no convincing quantitative data relating dietary exposure to PAHs in humans and risk of CRC. PAHs are found in many dietary sources, with PAHs being one of the lesser contributors to total PAH intake.

38. Comparison of the estimated human dietary exposure with animal studies indicates that there is a margin of exposure[4] (MOE) for BaP and defined groups of PAHs of 15, 900 -17,900 for average consumers but 10,000 or slightly less for high level consumers indicating possible concerns at high levels of intake (EFSA, 2008).

[4] The margin of exposure approach is a method by which the dose of a chemical known to cause tumours in a defined proportion of animals is compared to the level of human exposure. A margin of exposure of less than 10, 000 is considered to indicate a potential concern for human health.

Conclusions

Heterocyclic amines

39. HAs are carcinogenic in laboratory animals producing liver and other tumours, whether or not they have a role in human cancer is not clearly defined.

40. Meat is the only source of dietary HAs. The HA content tends to increase with cooking temperature, time and doneness. The most abundant HA is PhIP which tends to occur at higher concentrations in chicken than in red meat. Levels of HAs tend to be lower in meat products, such as sausages and ham, than in other meats. There is no evidence that meats treated with preservatives would have different levels of HAs than meats which had not been treated with preservatives.

41. Studies of CRC patients and controls indicate that HAs such as PhiP and MeIQx bind to DNA in the colon but that there was no correlation between DNA binding and cancer. In addition, variants in the genes that could lead to greater activation of HAs did not differ significantly between CRC patients and controls. The studies reported an increased risk of CRC associated with meat consumption but, did not indicate that HAs were a major contributor to the risk.

42. There is no clear basis for separating meat which contains preservatives from meat which does not on the basis of its HA content.

Polycyclic aromatic hydrocarbons

43. Some PAHs are known animal carcinogens producing a range of tumours, including tumours of the gastro-intestinal tract, when administered orally. Some PAH mixtures are also proven human carcinogens via inhalation and dermal exposure. However, the role of dietary PAHs in humans has been less studied.

44. Meat and meat products represent only a small proportion of dietary PaH intake. Barbecuing can increase the PAH content of meat, but this is only a limited part of the total diet. Therefore any contribution of PAHs to cancer risk associated with red or processed meats is likely to be minor. No direct comparisons of preserved to unpreserved meat have been made in respect of PAH levels. It is unclear whether preserved meats or meat products are more likely to be barbecued or grilled, thus there is no clear basis for separating such meats due to their PAH content. It is likely that meats which have been mechanically processed such as burgers may contain generate higher levels of PAHs through higher fat contents, but this would not be affected by preservative treatment.

45. Humans are exposed to PAHs from a range of dietary and non-dietary sources and it is not possible to determine whether PAHs in cooked meats contribute to CRC risk.

References

Alaejos, M.S., Gonzalez, V., Afonso, A.M. (2008). Exposure to heterocyclic aromatic amines from the consumption of cooked red meat and its effect on human cancer risk: A review. *Food Additives and Contaminants*, 25(1), 2-24.

Barrett, J.H., Smith, G., Waxman, R., Gooderham, N., Lightfoot T., Garner, R.C., Augustsson, K., Wolf, C.R., Bishop, D.T., Forman, D. and the Colorectal Cancer Study Group (2003). Investigation of interactions between *N*-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. *Carcinogenesis*, 24. 275-282.

Busquets, R., Mitjans, D., Puignou, L., Galceran, M.T. (2009). Quantification of heterocyclic amines form thermally processed meats selected from a small-scale population-based study. *Mol. Nutr. Food Res.*, 53, ahead of print DOI 10.1002/mnfr.200800048.

Cross, A.J. and Sinha, R. (2004). Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environmental and Molecular Mutagenesis*, 44, 44-55.

EFSA (2008) Polycyclic aromatic hydrocarbons in food. Scientific Opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal (2008) 724, 1-114.

Fontana, R.J., Lown, K.S., Paine, M.F., Fortkage, L., Santella, R.M., Felton, J.S., Knize, M.G., Watkins, P.B. (1999). Effects of a chargrilled meat diet on expression of CYP3A, CYP1A, and P-glycoprotein levels in healthy volunteers. *Gastroenterology*, 117, 89-98.

Garner, T01005 final project report. FSA, 2004. Summary can be obtained at http://www.food.gov.uk/science/research/researchinfo/foodcomponentsresearch/riskassessment/t01programme/t01projlist/t01003and4and5/

Gooderham and Boobis, T01003 final project report. FSA, 2004. Summary can be obtained at : http://www.food.gov.uk/science/research/researchinfo/foodcomponentsresearch/riskassessment/t01programme/t01projlist/t01003and4and5/

Gooderham, N.J., Murray, S., Lynch., A.M., Yadollahi-Farsani, M., Zhao, K., Boobis, A., Davies, D.S. (2001). Food-derived heterocyclic amine mutagens: variable metabolism and significance to humans. *Drug Metab Disp*, 2**9**, 529-534.

IARC (2006) Summary document from volume 92 (currently in preparation) http://monographs.iarc.fr/ENG/Meetings/92-pahs.pdf

Knize, M.G., Sinham R., Rothman, N., Brown, E.D., Salmon, C.P, Levander, O.A., Cunningham, P. A., Felton, J.S. (1995). Heterocyclic amine content in fast-food meat products. *Food Chem Toxicol*, 33, 545-551.

Knize, M.G., Salmon, C.P., Pais, P., Felton, J.S. (1999). Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. *Adv Exp Med Biol*, 459, 179-193.

López-Abente, G., Sanz-Anquela, J.M., González. C.A. (2001). Consumption of wine stored in leather bottles and incidence of gastric cancer. Arch Environ health, 56, 559-561.

Martinez, M.E., Jacobs, E.T., Ashbeck, E.L., Sinha, R., Lance, P., Alberts, D.S., Thompson, P.A. (2007). Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. *Carcinogenesis*, **28**(9),2019-2027.

Phillips, D.H. (1999). Polycyclic aromatic hydrocarbons in the diet. *Mutation Research*, 443, 139-147.

Sachse, C., Smith, G., Wilkie, M.J.V., Barrett, J.J.H., Waxman, R., Sullivan, F., Forman, D., Bishop, D.T., Wolf, C.R. and the Colorectal Study Group (2002). A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis*, 23, 1839-1849.

Santarelli, R.L., Pierre, F., Corpet, D.E. (2008). Processed meat and colorectal cancer: A review of epidemiologic and experimental evidence. *Nutrition and Cancer*, 60(2), 131-144.

SCF (2002). Opinion of the Scientific Committee on Food on the risk to human health of polycyclic aromatic hydrocarbons in Food. SCF/CS/CNTM/PAH/29 Final.

Sinha, R., Knize, M.G., Salmon, C.P., Brown. E.D., Rhodes, D., Felton, J.S., Levander, O.A., Rothman, N. (1998). Heterocyclic Amine Content of Pork Products Cooked by Different Methods and to Varying Degrees of Doneness. Food and Chemical Toxicology, 36, 289-297.

Sinha, R., Kulldorf, M., Chow, W-H, Denobile, J., Rothman, N. (2001). Dietary Intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev, 10, 559-562.

Sinha, R., Kulldorf, M., Gunter, M.J. Strickland, P., Rothman, N. (2005). Dietary Benzo[*a*]Pyrene Intake and Risk of Colorectal Adenoma. Cancer Epidemiol Biomarkers Prev, 14, 2030-2035.

Skog, K. (1993). Cooking procedures and food mutagens: A literature review. *Food and Chemical Toxicology*, 31(9), 655-675.

Skog, K, Johansson, M.A.E., Jaegerstad, M.I. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food and Chemical Toxicology*, 36, 879-896.

Skog K and Solyakov A (2002). Heterocyclic amines in poultry products: a literature review. *Food and Chemical Toxicology*, 40, 1213-1221.

Wu, K., Giovannucci, E., Byrne, C., Platz, E.A., Fuchs, C., Willett, W.C., Sinha, R. (2006). Meat and mutagens and risk of distal colon adenoma in a cohort of US men. *Cancer Epidemiol Biomarkers Prev*, 15(6), 1120-1125.

ANNEX 5

Appendix 2

N-nitroso compounds and cancer

Occurrence of N-nitroso compounds

1. N-nitroso compounds, including nitrosamines, can be formed in the bodies of healthy individuals following the reaction of dietary nitrate and nitrite with primary, secondary and tertiary amines via a nitric oxide intermediary (Rostkowska *et al*, 1998). Nitrates and nitrites can be found in a wide range of commonly consumed foods.

2. Studies in ileostomists have shown that nitrosamines are formed in the absence of colonic flora in the upper GI tract (Lunn *et al*, 2007) and are also produced endogenously in the stomach and colon of people who eat large amounts of red meat or take nitrate supplements. N-nitroso compounds can also be produced in meat during the curing process or during smoking, drying and salting of foods such as fish and meat (WCRF/AICR, 2007).

3. Exposure to N-nitrosodimethylamine (NDMA), the most widely consumed and widely studied dietary N-nitroso compound, has been found to be <1 µg/day whereas exposure to endogenously formed N-nitroso compounds (ENOC) was 93 µg/day (Jakszyn *et al*, 2006). Whilst NDMA is only one of many N-nitroso compounds in foods and represents a fraction of the total NOC exposure, it has been used as an indicator of dietary exposure to total exogenous NOCs.

Evidence for the carcinogenicity of nitrosamines

Animal studies

1. Nitrosamines have been shown to produce a range of tumours in 40 animal species (Bartsch, 1991). These tumours include liver, stomach and fore-stomach tumours and other tumours of the GI tract, but there is little evidence of a link to CRC in humans or animal models (although it cannot be assumed that there is concordance between tumour sites between animals and humans). Individual exposure to endogenous nitrosamines is highly dependent on dietary modifiers as well as disease status. G-A transition mutations in particular codons and DNA adducts characteristic of alkylating agents such as nitrosamines are commonly found in CRC. Two nitrosamines (N-nitrosodiethylamine and NDMA) have been classified as probably carcinogenic to humans (group 2A) based on data from many animal species with the main target organs being the liver, respiratory and upper digestive tracts and kidneys (IARC, 1998).

Human studies

2. In a cohort study in Finland, 189 gastro-intestinal cancers were identified in the 24 years follow-up period. Intakes of nitrate, nitrite and NDMA were estimated using a one year dietary history interview. NDMA was assumed to be provided by smoked and salted fish (51.9%) and cured meats and sausages (48.1%). A significant positive association was observed between intake of NDMA and subsequent occurrence of CRC [RR between highest and lowest quartiles of intake of 2.12; 95% CI 1.04-4.33]. Of the various sources, intakes of smoked and salted fish were significantly [RR = 2.58, 95% CI 1.21-5.51) associated with CRC whereas intakes of cured meat were non-significantly associated with CRC [RR = 1.84, 95% CI 0.98-3.47]. No significant associations were observed with other cancers of the GI tract or with nitrate and nitrite intake (Knekt *et al*, 1999).

3. A nested case-control study of patients with gastric cancer (GC) assessed the exposure to dietary NDMA and ENOC through a dietary and lifestyle questionnaire. Dietary intakes of nitrites and NDMA were estimated by matching food items on a country specific questionnaire with a food database of potential carcinogens. Dietary NDMA was not associated with an increased risk of GC (hazard ratio (HR) - 1.00; 95% CI 0.70-1.43 for an increment of 1.1 µg of NDMA. ENOC was positively but not statistically significantly associated with GC (HR - 1.18; 95% CI 0.99-1.39 for an increment of 40 µg of EN. When analysed by tumour site, ENOC was not associated with cardia cancer risk but was significantly associated with non-cardia cancer risk (HR- 1.42; 95% CI 1.14-1.78 for an increment of 40 µg of ENOC). Amongst individuals with *H. pylori* infection an association was found between non-cardia cancer (OR – 1.82; 95% CI 1.32-2.51) in all models but in non-infected individuals no association was observed. Individuals with elevated serum

vitamin C showed no association between ENOC and non-cardia GC where as those with low serum vitamin C showed a positive association (OR – 3.24; 95% CI 1.77-5.93) (Jakszyn *et al*, 2006).

4. Studies have suggested that the conditions found at the gastro-oesophageal junction of the stomach are optimal for the formation of nitrosamine compounds as the ascorbic acid to nitrite ratio was lowest at this point of the GI tract and the acidity and level of thiocyanate (a known catalyst for nitrosation of secondary amines by acidified nitrite) are sufficient to potentially promote this reaction (Suzuki *et al*, 2003).

5. Further studies have shown a higher percentage of faecal colonic exfoliated cells staining positive for O^6-methylguanine (O^6MeG) from individuals consuming a high red meat diet compared to those consuming a vegetarian diet. O^6MeG is a promutagenic DNA adduct which can be formed by many N-methyl-N-nitroso compounds. This particular adduct has been shown in *in vitro* assays to form G-A transitions and G-T transversions in adducted p53 cDNA and is not easily repaired by normal DNA repair mechanisms. This adduct may be a possible mechanism for the relationship between high red meat consumption and CRC (Lewin *et al*, 2006; Kuhnle *et al*, 2007) and it has been identified in many CRC cell lines (Nagasaka *et al*, 2008).

6. Further studies have identified a link between the consumption of cured and smoked meat and fish by pregnant mothers and young children with the development of childhood leukaemia and have shown a protective effect of vegetables and bean-curd foods (Liu *et al*, 2009). There are also a number of studies linking the consumption of preserved meats by pregnant women (along with other sources of exposure to N-nitroso compounds such as therapeutic drugs) with brain cancers in their children (McKean-Cowdin *et al*, 2003; Preston-Martin *et al*, 1982; Huncharek *et al*, 2003).

International reviews

7. The World Cancer Research Fund/American Institute for Cancer Research concluded in 2007 that there was limited evidence that processed[5] meats, but not red meats, were a cause of stomach cancer and that this was likely to be due to the formation of N-nitroso compounds both in the meat and in the stomach (WCRF/AICR, 2007).

8. The WCRF/AICR also concluded that Cantonese-style salted fish, which is relatively high in nitrosamine compounds, is a probable cause of nasopharyngeal cancer. This conclusion was supported by the evidence that CYP2E1 is expressed in the nasopharynx and is involved in the metabolic activation of nitrosamines *in vivo* and that individuals with a variant allele of CYP2E1 have been linked with an increased incidence of nasopharyngeal cancer.

9. With regards to oesophageal cancer, the WCRF/AICR concluded that for both red meat and processed meat, there was limited evidence to suggest a causal link.

10. Based on a substantial amount of data from cohort and case-control studies which showed a plausible dose-response relationship, the WCRF/AICR concluded that the evidence to suggest that processed and red meat are a cause of CRC was convincing but there are little data on the mechanism for this and whether this relates to the formation of nitrosamines.

Conclusion

11. There does not seem to be strong evidence to support a link between nitrosamine exposure (both exogenous and endogenous formation) and CRC. Although nitrosamine compounds may be more likely to be present in preserved meats (including smoked, cured and salted meats), evidence to link these foods with colorectal cancer is weak. There is a stronger association between preserved meats and stomach, oesophageal and other cancers than CRC. Exposure to N-nitroso compounds produced endogenously in the body appears to significantly exceed (~100 times for NDMA) that from N-nitroso compounds from foods.

[5] The WRCF/AICR acknowledges that the definition of processed meat varies between studies. They have classified processed meats at meats preserved by smoking, curing or salting, or addition of chemical preservatives.

References

Bartsch H. (1991) N-nitroso compounds and human cancer: where do we stand?
IARC Scientific Publications. 105 :1-10

Huncharek, M., Kupelnick, B. & Wheeler, L. (2003) Dietary cured meat and the risk of adult glioma: a meta-analysis of nine observational studies. Journal of Environmental Pathology, Toxicology and Oncology. 22 (2) 129-37

IARC (1998) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; Volume 17 : Some *N*-Nitroso
Lyon, France: IARC Press. http://monographs.iarc.fr/ENG/Monographs/vol17/volume17.pdf

Jakszyn, P., Bingham, S., Pera, G., Agudo, A., Luben, R., Welch, A., Boeing, H., Del Giudice, G., Palli, D., Saieva, C., Krogh, V., Sacerdote, C., Tumino, R., Panico, S., Berglund, G., Simán, H., Hallmans, G., Sanchez, M.J., Larrañaga, N., Barricarte, A., Chirlaque, M.D., Quirós, J.R., Key, T.J., Allen, N., Lund, E., Carneiro, F., Linseisen, J., Nagel, G., Overvad, K., Tjonneland, A., Olsen, A., Bueno-de-Mesquita, H.B., Ocké, M.O., Peeters, P.H., Numans, M.E., Clavel-Chapelon, F., Trichopoulou, A., Fenger, C., Stenling, R., Ferrari, P., Jenab, M., Norat, T., Riboli, E. & Gonzalez, C.A. (2006) Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study.
Carcinogenesis. 27(7):1497-501

Knekt, P., Jarvinen, R., Dich, J. & Hakulinen, T., (1999) Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. International Journal of Cancer. 80 (6) 852-6

Kuhnle G.G.C., Story G.W., Reda T., Mani A.R., Moore K.P., Lunn J.C. & Bingham S.A. (2007) Diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radical Biology and Medicine 43 1040-1047

Lewin, M.H., Bailey, N., Bandaletova, T., Bowman, R., Cross, A.J., Pollack, J., Shuker, D.E.G. & Bingham, S.A. (2006) Red meat enhances the colonic formation of the DNA adduct O^6-carboxymethyl guanine: Implications for colorectal cancer risk. Cancer Res. 66 (3) 1859-1865

Liu, C.Y., Hsu, Y.H., Wu, M.T., Pan, P.C., Ho, C.K., Su, L., Xu, X., Li, Y., Christiani, D.C., Klrg, K.L. (2009) Cured meat, vegetables, and bean-curd foods in relation to childhood acute leukemia risk: A population based case-control study. BMC Cancer. 2009 9 (1) 15.

Lunn, J.C., Kuhnle, G., Mai, V., Frankenfeld, C., Shuker, D.E.G., Glen, R.C., Goodman, J.M., Pollock, J.R.A., & Bingham, S.A. (2007) The effect of haem in red and processed meat on the endogenous formation of N-nitroso compounds in the upper intestinal tract. Carcinogenesis 28 (3) 685-690

McKean-Cowdin, R., Pogoda, J.M., Lijinsky, W., Holly, E.A., Mueller, B.A., & Preston-Martin, S. (2003) Maternal prenatal exposure to nitrosatable drugs and childhood brain tumours. International Journal of Epidemiology 32. 211-217

Nagasaka T, Goel A, Notohara K, Takahata T, Sasamoto H, Uchida T, Nishida N, Tanaka N, Boland CR, Matsubara N. (2008) Methylation pattern of the O6-methylguanine-DNA methyltransferase gene in colon during progressive colorectal tumorigenesis. Int J Cancer. 2008 Jun 1;122(11):2429-36

Preston-Martin, S., Yu, M.C., Benton, B. & Henderson, B.E. (1982) N-nitroso compounds and childhood brain tumors: a case-control study. Cancer Research 42 5240-5245

Rostkowska, K., Zwiez, K., Rozanski, A., Moniuszko-Jakoniuk, J. & Roszczenko, A. (1998) Formation and metabolism of N-nitrosamines. Polish Journal of Environmental Studies 7 (6) 321-325

Suzuki H., Lijima K., Moriya A., McElroy K., Scobie G., Fyfe V & McColl KEL (2003) Conditions for acid catalysed luminal nitrosation are maximal at the gastric cardia. Gut 52 1095-1101

WCRF/AICR (2007) Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington DC: AICR, 2007

Meat, haem iron and cancer

1. Evidence suggests that diets high in red and/or preserved meat are associated with a higher risk of CRC when compared with diets rich in chicken or fish. A major difference between red meat and white meat, such as chicken, is the levels of iron and haem which are approximately 4 and 10 times greater in red meat respectively (Cross *et al*., 2002, Santarelli *et al*., 2008; Sandhu *et al*., 2001; Norat *et al*., 2002, Larsson & Wolk, 2006, Norat *et al*., 2005; Lee *et al*, 2004; WCRF/AICR, 2007). There are some studies that have found no association between haem or iron intake and CRC (Kabat *et al*., 2007).

Foods containing iron

2. As noted in the draft SACN report, there is limited evidence suggesting that consumption of a diet high in total and haem iron may be associated with a higher risk of CRC. The WCRF concluded in 2007 that the evidence was "sparse, of poor quality and inconsistent" (WCRF/AICR, 2007). The definition of foods containing iron includes vegetables containing non-haem iron such as spinach.

Foods containing haem

3. The haem content of preserved and unpreserved meats from the same species appear to be comparable and therefore processing alone would not account for the different relative risks associated with non-preserved and preserved red meats. The majority of preserved meat products are of porcine origin and therefore contain lower levels of haem iron than beef-based products.

Proposed mechanisms – General studies

4. A number of mechanisms have been proposed for a possible relationship between haem/haemoglobin and CRC. These mechanisms include the formation of N-nitroso-compounds (see Appendix 2 for more information on the possible carcinogenic activities of N-nitroso compounds), the catalysis of lipid peroxidation by iron/haem in the gut and subsequent DNA adducts caused by lipid radicals and the nitrosylation of haem which may render it more cytotoxic than unnitrosylated haem. Some of the available studies are outlined below grouped by mechanism, prefaced by some studies looking at the carcinogenicity of haem and haemoglobin where the authors have proposed all of the above mechanisms but not specified which they think is most likely.

5. Rats fed a low calcium diet supplemented with haemin or haemoglobin and/or calcium, butylated hydroxyl anisole and rutin, and olive oil demonstrated that haemin and haemoglobin produced a statistically significant increase in numbers of total aberrant crypt foci (ACF) compared to controls. Calcium, antioxidants and olive oil reduced the total number of ACF found in the colons of these rats (Pierre *et al*., 2003). A similar study by the same author using meat instead of haemin/haemoglobin concluded that red meat in combination with a low calcium diet in rats promotes the number of ACF in the colons of rats. (Pierre *et al*, 2004).

6. Rats fed a purified control diet or a diet containing protoporphyrin IX, ferric citrate or bilirubin were compared to those consuming a purified diet containing haemin for 14 days. Rats on the haemin diet showed significant increases in proliferation of the colonic epithelium compared to control animals. Faecal water from the haemin consuming groups was significantly more cytotoxic to erythrocytes compared to controls (Sesink *et al*., 1999).

Proposed mechanisms - Nitrosation

7. In human volunteer dietary studies, apparent total N-nitroso compounds (ATNC) were found to be significantly elevated following consumption of a haem-supplemented and high red meat diet compared to a low meat diet. Iron supplementation did not have an effect. It was proposed that haem and not iron appears to enhance endogenous N-nitrosation to form mainly nitrosothiols under the acid conditions in the stomach which can then go on to form nitrosyl haem and other nitroso compounds in the alkaline and reductive conditions of the small and large intestines. However, not all nitroso compounds are carcinogenic. The analytical study was unable to characterise some of the nitroso compounds present in the ATNC (Cross *et al*., 2002; Kuhnle & Bingham, 2007; Kuhnle *et al*., 2007; Cross *et al*., 2003; Dennis & Clarke, 2006).

8. A number of animal studies have shown an increase in faecal ATNC following consumption of grilled bacon or other preserved meats, but this was not accompanied by an increase in aberrant crypt foci (precancerous cells in the epithelium of the colon) (Santarelli *et al*, 2008).

Proposed mechanisms - Lipid peroxidation and oxidative stress

9. A human volunteer study and rat study showed a significant increase in urinary markers of lipid peroxidation (4-hydroxynonenal metabolites or 8-*iso*-prostaglandin-F_2□) following consumption of high haem diets (Pierre *et al*., 2006).

10. Rats fed diets containing safflower oil (an oxidised refined PUFA) and haemoglobin at various levels for 36 weeks showed an increase in carcinoma of the colon compared to controls (it is noted that this study does not follow the standard time frame for carcinogenicity studies). Peroxyl radicals were shown to be produced *in vitro* from oxidised PUFA in the presence of haemoglobin and oxidised PUFA was found to cause single strand breaks in DNA in the presence of haematin (Sawa *et al*., 1998).

11. An Ames test carried out using S. typhimurium strains sensitive to oxidative mutagens (TA102 and TA104) suggested that iron may cause redox cycling of bile acids in the presence of vitamin K1 and S9 mixture (Blakeborough *et al*., 1989).

12. *In vitro* studies using colon cancer cell lines incubated with haemoglobin and haemin showed that iron from both compounds was released and rapidly absorbed by the cells and resulted in increased proliferation and production of reactive oxygen species (Lee *et al*., 2006; Glei *et al*., 2006).

Proposed mechanisms - Nitrosylation of haem

13. In raw cured meat, myoglobin is nitrosylated and further cooking releases nitrosyl haem from the myoglobin. Nytrosylated haem has been shown to be weakly mutagenic in the Ames test, but has not been tested *in vivo* (Santarelli *et al*, 2008).

14. In *in vitro* experiments, N-nitrosomorpholine (NMor) was formed in the presence but not absence of nitrosated haemoglobin at pH 6.8. In this study NMor was used as a marker for nitrosamine compounds in general (Lunn *et al*, 2007)

Conclusion

15. There is some evidence that consumption of a diet rich in iron (haem and non-haem) may be associated with a higher risk of CRC although this evidence is considered “sparse, of poor quality and inconsistent”. A clear mechanism demonstrating either the effect of haem or non-haem iron or any differences in risks between preserved or unpreserved red meats has not been demonstrated by the studies above. It is possible that a combination of the above mechanisms is occurring or that different mechanisms may produce N-nitroso compounds which are more potent in their carcinogenic activity than those produced endogenously from sources such as vegetables. There is currently insufficient evidence to support these mechanisms. These data do not provide a clear basis for separating preserved and unpreserved red meat for modelling purposes.

References

Blakeborough M.H., Owen R.W. & Bilton R.F. (1989) Free radical generating mechanisms in the colon: Their role in the induction and promotion of colorectal cancer? Free. Rad. Res. Comms. 6 (6) 359-367

Cross A.J., Pollock, J.R.A. and Bingham S.A. (2002) Red meat and colorectal cancer risk: the effect of dietary iron and haem on endogenous N-nitrosation. IARC Scientific Publications (156) 205-6

Cross A.J., Pollock, J.R.A. and Bingham S.A. (2003) Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red. Cancer Research 63 2358-2360

Dennis, M.J. & Clarke, D.B. (2006) The relevance of faecal ATNC to colon carcinogenesis. available at: http://www.foodbase.org.uk//admintools/reportdocuments/127-1-209_FD_Report_Final_Version__7Sept06_.pdf

Glei M., Klenow S., Sauer J., Wegewitz U., Richter K. & Pool-Zobel B.L. (2006) Hemoglobin and hemin induce DNA damage in human colon tumor cells HT29 clone 19A and in primary human colonocytes. Mutation research 594 162-171

Kabat G.C., Miller A.B., Jain M. & Roham T.E. (2007) A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. British Journal of Cancer. 97 118-122

Kuhnle G.G.C. & Bingham S.A. (2007) Biochemical Society Transactions (2007) 35 (5) 1355-1357

Kuhnle G.G.C., Story G.W., Reda T., Mani A.R., Moore K.P., Lunn J.C. & Bingham S.A. (2007) Diet-induced endogenous formation of nitroso compounds in the GI tract. Free Radical Biology and Medicine 43 1040-1047

Larsson S.C. & Wolk A. (2006) Meat consumption and risk of colorectal cancer: a metaanalysis of prospective studies. International Journal of Cancer 119 (11) 2657-64

Lee D.-H., Anderson, K.E., Harnack, L.J., Folsom, A.R. & Jacobs D.R. (2004) Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Study.

Lee R.-A., Kim H.-A., Kang B.-Y. & Kim K.-H. (2006) Hemoglobin induces colon cancer cell proliferation by release of reactive oxygen species. World J Gastroenterol 12 (35) 5644-5650

Lunn, J.C., Kuhnle, G., Mai, V., Frankenfeld, C., Shuker, D.E.G., Glen, R.C., Goodman, J.M., Pollock, J.R.A., & Bingham, S.A. (2007) The effect of haem in red and processed meat on the endogenous formation of N-nitroso compounds in the upper intestinal tract. Carcinogenesis 28 (3) 685-690

Norat T., Bingham S., Ferrari P., Slimani N., Jenab M., Mazuir M., Overvad K., Olsen A., Tjønneland A., Clavel F., Boutron-Ruault M.C., Kesse E., Boeing H., Bergmann M.M., Nieters A., Linseisen J., Trichopoulou A., Trichopoulos D., Tountas Y., Berrino F., Palli D., Panico S., Tumino R., Vineis P., Bueno-de-Mesquita H.B., Peeters P.H., Engeset D., Lund E., Skeie G., Ardanaz E., González C., Navarro C., Quirós J.R., Sanchez M.J., Berglund G., Mattisson I., Hallmans G., Palmqvist R., Day N.E., Khaw K.T., Key T.J., San Joaquin M., Hémon B., Saracci R., Kaaks R., Riboli E. (2005) Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. Journal of the National Cancer Institute 97 (12) 906-916

Norat T., Lukanova A., Ferrari P. & Riboli E. (2002) Meat consumption and colorectal cancer risk: dose response meta-analysis of epidemiological studies. International Journal of Cancer 98 (2) 241-56

Pierre F., Peiro G., Tache S., Cross A.J., Bingham S.A., Gasc N., Gottardi G., Corpet D.E. & Gueraud F. (2006) New marker of colon cancer risk associated with heme intake: 1,4-dihydroxynonane mercapturic acid. Cancer epidemiology biomarkers and prevention. 15 (11) 2274-2279

Pierre F., Freeman A., Tache S., Van der Meer R. & Corpet D.E. (2004) Beef meat and blood sausage promote the formation of axoxymethane-induced mucin-depleted foci and abherrant crypt foci in rat colons. The Journal of Nutrition. 134 (10) 2711-2716

Pierre F., Tache S., Petit, C.R., Van der Meer R. & Corpet D.E. (2003) Meat and Cancer: haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. Carcinogenesis 24 (10) 1683-1690

SACN (2008) Draft SACN report on iron.

Sandhu M.S., White I.R. & McPherson K. (2001) Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiology, Biomarkers and Prevention. 10, 439-446

Santarelli R.L., Pierre F. & Corpet D.E. (2008) Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. Nutrition and Cancer 60 (2) 131-144

Sawa T., Akaike T., Kida, K., Fukushima Y., Takagi K., & Maeda H. (1998) Lipid peroxyl radicals from oxidised oils and heme-iron: implication of a high-fat diet in colon carcinogenisis.Cancer epidemiology, biomarkers and prevention. 7 1007-1012

Sesink A.L.A. Termont D.S.M.L. Kleibeuker J.H., Van der Meer R. (1999) Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. Cancer Research. 59 5704-5709

WCRF/AICR (2007) Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington DC: AICR, 2007

ANNEX 5

Appendix 4

Preservatives and cancer

1. Fresh meat is not permitted to contain additives (including preservatives); additives can only be used in processed meats (including pre-packed fresh minced meat) and processed meat products[6]. Council Directive 95/2/EC on food additives other than colours and sweeteners details those additives approved for use in meat products. The table at the end of this Appendix contains a list of all additives that are approved for use in meat and meat products. The additives classed as preservatives are E200 to E252. Other permitted additives include anti-oxidants and colours (EC, 1995).

2. In order for an additive to be approved for use, the applicant must submit a large package of safety data to demonstrate than no adverse effects would be expected under the conditions of use. This package may include acute and chronic studies and reproductive toxicity studies in animals as well as *in vitro* studies to identify genotoxic activity. The data are reviewed by independent expert committees such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA). If a substance is found to cause cancer in laboratory animals by a genotoxic mechanism, it would not be permitted for use as an additive in foods.

3. Concerns have been expressed by some researchers regarding the potential carcinogenicity of nitrites/nitrates and benzoates and sorbates. These are considered below along with sulphates and sulphites. We are not aware of comparable concerns with respect to other preservatives or additives and so these have not been discussed.

Nitrate, nitrite and N-nitroso compounds:

Regulatory aspects

4. Potassium nitrite (E249), sodium nitrite (E250), sodium nitrate (E251) and potassium nitrate (E252) are permitted in "a range of meat products, cured hams, etc" at levels of between 10 and 300 mg/kg (EC, 1995). They are also permitted in a small number of cheeses and preserved fish. These substances are added as preservatives in meat products, particularly cured meats such as ham and bacon to reduce the growth of bacteria and therefore spoilage and health risks.

5. There is legislation in Europe limiting nitrate concentrations in vegetables (EC, 2005 and EC, 2001).

International reviews and derivation of the ADI

6. The acceptable daily intake (ADI) is the amount of an additive that expert bodies consider can be consumed on a daily basis for a lifetime without any appreciable adverse effects.

7. There is an ADI set for nitrate of 3.7 mg/kg bw/day (or 222 mg nitrate/day for an average 60 kg adult). This ADI was set by both the former Scientific Committee on Food (SCF) and the JECFA. JECFA reconfirmed the ADI in 2002 (WHO, 2003). At their meeting in 2002, JECFA also set an ADI for nitrite of 0.07 mg/kg bw (or 4.2 mg nitrite/day for an average 60 kg adult) (WHO, 2003).

8. The most recent assessments of the ADI for nitrate and nitrite were carried out in 2002 by the JECFA who confirmed the previous ADI for nitrate of 3.7 mg/kg bw/day and set an ADI for nitrite of 0.07 mg/kg bw/day. The ADI for nitrite was set using a 2 year rat study and a sub-chronic (125 day) dog study both of which showed growth restriction at intakes of the nitrate ion above 370 mg/kg bw/day. The ADI for nitrite was derived from a two year study in rats given sodium nitrite in their drinking water. Heart and lung toxicity were observed at doses of 100 mg/kg bw/day and above and the no observed adverse effect level (NOAEL) was

[6] Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin provides the following definitions:
'fresh meat' means meat that has not undergone any preserving process other than chilling, freezing or quick freezing including meat that is vacuum wrapped or wrapped in a controlled atmosphere;
'unprocessed products' means foodstuffs that have not undergone processing, and includes products than have been divided, parted, severed, sliced, boned, minced, skinned, ground, cut, cleaned, trimmed, husked, milled, chilled, frozen, deep-frozen or thawed;
'processed products' means foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics;
'processing' means any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of these processes.

10 mg/kg bw/day. No evidence of carcinogenic activity was observed in these studies (WHO, 2003). The EFSA Scientific Panel on Contaminants in the Food Chain concluded that there were no new data that would necessitate a revision in the ADI in the course of a recent evaluation of risks of nitrate in vegetables (EFSA, 2008).

9. The majority of people would not exceed the ADI for nitrate and nitrite (EFSA, 2008).

Natural occurrence and metabolism of nitrate and nitrite

10. Nitrate can be produced endogenously through the L-arginine-NO-synthase pathway and is found naturally in vegetables, especially leafy vegetables such as cabbage and spinach. Nitrate can be converted to nitrite, nitric oxide and N-nitroso compounds in the body and these metabolites of nitrate have the potential to cause adverse effects such as methaemoglobinaemia and carcinogenicity.

11. Nitrite may play a defensive role in the body through its anti-microbiological properties and nitric oxide may play a role in vasoregulation (EFSA, 2008), but these effects are not taken into account in the safety assessment of nitrate and nitrite in food.

12. In most individuals, 5-7% of dietary nitrate is converted to nitrite through the action of bacteria at the back of the tongue; for some individuals with a high rate of conversion, this may be up to 20%. Reduction of nitrate to nitrite in the mouth accounts for 70-80% of the total nitrite exposure in man, the remainder coming from cured meats and other routes of metabolism in the body. The nitrite formed in the oral cavity is swallowed along with saliva and food and enters the stomach where it can be converted to nitrogen oxides such as nitric oxide and subsequently may be metabolised to nitrosamines (EFSA, 2008).

Predicted exposures to nitrates and nitrites

13. An exposure assessment carried out by EFSA found that an individual consuming 400 grams of mixed vegetables per day containing a typical median nitrate level and taking into account other sources of nitrate including drinking water and animal products would not exceed the ADI for nitrate. The estimated intakes of nitrate were approximately 35-44 mg/person/day from sources other than vegetables (of which around 20 mg/person/day is contributed by water and the remainder from animal products such as preserved meats and fish and cheese) and 113 mg/person/day from vegetables to give a total intake of 157 mg/person/day (EFSA, 2008). From the results from the 1997 total dietary survey (TDS) it has been calculated that green vegetables, potatoes and other vegetables made the greatest contributions to nitrate exposure contributing 21%, 33% and 15% respectively. For dietary nitrite it was calculated that beverages made the greatest contribution at 36% with meat products contributing 15%, miscellaneous cereals 14% and milk 8% (MAFF, 1998).

14. The intake of nitrate and nitrite from consumption of vegetables is likely to considerably exceed that from preserved meats. Concomitant consumption of vitamin C appears to reduce the levels of nitrosamine produced by up to 50% when nitrate containing foods are consumed (EFSA, 2008).

Studies of carcinogenicity and genotoxicity of nitrate and nitrite

15. Sodium nitrate was found not to be mutagenic in *in vitro* studies. Similar studies carried out on sodium nitrite showed mutagenic potential in one out of the two strains of *S. typhimurium* tested but overall, in their assessment of genotoxicity, JECFA considered that neither nitrate nor nitrite should be regarded as genotoxic carcinogens (WHO, 2003). JECFA and EFSA also looked at a number of long term carcinogenicity studies on nitrate and nitrite. Both committees concluded that nitrate was not carcinogenic. The evidence for the carcinogenicity of nitrite was equivocal based on a trend in the incidence of squamous cell papilloma and carcinoma of the fore-stomach; an end-point that may not be relevant for humans.

16. Human epidemiology studies have shown no link between the incidence of cancer (multiple tissue sites) and nitrate intake from food and drinking water (EFSA, 2008; WHO, 2003).

Conclusion

17. Whilst the epidemiology studies may suggest that high consumption of preserved meats poses a greater relative risk of developing CRC, the evidence available does not support the hypothesis that this is due to the presence of nitrate and nitrite preservatives. This is because green vegetables and potatoes provide a

much greater source of nitrate than preserved meats and endogenous conversion of nitrate to nitrite provides a significantly greater source of nitrite than preserved meats.

Benzoates and sorbates

Regulatory aspects and international reviews

18. Sorbic acid (E200), potassium sorbate (E202), calcium sorbate (E203), benzoic acid (E210), sodium benzoate (E211), potassium benzoate (E212) and calcium benzoate (E213) are all approved additives which are permitted at levels "*quantum satis*" for the surface treatment of dried meat products. This category of meat products would include beef jerky and similar items and is not thought to form a large proportion of the UK diet. These preservatives are used to a greater extent in carbonated soft drinks, and meat products are unlikely to be a significant source of these preservatives (EC, 1995).

19. In 1996, the JECFA set a group ADI for benzoate salts and benzoic acid of 5 mg/kg bw/day which was reconfirmed in 2001. This was based on a multi-generation long term study in rats were no adverse effects were observed at intakes at or below 500 mg/kg bw/day. In 1973, the JECFA set a group ADI for sorbate salts and sorbic acid of 25 mg/kg bw/day. This was based on a multi-generation long term rat study where no treatment related adverse effects were observed at intakes at or below 2500 mg/kg bw/day.

COM assessment

20. In 2007, the Committee on Mutagenicity considered a study in modified yeast cells exposed to benzoate and sorbate preservatives that showed an increased level of DNA damage in exposed cells compared to controls. Members were of the opinion that direct extrapolation of the results from the mutant yeast cells used in this study to mammalian cells *in vivo* was not possible due to the fact that the yeast cells had attenuated anti-oxidant and DNA repair mechanisms. The Committee concluded that, taking into account the large package of toxicology data, including rodent carcinogenicity studies, this study did not suggest a need for a full evaluation of the mutagenicity data on benzoate and sorbate preservatives (COC, 2007).

Conclusion

21. This study along with the package of other data supplied prior to approval of these preservatives does not suggest a link between benzoate and sorbate preservatives and CRC.

Sulphates and sulphites

Regulatory aspects

22. Sodium sulphate and sodium hydrogen sulphate (E514), potassium sulphate and potassium hydrogen sulphate (E515) and calcium sulphate (E516) are permitted in all preserved meats but not fresh meats or poultry. Sodium sulphite (E221), sodium hydrogen sulphite (E222), sodium metabisulphite (E223), potassium metabisulphite (E224) and calcium sulphite (E226) are all permitted for use in burgers or breakfast sausages.

23. In 2000, JECFA assessed the safety of sodium sulphate and derived a temporary ADI "not specified" indicating that they had no concerns over the use of this additive in foods when used following good manufacturing practise (WHO, 2000). In 1999, JECFA reconfirmed the ADI set in 1986 of 0.7 mg/kg bw/day based on an 8-week animal study where gastric lesions were noted at doses in excess of 70 mg/kg bw/day.

24. As is the case with all additives, these substances have been rigorously assessed for safety by JECFA and other international experts. Sulphate compounds are endogenous in the body and long term carcinogenicity studies using both sulphates and sulphites have shown no carcinogenic effect. Some *in vitro* studies of the mutagenicity of sulphites have produced positive results but these are not confirmed by *in vivo* data (WHO, 1986; WHO 1999).

Conclusion

25. No data have been identified that support a hypothesis that sulphate preservatives used in preserved meats could be linked to CRC.

Overall conclusion on preservatives

26. The data do not suggest that commonly used food additives used in preserved meats are the basis for the link between processed meat intake and CRC.

References

COC (2007) can be found at: http://cot.food.gov.uk/pdfs/comsection07.pdf (page 134)

EC (2005) Commission Regulation (EC) No 1822/2005 of 8 November 2005 amending Regulation (EC) No 466/2001 as regards nitrate in certain vegetables:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:293:0011:0013:EN:PDF

EC (2001) Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32001R0466:EN:HTML

EC (1995) European Parliament and Council Directive No 95/2/EC of 20th February 1995 on food additives other than colours and sweeteners. Available at:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1995L0002:20060815:EN:PDF

EFSA (2008) Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission to perform a scientific risk assessment on nitrate in vegetables, The EFSA Journal (2008) 689, 1-79. Available at: http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/contam_ej_689_nitrate_en.pdf?ssbinary=true

MAFF (1998) Food survey Information Sheet 163 : 1997 Total diet Study – nitrate and nitrite. available at:
http://archive.food.gov.uk/maff/archive/food/infsheet/1998/no163/163tds.htm

WHO (2003) Safety evaluation of certain food additives, prepared by the fifty-ninth meeting (in 2002) of the Joint FAO/WHO Expert Committee on Food Additives. Available at:
http://www.inchem.org/documents/jecfa/jecmono/v50je06.htm and
http://www.inchem.org/documents/jecfa/jecmono/v50je05.htm

WHO (2000) Safety evaluation of certain food additives, prepared by the fifty-third meeting (in 1999) of the Joint FAO/WHO Expert Committee on Food Additives. Available at :
http://www.inchem.org/documents/jecfa/jecmono/v44jec07.htm

WHO (1999) Safety evaluation of certain food additives, prepared by the fifty-first meeting (in 1999) of the Joint FAO/WHO Expert Committee on Food Additives. Available at :
http://www.inchem.org/documents/jecfa/jecmono/v042je06.htm

WHO (1986) Safety evaluation of certain food additives, prepared by the thirtieth meeting (in 1986) of the Joint FAO/WHO Expert Committee on Food Additives. Available at:
http://www.inchem.org/documents/jecfa/jecmono/v21je15.htm

Name	E-Number	Authorisation	Maximum levels	Food Category
Food additives other than colours and sweeteners - Directive 95/2/EC				
Calcium carbonate	E170	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetic acid	E260	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium acetate	E261	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium acetates	E262	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Sodium acetate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Sodium hydrogen acetate (sodium diacetate)		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium acetate	E263	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Lactic acid	E270	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Carbon dioxide*	E290	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Malic acid	E296	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ascorbic acid	E300	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium ascorbate	E301	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium ascorbate	E302	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Fatty acid esters of ascorbic acid	E304	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Ascorbyl palmitate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Ascorbyl stearate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Tocopherol-rich extract	E306	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Alpha-tocopherol	E307	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Gamma-tocopherol	E308	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Delta-tocopherol	E309	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Lecithins	E322	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium lactate	E325	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium lactate	E326	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium lactate	E327	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Citric acid	E330	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium citrates	E331	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Monosodium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Disodium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(iii) Trisodium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium citrates	E332	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Monopotassium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Tripotassium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium citrates	E333	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry

(i) Monocalcium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Dicalcium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(iii) Tricalcium citrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Tartaric acid (L(+)-)	E334	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium tartrates	E335	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Monosodium tartrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Disodium tartrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium tartrates	E336	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Monopotassium tartrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Dipotassium tartrate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium potassium tartrate	E337	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium malates	E350	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Sodium malate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Sodium hydrogen malate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium malate	E351	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium malates	E352	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Calcium malate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Calcium hydrogen malate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium tartrate	E354	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Triammonium citrate	E380	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Alginic acid	E400	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium alginate	E401	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium alginate	E402	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ammonium alginate	E403	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium alginate	E404	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Agar	E406	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Carrageenan	E407	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Processed eucheuma seaweed	E407a	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Locust bean gum #	E410	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Guar gum #	E412	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Tragacanth	E413	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acacia gum (gum Arabic)	E414	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Xanthan gum #	E415	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Tara gum #	E417	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Gellan gum	E418	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Glycerol	E422	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Pectins	E440	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry

(i) Pectin				
(ii) amidated pectin				
Cellulose	E460	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Microcrystalline cellulose		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Powdered cellulose		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Methyl cellulose	E461	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ethyl cellulose	E462	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydroxypropyl cellulose	E463	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydroxypropyl methyl cellulose	E464	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ethyl methyl cellulose	E465	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Carboxy methyl cellulose	E466	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Sodium carboxy methyl cellulose		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Cellulose gum		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Enzymatically hydrolysed carboxy methyl cellulose	E469	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Enzymatically hydrolysed cellulose gum		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium, potassium and calcium salts of fatty acids	E470a	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Magnesium salts of fatty acids	E470b	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Mono- and diglycerides of fatty acids	E471	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetic acid esters of mono- and diglycerides of fatty acids	E472a	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Lactic acid esters of mono- and diglycerides of fatty acids	E472b	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Citric acid esters of mono- and diglycerides of fatty acids	E472c	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Tartaric acid esters of mono- and diglycerides of fatty acids	E472d	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids	E472e	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Mixed acetic and tartaric acid esters of mono- and diglycerides of fatty acids	E472f	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium carbonates	E500	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Sodium carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Sodium hydrogen carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(iii) Sodium sesquicarbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium carbonates	E501	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Potassium carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Potassium hydrogen carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ammonium carbonates	E503	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Ammonium carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry

(ii) Ammonium hydrogen carbonate				
Magnesium carbonates	E504	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Magnesium carbonate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Magnesium hydroxide carbonate (syn.: Magnesium hydrogen carbonate)		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydrochloric acid	E507	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium chloride	E508	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium chloride	E509	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Magnesium chloride	E511	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sulphuric acid	E513	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium sulphates	E514	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Sodium sulphate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Sodium hydrogen sulphate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium sulphates	E515	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(i) Potassium sulphate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
(ii) Potassium hydrogen sulphate		Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium sulphate	E516	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium hydroxide	E524	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium hydroxide	E525	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium hydroxide	E526	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ammonium hydroxide	E527	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Magnesium hydroxide	E528	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium oxide	E529	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Magnesium oxide	E530	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Fatty acids	E570	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Gluconic acid	E574	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Glucono-delta-lactone	E575	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Sodium gluconate	E576	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Potassium gluconate	E577	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Calcium gluconate	E578	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Glycine and its sodium salt	E640	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
L-Cysteine (1)	E920	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Argon*	E938	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Helium*	E939	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Nitrogen*	E941	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Nitrous oxide*	E942	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Oxygen*	E948	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydrogen *	E949	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry

Invertase	E1103	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Polydextrose	E1200	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Oxidized starch	E1404	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Monostarch phosphate	E1410	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Distarch phosphate	E1412	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Phosphated distarch phosphate	E1413	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetylated distarch phosphate	E1414	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetylated starch	E1420	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetylated distarch adipate	E1422	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydroxy propyl starch	E1440	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Hydroxy propyl distarch phosphate	E1442	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Starch sodium octenyl succinate	E1450	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Acetylated oxidised starch	E1451	Annex I - 95/2	Quantum S	All processed meat - not fresh meat or poultry
Ascorbic acid	E300	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Sodium ascorbate	E301	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Calcium ascorbate	E302	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Citric acid	E330	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Sodium citrates	E331	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Potassium citrates	E332	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Calcium citrates	E333	Annex II - 95/2	Quantum S	Pre-packed preparations of fresh minced meat
Sorbic acid	E200	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Potassium Sorbate	E202	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Calcium sorbate	E203	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Benzoic acid	E210	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Sodium benzoate	E211	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Potassium benzoate	E212	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Calcium benzoate	E213	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Ethyl-p-hydroxybenzoate	E214	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Sodium ethyl p-hydroxybenzoate	E215	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Methyl p-hydroxybenzoate	E218	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Sodium methyl p-hydroxybenzoate	E219	Annex III - A 95/2	Quantum S	Surface treatment of dried meat products
Sulphur dioxide	E220	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Sodium sulphite	E221	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"

Sodium hydrogen sulphite	E222	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Sodium metabisulphite	E223	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Potassium metabisulphite	E224	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Calcium sulphite	E226	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Calcium hydrogen sulphite	E227	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Potassium hydrogen sulphite	E228	Annex III - B 95/2	450 mg/kg	"Burger meat with minimum vegetable and/or cereal content of 4%" & "Breakfast sausages"
Potassium nitrite	E249	Annex III - C 95/2	Ranging Between 10 - 300 mg/kg	Range of meat products, cured hams, etc.
Sodium nitrite	E250	Annex III - C 95/2	Ranging Between 10 - 300 mg/kg	Range of meat products, cured hams, etc.
Sodium nitrate	E251	Annex III - C 95/2	Ranging Between 10 - 300 mg/kg	Range of meat products, cured hams, etc.
Potassium Nitrate	E252	Annex III - C 95/2	Ranging Between 10 - 300 mg/kg	Range of meat products, cured hams, etc.
Erytorbic acid	E315	Annex III - D 95/2	500 mg/kg	Cured meat products and preserved meat products
Sodium erythorbate	E316	Annex III - D 95/2	500 mg/kg	Cured meat products and preserved meat products
Sodium stearoyl-2-lactylate	E481	Annex IV - 95/2	4 mg/kg	Minced and diced canned meat products
Calcium stearoyl-2-lactylate	E482	Annex IV - 95/2	4 mg/kg	Minced and diced canned meat products
Neohesperidine	E959	Annex IV - 95/2	5 mg/kg	Meat products
Colours - Directive 94/36/EC				
Curcumin	E100	Annex III - 94/36	20 mg/kg	Sausages, pates and terrines
Cochineal	E120	Annex III - 94/36	100 mg/kg	Sausages, pates and terrines
Plain caramel	E150a	Annex III - 94/36	Quantum S	Sausages, pates and terrines
Caustic sulphite caramel	E150b	Annex III - 94/36	Quantum S	Sausages, pates and terrines
Ammonia caramel	E150c	Annex III - 94/36	Quantum S	Sausages, pates and terrines

Sulphite ammonia caramel	E150d	Annex III - 94/36	Quantum S	Sausages, pates and terrines
Carotenes	E160a	Annex III - 94/36	20 mg/kg	Sausages, pates and terrines
Paprika extract	E160c	Annex III - 94/36	10 mg/kg	Sausages, pates and terrines
Beetroot Red	E162	Annex III - 94/36	Quantum S	Sausages, pates and terrines
Allura Red AC	E129	Annex III - 94/36	25 mg/kg	Luncheon meat
Allura Red AC	E129	Annex III - 94/36	25 mg/kg	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Cochineal	E120	Annex III - 94/36	100 mg/kg	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Plain caramel	E150a	Annex III - 94/36	Quantum S	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Caustic sulphite caramel	E150b	Annex III - 94/36	Quantum S	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Ammonia caramel	E150c	Annex III - 94/36	Quantum S	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Sulphite ammonia caramel	E150d	Annex III - 94/36	Quantum S	"Breakfast sausages with a minimum cereal content of 6%" & "Burger meat with a minimum vegetable and/or cereal content of 4%"
Cochineal	E120	Annex III - 94/36	200 mg/kg	Chlorizo sausage - salchichon
Ponceau 4R	E124	Annex III - 94/36	250 mg/kg	Chlorizo sausage - salchichon
Sunset yellow FCF	E110	Annex III - 94/36	135 mg/kg	Sobrasada - sausage
Ponceau 4R	E124	Annex III - 94/36	200 mg/kg	Sobrasada - sausage
Curcumin	E100	Annex III - 94/36	Quantum S	Pasturmas (edible external coating) - A spicy meat
Riboflavin	E101 (i) (ii)	Annex III - 94/36	Quantum S	Pasturmas (edible external coating) - A spicy meat
Cochineal	E120	Annex III - 94/36	Quantum S	Pasturmas (edible external coating) - A spicy meat
All Colours	N/A	Annex V - 1 94/36	Quantum S	Meat and fish analogues based on vegetable proteins
All Colours	N/A	Annex V - 2 94/36	100 mg/kg	Meat and fish analogues based on vegetable proteins

Annex 6

Iron intakes and status of the UK population

Table 9.3: NDNS - Contribution (%) of food types to average daily intake of total iron

	Children 1½-4½		Young people 4-18y		Adults19-64y		Adults 65+ Free-living		Adults 65+ Institutions	
	Males	Females	Male	Females	Males	Females	Males	Females	Males	Females
Cereals	49	48	55	51	44	45	48	47	50	50
white bread	8	8	11	11	*10*	*8*	10	9	10	10
wholemeal bread	3	3	2	2	*3*	*3*	7	7	5	6
soft grain & other bread	-	-	-	-	*3*	*3*	-	-	-	-
whole grain & high fibre breakfast cereals	11	10	12	9	*12*	*13*	10	12	8	9
other breakfast cereals	10	10	17	14	*6*	*7*	5	5	9	8
biscuits, buns, cakes, pastries	5	5	6	8	*4*	*5*	8	8	10	10
Milk & milk products	6	6	3	3	1	1	3	4	4	5
Eggs & egg dishes	2	3	2	2	3	3	3	3	4	4
Fat spreads	0	0	0	0	0	0	0	0	0	0
Meat and meat products	14	14	14	13	19	**15**	**18**	16	17	15
Fish & fish dishes	2	2	1	2	2	3	3	2	2	2
Vegetables (excluding potatoes)	7	7	7	8	9	11	8	10	8	8
Potatoes & savoury snacks	7	7	7	7	7	8	7	7	5	5
Fruit and nuts	3	3	1	2	2	3	3	3	3	3
Sugars, preserves, confectionery	4	3	4	4	2	2	1	1	1	1
Drinks	3	3	1	2	7	6	3	3	1	1
Misc	2	2	2	2	3	3	3	4	5	6
Total no respondents (w)	-	-	-	-	*833*	*891*	*540*	*735*	*93*	*319*
Total no respondents (unw)	*848*	*827*	*856*	*845*	*766*	*958*	*632*	*643*	*204*	*208*

* includes soft drinks, alcoholic drinks, tea, coffee and water

** includes powdered beverages (except tea and coffee), soups, sauces, condiments and artificial sweeteners

Table 9.4: LIDNS - Contribution (%) of food types to average daily intake of total iron

	Children (2-10 y)		Adults (19 y and over)	
	Boys	**Girls**	**Men**	**Women**
Cereals and cereal products	53	49	40	41
Milk and milk products	2	2	1	1
Eggs and egg dishes	2	2	4	3
Fat spreads	0	0	0	0
Meat and meat products	17	16	23	21
Fish and fish dishes	1	2	2	3
Vegetables excluding potatoes	7	8	10	10
Potatoes and savoury snacks	10	11	8	9
Fruit and nuts	1	2	2	2
Sugars, preserves and confectionery	3	3	2	2
Drinks*	1	2	5	4
Miscellaneous**	2	2	3	4
Total no. respondents (unw)	*439*	*493*	*946*	*1850*

* includes soft drinks, alcoholic drinks, tea, coffee and water
** includes powdered beverages (except tea and coffee), soups, sauces, condiments and artificial sweeteners

Table 9.5: NDNS - Total mean (median) iron intake from all sources and food sources

	MALE				FEMALE			
Age (years)	Mean (median) intake - all sources, mg/d	Mean (median) intake - food sources, mg/d	*Base (w)*	*Base (unw)*	Mean (median) intake from all sources, mg/d	Mean (median) intake food sources, mg/d	*Base (w)*	*Base (unw)*
Children 1.5-4.5	**5.7** (5.4)	**5.5** (5.4)	-	*848*	**5.4** (5.0)	**5.2** (5.0)	-	*827*
1.5-2.5*	5.0 (4.7)	4.9 (4.7)	-	*288***	5.0 (4.7)	4.9 (4.7)	-	*288***
2.5-3.5*	5.6 (5.4)	5.4 (5.3)	-	*303***	5.6 (5.4)	5.4 (5.3)	-	*303***
3.5-4.3	6.2 (5.9)	6.1 (5.9)	-	*250*	5.9 (5.5)	5.6(5.5)	-	*243*
Young people 4-18	**10.5** (9.9)	**10.4** (9.8)	*3331*	*856*	**8.5** (8.0)	**8.3** (7.9)	*3159*	*845*
4-6	8.3 (8.0)	8.2 (7.9)	*1134*	*184*	7.4 (7.1)	7.3 (7.1)	*656*	*171*
7-10	9.8 (9.3)	9.7 (9.3)	*912*	*256*	8.5 (8.2)	8.4 (8.2)	*866*	*226*
11-14	10.8 (10.4)	10.8 (10.4)	*870*	*237*	9.1 (8.6)	8.8 (8.4)	*821*	*238*
15-18	12.6 (11.7)	12.5 (11.6)	*861*	*179*	8.9 (8.2)	8.7 (8.0)	*816*	*210*
Adults 19-64	**14** (12.9)	**13.2** (12.6)	*833*	*766*	**11.6** (10.0)	**10.0** (9.6)	*891*	*958*
19-24	11.5 (11.3)	11.4 (11.2)	*108*	*61*	10.0 (9.3)	8.8 (9.1)	*104*	*78*
25-34	13.9 (12.8)	13.0 (12.5)	*219*	*160*	9.8 (9.0)	9.2 (9.0)	*210*	*211*
35-49	14.1 (13.2)	13.7 (13.1)	*253*	*303*	12.9 (10.5)	10.2 (10.1)	*318*	*379*
50-64	15.2 (13.6)	13.6 (13.3)	*253*	*242*	12.3 (11.0)	10.9 (10.6)	*259*	*290*
Adults 65 & over								
Free-living	**11.6** (10.6)	**11.0** (10.5)	*540*	*632*	**8.9** (8.4)	**8.6** (8.3)	*735*	*643*
65-74	11.9 (10.6	11.1(10.5)	*353*	*271*	9.3 (8.7)	9.0 (8.6)	*409*	*256*
75-84	11.1 (10.7)	10.8 (10.5)	*160*	*265*	8.5 (8.1)	8.4 (8.1)	*249*	*217*
85 +	10.6 (9.7)	10.4 (9.7)	*26*	*96*	7.9 (7.6)	7.7 (7.5)	*77*	*170*
Institutionalised	**9.6** (9.3)	**9.6** (9.3)	*93*	*204*	**8.3** (7.9)	**8.2** (7.9)	*319*	*208*
65-84	9.6 (9.2)	9.6 (9.2)	*57*	*128*	8.7 (8.1)	8.6 (8.1)	*144*	*91*
85 +	9.7 (9.3)	9.6 (9.3)	*36*	*76*	8.0 (7.7)	7.8 (7.6)	*174*	*117*

** half of the base figure for the sum of boys and girls as data combined in report

Table 9.6: LIDNS - Total mean (median) daily intake of iron (mg) from food sources only

Age (years)	**Male**	*Base(unw)*	**Female**	*Base (unw)*
Children aged 2-10 y	9.0 (8.6)	*239*	7.9 (7.7)	*278*
Young people 11-18 y	11.4 (10.6)	*200*	9.3 (9.0)	*215*
Adults 19-64 y				
19-34y	11.6 (11.0)	*194*	8.5 (8.0)	*483*
35-49y	10.8 (10.4)	*226*	8.7 (7.8)	*494*
50-64y	11.5 (11.4)	*258*	8.8 (8.5)	*336*
Adults 65 y and over	10.2 (9.8)	*268*	9.0 (8.6)	*537*

Table 9.7: NDNS - Contribution of haem iron to total iron intake mg/d (%)/median

Age (years)	**Males**	**Females**
Children 1.5-4.5	0.2 (3.5%)/0.2	0.3 (5.6%)/0.2
Young people 4-18	0.4 (3.8%)/0.4	0.3 (3.5%)/0.3
Adults 19-64	0.8 (5.7%)/0.7	0.5 (4.3%)/0.5
Adults 65 & over		
Free-living	0.7(6%)/0.6	0.5 (5.6%)/0.4
Institutionalised	0.6 (6.3%)/0.5	0.4 (4.8)/0/4

base numbers as in table 9.4

Table 9.8: LIDNS - Contribution of haem iron to total iron intake mg/d (%)/median

Age (years)	**Males**	**Females**
Children 2-10	0.4 (4.4%)/0.3	0.4 (5.1%)/0.0.3
Young people 11-18	0.6 (5.3%)/0.4	0.5 (5.4%)/0.4
Adults 19-64	0.88 (7.8%)	0.54 (6.2%)
19-34	0.8 (6.9%)/0.7	0.5 (5.9%/0.5)
35-49	0.8 (7.4%)/0.6	0.6(6.9%)/0.5
50-64	1.0 (8.7%)/0/8	0.5 (5.7%)/0.4
Adults 65 & over	0.7 (6.9%)/0.6	0.6 (6.6%)/0.5

base numbers as in table 9.5

Table 9.9: NDNS - Average total daily iron intakes (from all sources) as a proportion of DRVs

	Male		Female	
	%<LRNI	Mean intake as % of RNI	%<LRNI	Mean intake as % of RNI
Children 1.5-4.5 y				
1.5-2.5 y	24*	73	24*	73*
2.5-3.5 y	12*	81	12*	81*
3.5-4.5 y	4	95	4	92
Young people 4-18 y				
4-6 y	0	136	1	121
7-10 y	1	112	3	97
11-14 y	1	96	44	61
15-18 y	0	111	48	60
Adults 19-64y				
19-24y	3	133	40	68
25-34y	0	160	40	66
35-49y	1	163	25	87
50-64y	1	174	4	137
Adults 65 y +				
Free-living				
65-74 y	0	133	4	102
75- 84 y	2	133	6	102
85+	4	133	10	102
Institutions				
65-84 y	4	111	3	95
85+ y	5	111	8	95

* boys and girls combined
base numbers as in table 9.4

Table 9.10: LIDNS - Average total daily iron intakes (from food sources) as a proportion of DRVs

	Male		Female	
	%<LRNI	Mean intake as % of RNI	%<LRNI	Mean intake as % of RNI
Children 2-18 y				
2-10 y	2	120	2	107
11-18 y	14	101	39	63
Adults 19-65+y				
19-34y	5	134	49	58
35-49y	5	124	52	59
50-64y	4	133	13	99
65+y	3	117	5	103

base numbers as in table 9.5

Table 9.11: NDNS - Proportion with Hb concentration below WHO cut-offs*

Age group (y)	Male			Female		
	Hb cut-off (g/L)	*Base*	% below cut-off	Hb cut-off (g/L)	*Base*	% below cut-off
1.5-4.5	110	*475 (unw)*	8.1	110	*476 (unw)*	9.1
4-6	110	*81 (unw)*	2.5	110	*76 (unw)*	9.1
	115		7.8	115		15.2
7-10	115	*176 (unw)*	1.4	115	*133 (unw)*	4.6
11-14	115	*166 (unw)*	3.1	115	*157 (unw)*	1.8
	120		8.1	120		4.3
15-18	130	*140 (unw)*	1.1	120	*156 (unw)*	9.1
19-24	130	*45 (w)*	0.0	120	*44 (w)*	6.9
25-34	130	*107 (w)*	2.1	120	*146 (w)*	9.1
35-49	130	*213 (w)*	3.7	120	*278 (w)*	9.0
50-64	130	*168 (w)*	3.4	120	*191 (w)*	6.7
65-74 free-living	130	*284 (w)*	7.0	120	*311 (w)*	5.9
75-84 free-living	130	*125 (w)*	16.0	120	*190 (w)*	13.1
85+ free-living	130	*20 (w)*	37.5	120	*53 (w)*	16.0
65+ Institutionalised	130	*147 (w)*	52.2	120	*135 (w)*	38.6

**Except for children aged 1½-4½y, weighting factors were used to adjust for over- or under- sampling of subgroups of the population, based on age, sex, and socio-demographic factors*

Table 9.12: LIDNS - Proportion with Hb concentration below WHO cut-offs*

Age group (y)	Male			Female		
	Hb cut-off (g/L)	*Base (unw)*	% below cut-off	Hb cut-off (g/L)	*Base (unw)*	% below cut-off
19-34	130	*67*	1	120	*205*	12
35-49	130	*99*	0	120	*250*	18
50-64	130	*135*	5	120	*181*	6
65+	130	*155*	20	120	*270*	9

**Children aged 8-18 not included as sample sizes were too small*

Table 9.13: NDNS - Proportion with serum ferritin concentrations below WHO cut-offs

Age group (y)	SF cut-off (µg/L)	Male	*Base*	Female	*Base*
1.5-4.5	12	33.5	*467 (unw)*	25.1	*463 (unw)*
4-6	12	4.1	*65 (unw)*	6.3	*57 (unw)*
	15	9.8		9.2	
7-10	15	6.7	*141 (unw)*	2.9	*93 (unw)*
11-14	15	5.0	*137 (unw)*	11.5	*121 (unw)*
15-18	15	2.7	*110 (unw)*	23.8	*127 (unw)*
19-24	15	0.0	*45 (w)*	16.0	*53 (w)*
25-34	15	0.0	*119 (w)*	8.2	*157 (w)*
35-49	15	2.5	*245 (w)*	12.5	*298 (w)*
50-64	15	2.3	*191 (w)*	8.6	*21 (w)0*
65-74 (free-living)	15	5.2	*272 (w)*	6.2	*306 (w)*
75-84 (free-living)	15	5.8	*121 (w)*	12.3	*177 (w)*
85+ (free-living)	15	7.4	*19 (w)*	14.3	*49 (w)*
65+ (Institutions)	15	8.0	*63 (w)*	10.2	*122 (w)*

Table 9.14: LIDNS - Proportion with serum ferritin concentrations below WHO cut-offs

Age group (y)	SF cut-off (µg/L)	Male	Female
19-34	15	0	21
35-49	15	0	14
50-64	15	4.9	5
65+	15	2.3	4

Base numbers as for table 9.

Table 9.15: NDNS: Proportion with Hb and SF concentrations below WHO cut-offs

Age group (y)	Male			Female		
	Hb (g/L) cut-off	SF (µg/L) cut-off	% below Hb & SF cut-offs	Hb (g/L) cut-off	SF (µg/L) cut-off	% below Hb & SF cut-offs
1.5-2.4	110	12	5.8	110	12	6.0
2.5-3.4	110	10	2.3	110	10	3.0
	110	12	3.5	110	12	3.6
3.5-4.5	110	10	2.2	110	10	2.8
	110	12	2.2	110	12	2.8
4-6	110	12	0.0	110	12	1.7
	110	15	0.0	110	15	1.7
	115	12	0.0	115	12	2.5
	115	15	0.0	115	15	1.7
7-10	115	15	0.6	115	15	0.0
11-14	115	15	1.2	115	15	1.9
15-18	130	15	0.0	120	15	5.0
19-24	130	15	0.0	120	15	3.8
25-34	130	15	0.0	120	15	2.0
35-49	130	15	0.9	120	15	4.8
50-64	130	15	0.5	120	15	2.9
65-74 (free-living)	130	15	1.4	120	15	1.6
75-84 (free-living)	130	15	0.5	120	15	3.3
85+ (free-living)	130	15	5.7	120	15	5.6
65+ (Institutions)	130	15	5.0	120	15	3.3

Table 9.16: LIDNS: Proportion with Hb and SF concentrations below WHO cut-offs

Age group (y)	Male			Female		
	Hb (g/L) cut-off	SF (µg/L) cut-off	% below Hb & SF cut-offs	Hb (g/L) cut-off	SF (µg/L) cut-off	% below Hb & SF cut-offs
19-34	130	15	0.0	120	15	9.1
35-49	130	15	0.0	120	15	10.5
50-64	130	15	0.0	120	15	2.9
65+	130	15	2.3	120	15	6.3

Annex 7

Modelling the impact of reductions in total re meat consumption on iron, zinc, and vitamin d intakes

ANNEX 7

Modelling the impact of reduced red meat consumption on iron, zinc, and vitamin D intakes

Summary

1. The aim of this analysis was to estimate current consumption of total red meat and to use this information to model the impact of reducing total red meat consumption on iron and zinc intakes. An assessment was also made of the effect of reducing total red meat consumption on vitamin D intakes.
2. The analysis showed that amongst red meat consumers mean current consumption of total red meat is70 g/d (88g/d for men and 52g/d for women). The modelling assessed the impact of reducing total red meat intakes to different maximum levels down to 50g/d. It showed reducing total red meat consumption to these levels had very little effect with respect to iron and vitamin D intakes. For zinc however, the proportion of the population with intakes below the lower reference intake[7] (LRNI) (DH, 1991) may be of concern[8] for men at intakes of 70g red meat per day.[9]

Background

3. Epidemiological studies suggest a link between red and processed meat consumption and risk of colorectal cancer (CRC). The available data suggest that processed meat consumption is associated with CRC risk independently of red meat. As these meats are a source of iron and zinc in the diets of the UK population, any recommendation to reduce consumption could increase the proportion of the population with intakes below the LRNI for these nutrients. Red meat is also a dietary source of vitamin D, so reduced consumption could also reduce dietary vitamin D intake.

Methods

Defining categories for analysis

4. Processed meat typically refers to meat (usually red meat) that has been preserved by smoking, curing, salting, or by the addition of preservatives (WCRF, 2007), however there is considerable inconsistency in the definition and categorisation of “processed meat” in epidemiological studies.
5. Following a review of the evidence by Food Standards Agency (FSA) toxicologists, it was advised that the available data do not support an association between increased CRC risk and the presence of preservatives (see Annex 5). The modelling exercise therefore considered the impact of a reduction in total red meat consumption (as defined in appendix 1) as there was no clear basis for separating red and processed meat.

[7] The amount of a nutrient that is likely to meet the needs of only 2.5% of the population.
[8] 5% of the population failed to meet the LRNI.
[9] It should be noted that the results observed are dependent on the modelling approach taken. It is assumed: (a) that low red meat consumers are not affected by a reduction in red meat intakes and (b) that the red meat removed from the diet is not replaced by other food sources of iron, zinc or vitamin D.

Estimates of total red meat consumption

6. Currently there are no accurate estimates for total red meat consumption in the UK. The National Diet and Nutrition Surveys (NDNS) provide estimates of total meat and meat dishes consumed. Red meat reported as consumed on its own, e.g. roast beef, can be isolated, but red meat products and meals containing red meat are composite dishes that also contain non-meat components (e.g. sausage rolls, lasagne and pies). These composite dishes are reported as total amount consumed, resulting in an overestimation of red meat consumption.
7. In order to obtain more realistic estimates of total red meat consumption, composite dishes were disaggregated i.e. the actual amount of red meat within these products was identified. For recipe data collected in the survey this was simply a case of identifying the meat component within the recipe. For manufactured meat products and dishes reported within the NDNS, composition data and ingredient declaration was used to establish the percentage red meat content of brand leaders (appendix 2).
8. The analysis focused on adults aged 19-64 years (Henderson *et al,* 2002) (data collected 2000/01). Data for the NDNS adults aged over 65 years was collected in 1994/5 and was not included within the analysis as it was considered of limited validity.

Assigning values for iron and zinc content of types of red meat and products

9. Typical iron and zinc values, obtained from *McCance & Widdowson's The Composition of Foods* meat supplement publications (Chan *et al,* 1995; Chan *et al,* 1996), were assigned to each meat type (appendix 1, table 2). These values were used, together with estimates of consumption of each meat type within all red meat containing foods, to estimate current iron and zinc intakes from total red meat.

Estimation of current total iron and zinc intakes

10. In order to assess current total iron and zinc intakes from all foods, data from trade associations[10] as well as retail label data were checked for details of fortified foods currently available on the market, to reflect current fortification practices i.e. practices of voluntarily fortifying products with micronutrients may have changed since the survey was carried out and iron and zinc values for specific products may therefore have changed. However the amendments made to nutrient databank values made no difference to overall population iron and zinc intakes.

Modelling the effect of reduced total red meat consumption on iron and zinc intakes

11. The aim of the modelling exercise was to consider the effect of reducing total red meat consumption on a) mean intakes of iron and zinc and b) the proportion of adults with iron and zinc intakes below the lower reference nutrient intakes (LRNI) for iron and zinc. The LRNI represents the amount of a nutrient likely to meet the needs of only 2.5% of the population (DH, 1991). This means that, in a healthy replete population, about 2.5% of the population would be expected to have intakes below the LRNI. Intakes below the

[10] Personal communication with The Food and Drink Federation (FDF) October 2008 in relation to foods currently fortified with iron. The British Retail Consortium (BRC) was also contacted requesting information on foods fortified with iron.

LRNI are almost certainly inadequate for most individuals.[11] It was considered a matter of concern if 5% or more of the population had intakes below the LRNI.

12. The potential effect of reducing total red meat consumption was assessed for the following maximum levels per day: 180g, 160g, 140g, 120g, 100g, 90g[12], 80g, 70g[13], 60g, 50g and 0g (see tables 2 & 3). Consumers exceeding the maximum consumption of red meat were reduced to the maximum, consumers below the maximum were left unchanged.

Results

Current total red meat consumption, and iron and zinc intakes

Table 1: Current total red meat consumption (consumers of red meat only, 94% of survey participants) (2 significant figures).

	Mean (95% CI)	**Median**	**97.5%ile**	**Max**
Total red meat (g/d)				
Men	88 (84.3-91.1)	81	209	323
Women	52 (49.7-54.4)	48	133	249
Iron from total red meat (mg/d)				
Men	1.5 (1.5-1.6)	1.3	4.5	8.9
Women	0.9 (0.8-1)	0.8	2.7	7.5
Zinc from total red meat (mg/d)				
Men	3.3 (3.2-3.4)	3.0	7.8	12.8
Women	2.0 (1.9-2.1)	1.8	5.8	10.5

13. Table 1 illustrates:

- Current estimated mean total red meat consumption (consumers of red meat only) is 70 g/d (88g/d for men and 52g/d for women)[14]. As previous estimates of meat consumption included non-meat components, there are no data available for simple comparison. However the adults NDNS estimated mean daily consumption (for meat consumers only) of all meat, meat dishes and meat products (including poultry and other white meat) at 204g for men and 135g for women.

[11] The effect of reducing red meat consumption on the percentage of adults with intakes below the reference nutrient intake (RNI) was not considered. This is because the RNI represents the amount of a nutrient likely to meet the needs of 97.5% of the population (DH, 1991); therefore in a healthy, replete population there would be no cause for concern if up to 50% of adults had intakes below the RNI.

[12] Daily recommendations for total red meat consumption (maximum 90g per day) - Committee on Medical Aspects of Food Policy (COMA) (DH, 1998). The COMA figure is based on almost no disaggregation.

[13] Based on weekly recommendations for red meat consumption (maximum 500g per week) - The World Cancer Research Fund (WCRF) (WCRF, 2007). The WCRF committee did not address the issue of disaggregation, however the most recent studies included in their meta-analysis (i.e. EPIC studies) were based on some disaggregation.

[14] Average consumption of total red meat for the UK population is 66 g/d (94% of consumers). Average consumption of red meat is 85 g/d for males and 47 g/d for females (i.e. 97% of males and 91% of females are consumers of red meat).

- Estimated mean intake of iron from total red meat consumption is 1.5mg/d for men and 0.9mg/d for women.
    - Therefore the estimated amount of iron obtained per gram of total red meat is 0.017mg.
- Estimated mean intake of zinc from total red meat consumption is 3.3mg/d for men and 2.0mg/d for women.
    - Therefore the estimated amount of zinc obtained per gram of total red meat is 0.038mg.

ANNEX 7

Modelling the effect of reduced total red meat consumption on mean intakes of iron and zinc

Table 2: Mean iron and zinc intakes from all foods (excluding contribution from supplements) at current levels of total red meat consumption, and at various thresholds of maximum consumption. Data illustrated for consumers only (94% of survey participants) (2 significant figures).

Iron intake from ALL FOODS		**Current consumption**	**Max 180g/d**	**Max 120g/d**	**Max 100g/d**	**Max 90g/d**	**Max 80g/d**	**Max 70g/d**	**Max 60g/d**	**Max 50g/d**	**0g/d**
Men	**Mean (mg/d)**	13	13	13	13	13	13	13	13	13	12
	95% CI	(12.9-13.5)	(12.8-13.5)	(12.7-13.4)	(12.6-13.3)	(12.6-13.2)	(12.5-13.1)	(12.4-13)	(12.3-12.9)	(12.2-12.8)	(11.4-12.1)
Women	**Mean (mg/d)**	10	10	10	10	10	10	9.9	9.9	9.8	9.2
	95% CI	(9.8-10.3)	(9.8-10.3)	(9.8-10.3)	(9.8-10.2)	(9.7-10.2)	(9.7-10.2)	(9.7-10.2)	(9.6-10.1)	(9.6-10.1)	(9-9.5)
Zinc intake from ALL FOODS											
Men	**Mean (mg/d)**	10	10	9.9	9.7	9.5	9.4	9.2	8.9	8.7	7.0
	95% CI	(10-10.4)	(9.9-10.4)	(9.7-10.1)	(9.5-9.9)	(9.4-9.7)	(9.2-9.6)	(9-9.4)	(8.7-9.1)	(8.5-8.9)	(6.8-7.2)
Women	**Mean (mg/d)**	7.5	7.4	7.4	7.4	7.3	7.2	7.1	7.0	6.9	5.6
	95% CI	(7.2-7.5)	(7.2-7.5)	(7.2-7.5)	(7.2-7.4)	(7.1-7.4)	(7.1-7.3)	(7-7.3)	(6.9-7.2)	(6.8-7)	(5.5-5.7)

14. Table 2 illustrates that:

- Current mean iron intake from all foods (excluding supplements) for consumers of red meat is 13mg/d for men and 10mg/d women. As current mean intake of iron from total red meat consumption is 1.5mg/d for men and 0.9mg/d for women (see table 1), the contribution of iron from total red meat to total iron intake is approximately 12% and 9% for men and women respectively. [15]
- Current mean zinc intake from all foods (excluding supplements) for consumers of red meat is 10mg/d for men and 7.5mg/d women consumers. As current mean intake of zinc from total red meat consumption is 3.3mg/d for men and 2mg/d for women (see table 1), the contribution of zinc from total red meat to total zinc intake is approximately 32% and 27% men and women respectively. [9]
- Reducing total red meat consumption to a maximum of **100g/d** would cause (for consumers of red meat only):
  - No change in total iron intake (13mg/d for men and 10mg/d for women).
  - A reduction in total zinc intake from 10mg/d to 9.7mg/d for men and 7.5mg/d to 7.4mg/d for women.

[15] Non rounded figures were used in calculations.

- Reducing total red meat consumption to a maximum of **90g/d** (COMA recommendation) would cause (for consumers of red meat only):
  - No change in total iron intake (13mg/d for men and 10mg/d for women).
  - A reduction in total zinc intake from 10mg/d to 9.5mg/d for men and 7.5mg/d to 7.3mg/d for women.

- Reducing total red meat consumption to a maximum of **80g/d** would cause (for consumers of red meat only):
  - No change in total iron intake (13mg/d for men and 10mg/d for women).
  - A reduction in total zinc intake from 10mg/d to 9.4mg/d for men and 7.5mg/d to 7.2mg/d for women.

- Reducing total red meat consumption to a maximum of **70g/d** (WCRF recommendation would cause (for consumers of red meat only):
  - No change in total iron intake for men (13mg/d) and a reduction in total iron intake from 10mg/d to 9.9mg/d for women.
  - A reduction in total zinc intake from 10mg/d to 9.4mg/d for men and 7.5mg/d to 7.2mg/d for women.

ANNEX 7

Modelling the effect of reduced total red meat consumption on % of the population below LRNI for iron and zinc

Table 3: Effect of reducing maximum total red meat consumption on the proportion of adults with iron and zinc intakes below the LRNI, including non consumers of red meat, excluding contribution from supplements (% to 2 significant figures).

		Current consumption	Max 180g/d	Max 120g/d	Max 100g/d	Max 90g/d	Max 80g/d	Max 70g/d	Max 60g/d	Max 50g/d	0g/d
Men	% population exceeding each		5	20	33	42	50	58	66	74	97
Women			0.3	4.1	8.3	12	17	23	33	43	91
Iron intake											
Men	**%<LRNI**[16]	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.8
	95% CI	(0.2-1.5)	(0.2-1.5)	(0.3-1.7)	(0.3-1.7)	(0.3-1.7)	(0.3-1.7)	(0.3-1.7)	(0.3-1.7)	(0.3-1.7)	(1.7-3.9)
Women	**%<LRNI**	25	25	25	25	25	26	26	27	27	32
	95% CI	(22-27.7)	(22-27.7)	(22.4-28.1)	(22.6-28.3)	(22.6-28.3)	(22.7-28.4)	(23.3-29.1)	(23.7-29.5)	(24.3-30.1)	(29.3-35.4)
Zinc intake											
Men	**%<LRNI**[17]	3.7	3.9	3.9	4.0	4.1	4.4	5.5	6.1	9.5	29
	95% CI	(2.4-5)	(2.6-5.2)	(2.6-5.2)	(2.7-5.3)	(2.8-5.5)	(3-5.8)	(3.9-7)	(4.5-7.8)	(7.3-11.2)	(26.3-32.5)
Women	**%<LRNI**	3.8	3.9	3.9	3.9	3.9	3.9	3.9	4.1	5.0	20
	95% CI	(2.5-5.1)	(2.6-5.1)	(2.6-5.1)	(2.6-5.1)	(2.6-5.1)	(2.6-5.1)	(2.6-5.1)	(2.8-5.4)	(3.6-6.4)	(17.3-22.5)

15. Table 3 illustrates that:

- Reducing total red meat consumption to a maximum of **100g/d** would cause:
  - The proportion of adults with intakes below the LRNI for iron to increase from 0.9% to 1% for men and remain at 25% for women.

[16] The LRNI for iron is 8mg/d females aged 11-50 years; 6.1mg/d males aged 11-18years; 4.7mg/d for males aged 19 years above and females aged 50 years and above (DH, 1991).
[17] The LRNI for zinc is 5.3mg/d for males and females aged 11-14years; 4mg/d for females aged 15 years and above and 5.5mg/d for males aged 15years and above (DH, 1991).

- The proportion of adults with intakes below the LRNI for zinc to increase from 3.7% to 4.0% for men and from 3.8% to 3.9% for women.[18]

➢ Reducing the total red meat consumption to a maximum of **90g/d** (COMA recommendation) would cause:

- The proportion of adults with intakes below the LRNI for iron to increase from 0.9% to 1% for men and remain at 25% for women.
- The proportion of adults with intakes below the LRNI for zinc to increase from 3.7% to 4.1% for men and from 3.8% to 3.9% for women.

➢ Reducing the total red meat consumption to a maximum of **80g/d** would cause:

- The proportion of adults with intakes below the LRNI for iron to increase from 0.9% to 1% for men and from 25% to 26% for women.
- The proportion of adults with intakes below the LRNI for zinc to increase from 3.7% to 4.4% for men and from 3.8% to 3.9% for women.

➢ Reducing the total red meat consumption to a maximum of **70g/d** (WCRF recommendation) would cause:

- The proportion of adults with intakes below the LRNI for iron to increase from 0.9% to 1% for men and from 25% to 26% for women.
- The proportion of adults with intakes below the LRNI for zinc to increase from 3.7% to 5.5% for men and from 3.8% to 3.9% for women.

[18] Females aged 19-24 years specifically (i.e. those considered most at risk from low iron intakes within this population group), had a similar pattern of change compared to all women, for example reducing the total red meat consumption to a maximum of **100g/d**, increased the proportion failing to meet the LRNI for iron from 42% to 44% however proportion failing to meet the LRNI for zinc remained at 5.4%.

Modelling the effect of reduced total red meat consumption – Mean intakes for vitamin D

16. The modelling exercise also considered the effect of reducing total red meat consumption on mean vitamin D intake. Vitamin D is found in a few foods, such as oily fish, fortified margarines and breakfast cereals; smaller amounts are found in red meat and egg yolk (Food Standards Agency, 2002). There are currently no dietary recommendations for vitamin D intake for the general population in the UK, as the main source of vitamin D is from the action of sunlight on the skin (DH, 1991).[19]

17. Low vitamin D status is apparent in some groups of the UK population[20] (Ruston *et al,* 2004). As meat is a dietary source of vitamin D (Henderson *et al*, 2003), the effect of reducing consumption of total red meat to down to a maximum of 50g/d on mean intakes of vitamin D was assessed. Typical vitamin D values obtained from *McCance & Widdowson's The Composition of Foods* meat supplement publications (Chan *et al,* 1995; Chan *et al,* 1996) were assigned to each meat type (appendix 2 table 2).

18. The results of the analysis (for consumers of red meat only) were as follows:

    Estimated mean intake of vitamin D from the diet (excluding supplements) is 3.7µg/d for men and 2.9µg/d for women.

    The estimated mean intake of vitamin D from red meat is 0.68µg/d for men and 0.39µg/d for women, which accounts for about 1/6 of dietary vitamin D.

    Reducing total red meat consumption to a maximum of 100g/d or 90g/d would cause a reduction in mean vitamin D intakes: from a current intake of 3.7µg/d for men to 3.6µg/d; and from 2.9µg/d to 2.8µg/d for women.

    Reducing total red meat consumption to a maximum of 80g/d or 70g/d would cause a reduction in mean vitamin D intakes: from a current intake of 3.7µg/d for men to 3.5µg/d; and from 2.9µg/d to 2.8µg/d for women.

    Reducing total red meat consumption to a maximum of 50g/d would cause a reduction in mean vitamin D intakes: from a current intake of 3.7µg/d for men to 3.4µg/d; and from 2.9µg/d to 2.7µg/d for women.

Limitations of the Modelling

19. The analyses carried out in the modelling exercise on the effect of reducing total red meat consumption on the proportion of adults with intakes below the LRNI for iron and zinc, and on overall iron, zinc and vitamin D intakes, represent a worse case scenario and overestimate the likely real-life effect. The modelling exercise did not attempt to incorporate the potential intakes of iron, zinc and vitamin D that might be obtained by replacing the reduction in total red meat consumption

[19] Specific population groups at risk from low vitamin D status, i.e. those confined to the indoors, Asian women and children living in UK, pregnant and lactating women, and the elderly are recommended to consume 10µg/d vitamin D in the form of a vitamin supplement (DH, 1991).

[20] Data from the NDNS suggest low vitamin status in 3% of children aged 4-6 years, 11% in 11-14 years, 13% in 15-18 years, 15% of adults aged 19-64 years, and 8% of adults over 65 years.

with other foods, such as white meat/poultry or other food groups, some of which are likely to contain iron, zinc and vitamin D.

20. The modelling did not look at the effect of reducing total red meat intakes on low red meat consumers, as it was assumed advice to reduce high levels of red meat consumption would be taken up by high red meat consumers only.

21. The analyses assessed the impact on iron, zinc, and vitamin D intakes, however it is also possible that reducing total red meat consumption may have impact on other nutrients such as total energy, fat and saturated intake, depending on the nutrient profile of foods replacing red meat in the diet. This may be of public health benefit given energy balance concerns about excesses of energy and saturated fat in the diet.

22. The modelling focuses on the impact of a reduction in red meat consumption on the iron, zinc and vitamin D intakes and does not consider at status. Iron status is considered in the main report.

Summary of Results

23. Current estimated mean total red meat consumption (consumers of red meat only) is 88g/d for men and 52g/d for women.

24. Red meat makes a greater contribution to total zinc intake from all foods than to total iron intake: the contribution of iron from total red meat to total iron intake is 12% for men and 9% for women; the contribution of zinc from total red meat to total zinc intake is 32% for men and 27% for women.

25. Reducing red meat to a maximum of 100g/d or 90g/d would have a minimal effect on the proportion of people with intakes below the LRNI for iron or zinc.

26. Reducing total red meat to a maximum of 80g/d or 70g/d would have a minimal effect on the proportion of people with intakes below the LRNI for iron, but would increase the proportion of men with intakes below the LRNI for zinc from 3.7% to 4.4% and over 5% respectively.

27. Red meat contributes approximately one-sixth of dietary vitamin D intake. Reducing total red meat to a maximum of 100g/d, 90g/d, 80g/d or 70g/d would have little effect on dietary vitamin D intakes of men or women.

References

Chan W, Brown J, Lee S, and Buss DH. *Meat, Poultry and Game. Fifth supplement to 5th edition of McCance and Widdowson's The Composition of Foods*. Royal Society of Chemistry, Cambridge, 1995.

Chan W, Brown J, Church SM and Buss DH. *Meat Products and Dishes. Sixth supplement to 5th edition of McCance and Widdowson's The Composition of Foods*. Royal Society of Chemistry, Cambridge, 1996.

Department of Health. *Dietary Reference Values for Food, Energy and Nutrients in the United Kingdom. (Report on Health and Social Subjects, No. 41).* London: HSMO, 1991.

Department of Health. *Nutritional Aspects of the Development of Cancer. Report on Health and Social Subjects, No.48 .* London: HMSO, 1998.

Henderson L, Gregory J, & Swan G. *National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 1: Types and quantities of foods consumed*. London: TSO, 2002.

Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G & Farron M. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes. TSO London: TSO, 2003.

Food Standards Agency *McCance and Widdowson's The Composition of Foods, Sixth summary edition.* Cambridge: Royal Society of Chemistry, Cambridge, 2002.

Ruston D, Hoare J, Henderson L, Gregory J, Bates CJ, Prentice A, Birch M, Swan G & Farron M. National Diet and Nutrition Survey: adults aged 19-64 years. Volume 4: Nutritional Status (anthropometry and blood analytes), blood pressure and physical activity. London:TSO, 2004.

World Cancer Research Fund. *Food, Nutrition, Physical Activity and the Prevention of Cancer: a global perspective*. 2007 [Available online] www.dietandcancerreport.org/

ANNEX 7

Appendix 1

Estimation of current red meat consumption and modelling the effect of reduced consumption on iron, zinc and vitamin D intakes

Table 1: Assumptions made in order to estimate current total red meat consumption in relation to colorectal cancer risk and the effect of reducing red meat consumption on iron, zinc and vitamin D intakes in UK adults.

Subject	**Assumption**	**Comments**
	Estimation of red meat consumption	
Categorisation of total red meat	**Risk factors for red and processed meat CRC risk** • The epidemiological evidence indicates that increased consumption of red and processed meat increases the risk of colorectal cancer (CRC). • By contrast, an increased risk of CRC is not observed with increased total meat and therefore increased chicken or fish intakes are not considered to pose a risk. • The data suggests that the CRC risk is associated with "processed meat" independently of red meat consumption (see main report for details). • From a toxicological perspective, there are three possible mechanisms that may explain a link between red and processed meat and CRC: • The first is related to haemoglobin levels, the second to compounds created on cooking and the third is in relation to preservatives present in processed meats (see main report for details). • However there seems to be no clear evidence to identify the most likely mechanism. • This modelling work therefore focused on total red meat consumption. • It was considered prudent however not to rule out any affect the presence of preservatives (excluding sodium chloride) may have on CRC risk. An exercise was therefore carried out to identify red meat and red meat products that do and do not contain preservatives. The details of this categorization are not included within this annex. However it should be noted that for composite meat products identified as containing preservatives (e.g. sausages) the weight of the whole product consumed was estimated rather than the weight of the meat within the product, to account for the role that the preservative-containing part of the product has on CRC risk. This is explained further below.	
	Total red meat The following types of meat were included within the estimation of total red meat consumption: **Carcass meats:** Beef, lamb, pork, veal, mutton, venison, hare, goat including kebab meat (doner, shish, kofte), sliced meats (including ham and similar products), grill steaks, rib steaks. **Offal**: Offal and offal component of meat products (including haggis, black pudding, brawn and faggots) from corresponding red meat animals.	In order to decide whether certain meat products should be included within the analysis as the weight of the meat components of the products, or the total weight of the meat products themselves, it was necessary to identify whether the products contain preservatives. **Beef burgers** The World Cancer Research Fund (WCRF) report on cancer (2007)[1] states that minced meat and hamburgers are sometimes considered processed if they are preserved chemically. Recent retail and manufacturer research indicates: • The majority of retail chilled burgers contain preservatives.[2] Retail frozen burgers generally do not contain preservatives.[2] • Market share data[3] indicates that the majority of burgers sold in retail are frozen. • Burgers sold in large fast food chains (McDonalds, Burger King) do not contain preservatives.[2] • Burgers sold through catering suppliers such as 3663 and Brakes are mostly frozen and some do contain preservatives[4] -estimated at about 50% of product types, although there are plans towards removing all preservatives from frozen burgers. The National Diet and Nutrition Survey (NDNS) nutrient databank does not classify burgers according to their preservative content, so it is necessary to make assumptions about the likelihood of burgers containing and not

	Red meat component of meat products: meat balls, beef paste, Cornish pasties, porkpies, sausage rolls, Scotch eggs. some beef burgers (purchased for home cooking from retail and from McDonalds and Burger King). **Red meat products:** The total weight of the following red meat products was included in the analysis as they were identified as containing preservatives: sausages, salami, frankfurters, corned beef, spam, pâté, meat pie, and beef burgers eaten out of the home (except McDonalds and Burger King).	containing preservatives being classified in particular food codes. It is possible to identify food codes that are more likely to be used for retail burgers or those more likely to be used for burgers purchased for consumption out of the home, for example: the code for *Beef Burgers Economy Frozen Raw* is likely to be purchased in retail and consumed at home, whereas the code for *Cheeseburger Takeaway*, will have been consumed out of the home. In addition Burger King and McDonalds products are coded separately. For the purposes of the modelling work, it was assumed that: • All burgers purchased for home cooking from retail outlets do not contain preservatives. • All those consumed out of the home (excluding McDonalds and Burger King) do. • It is appreciated this was an over estimation as many burgers consumed out of the home do not contain preservatives. • However assuming that all burgers purchased for home cooking from retail do not, was an under estimation so these should have balanced each other out during the modelling. • These assumptions related to beef burgers only, all other burgers (e.g. lamb) were assumed not to contain preservatives and the weight of the meat content alone was estimated. **Meatballs** • Meatballs were assumed not to contain preservatives as the majority available in retail do not. Therefore consumption of the meat within meatballs was estimated.[2] **Sausages** The WCRF report classifies *sausages and Frankfurters, to which nitrates, nitrites or other preservatives are added*, as processed. [1] Most sausages available in the UK contain preservatives except for some frozen sausages. [2] Market share data[3] suggests more chilled sausages are purchased compared to frozen. As with burgers, the nutrient databank does not classify sausages according to preservative content, however unlike burgers there does not seem to be a difference between sausages eaten in the home or out of the home. As only a small proportion of frozen sausages are preservative free, it was assumed that all sausages contain preservatives.[2] It is appreciated that this may have been a slight over estimation. "Sausages" refers to all meat sausages excludes frankfurters and salami. **Cornish pasties, porkpies, sausage rolls, Scotch eggs** • A few brands of sausage rolls do contain preservatives however the majority do not. [2] • Therefore these products were all considered not to contain preservatives and the weight of meat within these products was estimated. • Although pork and egg pies tend to contain preservatives[2] the weight of the pork within the pie was used in the calculation rather than the preservative containing part, as this was difficult to estimate from data provided within the ingredients list.
		Offal • Offal from red meat animals was included within the red meat category, including offal within products:

		haggis, black pudding, faggots and brawn. These products generally do not contain preservatives.[5] Some black pudding (Tesco) contains bacon, [2] and therefore contains preservatives, however the majority do not contain bacon and therefore all black pudding was assumed not to contain preservatives and the consumption of the blood within the black pudding was estimated rather than consumption of the black pudding itself.
	Other meat Products **not** included in the estimation of total red meat: Chicken, turkey, goose, duck, other wild birds e.g. pheasant, guinea fowl, partridge, pigeon, and rabbit, chicken and turkey burgers/sausages, poultry offal. Also not included are: bacon flavour crisps, pork scratchings, lard, beef dripping, Bovril, gravy, gelatine, meat wastage (bones, skin etc.)	**Poultry Burgers/Sausages** • Although these products may be considered as "processed meat", poultry and other white meat are not considered a risk factor for CRC. • These products were not included within the analysis of total red meat. **Poultry Offal** • Offal from poultry was not included within the estimation of total red meat. • Although likely to be very similar in composition to offal from red meat, it was considered more appropriate to be classified with poultry. • As it was likely to represent a small proportion of total offal consumed, this was not likely to have greatly affected the results.
Estimating meat content of composite foods and dishes	All products and dishes containing red meat were included in the analysis. Although items such as meat stocks and beef extract were excluded.	• Within the nutrient databank there are a number of composite meat containing products, which do not have recipes assigned to them (generally retail or takeaway products or dishes). • Recipes have therefore been assigned to these products. This was done as follows: • Where known, the brand leader[3] was chosen for each product type and the stated % meat content used. e.g. for a lasagne ready meal, the meat content of the best selling lasagne was used.[2,3] • Products for which market share data was not available, the % meat content from an equivalent known top selling brand or retailer was used.[2] • Meat content was established from the label data obtained from retailer and manufacturer websites[2] or from supermarket searches where necessary. • Products that were no longer available on the market were compared with similar equivalent products. • As mentioned above for meat products containing preservatives, rather than estimating the meat content only, the content of the preservative containing part of the meat product was estimated. Thus including non-meat components. For example, consumption of *sausages* was estimated not the consumption of *pork* within the sausages. • Similarly for burgers, those assumed to contain preservatives were considered 100% *beef burger,* whereas for those burgers assumed not to contain preservatives, % *beef* within the beef burger was estimated. This was also done for other meat products containing preservatives including, frankfurters, salami, corned beef, meat loaf, Spam and pâté were all assumed to be 100% preservative-containing meat. • See appendix 2 for estimated meat content of manufactured/retail food codes within the NDNS. • The values obtained for meat content were broadly compared with TNS purchasing data 2008 provided by the Agriculture and Horticulture Development Board (AHDB).[6] Any major discrepancies were discussed with AHDB and the most appropriate figure was used.

	The % meat content was assumed to be % cooked weight except for updated recipes which uses retail label data.	• The % meat content used was either established from recorded recipes within NDNS diaries and was therefore the equivalent % meat in the cooked dish (water loss on cooking has been taken into account). Alternatively, as described above, it was taken from retail label information. No attempt was made to adjust the meat content of these products for water losses on cooking.
Iron content of foods		
Natural iron content of foods	The analytical values within the nutrient databank for iron naturally present within foods reflect current values.	• The natural iron content of foods, including meat, was assumed not to have changed since analysis (early-mid 1990s) and inclusion within the NDNS nutrient databank. • There may have been recent changes within the meat industry towards leaner cuts of meat, or towards increased sales of organic less lean meats, which would reduce/increase the fat content and therefore increase/reduce the iron content per 100g, respectively. • However changes such as these were not accounted for in the modelling due to lack of data on which to base any changes and the impact on overall iron intake would be small.
Iron content of types of red meat	The iron content of meat cuts/products were assumed to be standard average cooked values.	• The iron content of meat cuts used within the NDNS vary, • For the purposes of the modelling, standard average values for the iron content of cooked meat types were estimated using ranges of values from *McCance and Widdowson's Composition of Foods* meat supplements.[7,8] • Estimated standard iron values for each meat type used the modelling are listed in table 2.
Foods fortified with iron – white and brown flour	Iron values for products containing flour within the nutrient databank were assumed to reflect current levels.	• Mandatory fortification of wheat flour with iron in the UK results in flour containing products contributing to total iron intake. • The NDNS nutrient databank is updated as and when new analytical data becomes available. • Some food groups were last updated over 6 years ago and the proportion of flour within food products may have changed since analysis. • Data for flour within the databank is based on analysis carried out in 2003, but data for some flour containing products e.g. cakes and biscuits are older.
	Potential for overage[9] of iron within flour containing products was not taken into account.	As iron values for flour within the nutrient databank are analytical, the potential for overage would already have been captured within the analysis.
Foods fortified with iron - breakfast cereals	The number and types of breakfast cereals available on the market were assumed not to have changed since the NDNS.	• Current fortification practices were researched through retail and manufacturer websites[10] as well as information provided from trade organisations.[11] • Iron values were compared to those within the NDNS nutrient databank. Iron values for products within the databank for which the iron values have changed, were updated to reflect current fortification practices. • It was not possible to account for new products available on the market since the NDNS survey was carried out (see above).
	For breakfast cereals a typical overage of 20% was applied.	• An overage is applied to breakfast cereals by manufacturers to account for raw material and process variability in order to meet the typical content as labelled. • The labelled value for iron is the total iron content of the product - natural and added.[12] As this overage is not added to account for degradation over time (as iron is stable and not prone to degradation), the full overage (20%) was assumed at the point of consumption.
Foods fortified	The number and types of foods	• Other fortified foods within the nutrient databank were compared with recent commercial data.[10]

with iron - other	fortified with iron were assumed not to have changed since the NDNS. No adjustments were made for fortified foods introduced into the market since the NDNS, such as cereal bars, fruit juices etc.	• Any products already within the nutrient databank, i.e. consumed within the NDNS, which have recently become fortified or the level of fortification has changed, were updated. Although any changes made had a minimal affect on population iron intakes. **New products and changes in consumption patterns** • Each NDNS survey is accompanied by a nutrient databank which contains nutrient information for each food consumed within each survey. • The nutrient information in each databank is contemporaneous with the date of the survey so it is necessary to update the databank to take account of changes in fortification practices. • Some fortified foods such as cereal bars are more abundant than they were during data collection for the NDNS. • It is also not known in what quantity or frequency they are consumed, by which individuals or what foods they have taken the place of in the diet. • It is appreciated that excluding foods such as these may have resulted in an under estimation in consumption of voluntary fortified products. • Conversely, consumption of organic, simple foods (which are less likely to be fortified) is also likely to have increased, which may balance out any under estimation.
	Apart from for breakfast cereals The potential for overage was not accounted for.	• Analytical values for iron were used where available in the nutrient databank. • For some fortified foods, only label data was available, which may have been a slight under estimation of iron content as an overage may be applied by manufacturers in order to ensure the label value for iron content is reached at time of consumption.
Zinc content of foods		
Zinc	Although the main focus of this modelling was to support the SACN report on iron and health, and therefore looked at the impact of reduced red meat consumption on iron intakes, red meat is also a source of zinc. Therefore an analysis of the impact of a reduction in red meat consumption was also assessed for zinc intake.	
Natural zinc content of foods	The analytical values within the nutrient databank for iron naturally present within foods reflect current values.	• As with iron, the natural zinc content of foods, including meat, was assumed not to have changed since analysis (early-mid 1990s) and inclusion within the National Diet and Nutrition Survey (NDNS) nutrient databank.
Zinc content of types of red meat	The zinc content of meat cuts/products was assumed to be standard average cooked values.	• The zinc content of meat cuts used within the NDNS vary, • For the purposes of the modelling, standard average values for the zinc content of cooked meat types were estimated using ranges of values obtained from *McCance and Widdowson's Composition of Foods* meat supplements.[7,8] • Estimated standard zinc values for each meat type used in the modelling are listed in table 2.
Foods fortified with zinc	The number and types of foods fortified with zinc on the market were assumed not to have changed since the NDNS.	• The NDNS nutrient databank was checked for foods fortified with zinc. • Databank values were compared to label values on retailer and manufacturer websites[12] and the nutrient databank values were updated where appropriate. • Any fortified foods not contained within the databank were not accounted for. • Overage was not taken into account for foods fortified with zinc. •

Vitamin D content of foods		
Vitamin D	As meat is also a dietary source of vitamin D, the effect of reducing red meat consumption was also assessed for vitamin D intakes.	
Natural vitamin D content of foods	The analytical values within the nutrient databank for vitamin D naturally present within foods reflect current values.	• As with iron and zinc the natural vitamin D content of foods, including meat, was assumed not to have changed since analysis (early-mid 1990s) and inclusion within the NDNS nutrient databank.
Vitamin D content of types of red meat	The vitamin D content of meat cuts/products was assumed to be standard average cooked values.	• The vitamin D content of meat cuts used within the NDNS vary, • For the purposes of the modelling, standard average values for the vitamin D content of cooked meat types were estimated using ranges of values obtained from *McCance and Widdowson's Composition of Foods* meat supplements [7, 8] and the NDNS nutrient databank. • Estimated standard vitamin D values for each meat type used in the modelling are listed in table 2.
Other assumptions		
Other nutrients	The effect of reducing red meat consumption on the intake of other nutrients was not considered.	• Red meat is also considered a good source of protein and B vitamins (in particular B12)[13], however intakes of these nutrients are well above the Dietary Reference Values (DRVs).[14,15,16] • Red meat is also a source of selenium[15] however selenium was not included in the nutrient databank for NDNS adults 2000/01 as there was insufficient selenium composition data available at that time, so no estimate of selenium intake is available for that survey.
Processing losses	The potential for losses/gains in iron content as a result of food processing was not accounted for.	• Although iron can be both removed (through leaching) and added during processing (from equipment/instruments) (see main report for more details) this was not taken into account. • Where recipes contain ingredients fortified with iron such as flour and fortified breakfast cereals, additions/removal through cooking/processing were not taken into account. • The reason for this is that there is no standard factor that could be applied across products to take these losses of gains into account.
Bio availability Speciation	Changes in the bioavailability of iron as a result of food processing have not been accounted.	• Cooking may increase iron bioavailability, heat processing can affect iron solubility and can denature haem iron. • Cooking and baking can also destroy ascorbic acid and reduce iron bioavailability (see main report for details). • These factors were not accounted for in the modelling.
	Iron intake was not split into haem and non-haem iron, but measured as total iron intake.	• In general haem iron is absorbed more efficiently than non-haem iron (see main report for details), • However bioavailability was taken into account in setting the DRVs.[16] • Therefore for the purposes of this modelling total iron intake was estimated, rather than splitting into haem and non-haem iron.
	The potential for oxidation/reduction of iron species and thus a change in bioavailability was not taken into account within this modelling.	
Under reporting	Dietary intake data from NDNS series were assumed to represent usual intake. Potential for underreporting was not considered.	• Dietary surveys such as the NDNS are prone to bias in reporting. • No attempt was made to adjust the energy and nutrient intakes presented in the NDNS report to take account of under-reporting.[14,15]
Population sample	analysis used consumption data from the NDNS of adults aged 19-64 years[14.15] (data collected 2000/01) only. Data for the NDNS adults aged over 65 years[17] was collected in 1994/5 and considered of limited validity.	

Table 2: Standard iron, zinc and vitamin D values used for types of cooked meat [9, 10]

Meat	Iron (mg/100g) Cooked value	Zinc (mg/100g) Cooked value	Vitamin D (µg/100g) Cooked value
Beef	2.3	5.8	0.6
Lamb (1)	1.8	4.1	0.5
Pork	1.0	2.9	0.8
Veal	1.0	3.5	1.5
Venison	5.1	3.9	0.5*
Ham(2)	0.9	2.1	0.8
Bacon	0.7	2.2	0.7
Sausages	1.1	1.3	1.1
Frankfurter (3)	1.1	1.4	0.5*
Salami (4)	1.3	3	0.5*
Haslet	1.9	1.5	0.2*
Polony	1.3	1.2	0.5*
Beef burger	2.7	6.2	1.9
Meat loaf	1.5	2.4	0.6*
Corned beef	2.4	5.5	1.3
Liver	12	8.6	0.8
Kidney	9	4	0.6*
Blood (5)	33	1.9	1.6
Pâté	0.5	2.7	1.2
Other offal (6)	3.4	3.6	Trace

(1) Assume values for goat are the same as for lamb as no nutrient data available
(2) Includes gammon, canned, Parma ham and pork shoulder
(3) Frankfurter only (not including bun, ketchup or mustard)
(4) "Salami" includes Pepparami and chorizo
(5) Based on the figures for cooked black pudding, assuming black pudding is 37% blood and the non-blood components of black pudding do not contribute to iron, zinc and vitamin D content.
(6) Offal encompasses brain, heart, tripe, lung, head, oxtail, tongue, sweetbread, trotters and tails
* Vitamin D values for these products are denoted by "N" within *McCance and Widdowson's Composition of Foods* [9, 10] as the nutrient is present in significant quantities, but there is no reliable information on the amount. For the purposes of this modelling therefore values have been taken from the NDNS nutrient databank, where estimates for vitamin D content of these products have been assigned, for purposes of dietary analysis.

References

1. World Cancer Research Fund. Food, Nutrition, Physical Activity and the Prevention of Cancer: a global perspective. 2007. [Available online] www.dietandcancerreport.org/.

2. Retail and Manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.burgerking.com; www.mcdonalds.com; www.birdseye.co.uk; www.findus.co.uk; www.ginsters.com; www.turkeyfortoday.com; (Accessed August 2008)

3. Taylor Nelson Sofres (TNS) market share data (2006).

4. Personal communication with 3663 & Brakes Food Service (August 2008).

5. Personal communication with The Bury Black Pudding Company (October 2008) and Retailer and Manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.farmingfriends.com; (Accessed October 2008).

6. The Agriculture and Horticulture Development Board (AHDB) provided FSA with Taylor Nelson Sofres (TNS) estimates of % meat content based on purchasing figures for GB 2008.

7. Chan, W., Brown, J., Lee, S, and Buss, D.H. Meat, Poultry and Game. *Fifth supplement to 5th edition of McCance and Widdowson's The Composition of Foods.* Royal Society of Chemistry, Cambridge, 1995.

8. Chan, W., Brown, J., Church, S.M. and Buss, D.H. Meat Products and Dishes. *Sixth supplement to 5th edition of McCance and Widdowson's The Composition of Foods*. Royal Society of Chemistry, Cambridge, 1996.

9. Manufacturers often add an "overage" during fortification to ensure the label value is achieved at point of consumption. This is done as some vitamins and minerals are prone to degradation over time or losses during processing.

10. Retail and Manufacturer websites: www.asda.com; www.ocado.com; www.sainsburys.com; www.tesco.com; www.horlicks.co.uk; www.ovaltine.co.uk; www.slimfast.co.uk; (Accessed August 2008).

11. Personal communication with the Food and Drink Federation (FDF). The British Retail Consortium (BRC) were also contacted requesting data (October 2008).

12. Personal communication with Kellogg's (August 2008).

13. Food Standards Agency advice [available online] http://www.eatwell.gov.uk (Accessed March 2009).

14. Henderson L, Gregory J, Irving K & Swan G. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 2: Energy, protein, carbohydrate, fat and alcohol intake. London:TSO, 2003.

15. Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G & Farron M. National Diet and Nutrition Survey: adults aged 19 to 64 years. Volume 3: Vitamin and mineral intake and urinary analytes. London:TSO, 2003).

16. Department of Health. Dietary Reference Values for Food, Energy and Nutrients in the United Kingdom. (Report on Health and Social Subjects, No. 41). London: HSMO, 1991.

17. Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G & Clarke PC. National Diet and Nutrition Survey: people aged 65 years or over. Volume 1: Report of the diet and nutrition survey. London:TSO, 1998.

ANNEX 7

Appendix 2

Total red meat content of manufactured/retail foods within NDNS

Table 1 below lists red meat containing food codes for retail/manufactured foods consumed within the NDNS and their corresponding estimated total red meat content. The methodology for estimating the meat content of these foods is explained in detail in appendix 1. It should be noted that these are estimates based on data available from retailer and manufacturer websites, as well as food ingredient labels. Products that were no longer available or could not be found on the market were compared with similar equivalent products. These data were agreed in consultation with the Agriculture and Horticulture Development Board (AHDB).

Table 1: Estimated red meat content of manufactured/retail foods within the NDNS. The type of red meat within the food codes corresponds to the categories of red meat listed in table 2 of appendix 1.

NDNS Food Code Name	% Red meat content
PASTA RAVIOLI CANNED IN TOMATO SAUCE	7% Beef
PASTA SPAGHETTI CANNED IN BOLOGNAISE SAUCE	4% Beef
PIZZA HAM DEEP PAN	6% Ham
PIZZA HAM THIN BASE	9% Ham
SCOTCH EGG	26% Pork
PIZZA HAM MUSHROOM CHICKEN GREEN PEPPER, DEEP PAN	5% Ham
LIVER & ONION GRAVY RETAIL	37% Liver
BEEFMINCED IN GRAVY CANNED	75% Beef
BEEF MINCED PIE FILLING CANNED	75% Beef
BEEF MINCED REFORMED PIE FILLING + ONION CANNED	75% Beef
BEEF STEWING REFORMED PIE FILLING CANNED	75% Beef
HAM AND PORK CHOPPED CANNED	50% Pork, 50% Ham
IRISH STEW CANNED	30% Lamb
BAKED BEANS IN TOMATO SAUCE WITH PORK SAUSAGES	17% Sausage
STEAK AND KIDNEY PIE CANNED	19% Beef, 15% Kidney
STEAK AND KIDNEY PUDDING CANNED	16% Beef, 13% Kidney
STEWING STEAK IN GRAVY PIE FILLING CANNED	77% Beef
STEWING STEAK / MEATBALLS IN GRAVY CANNED	20% Pork
BLACK PUDDING FRIED	37% Blood
VEAL JELLIED CANNED	85% Veal
FAGGOTS IN GRAVY READY MEAL	11% Pork
LIVER SAUSAGE	100% Sausage
MEAT LOAF DELICATESSEN	100% Meat loaf
MEAT PASTE CANNED	36% Beef
MEAT PASTE NOT CANNED	37% Beef
BEEFBURGERS IN GRAVY CANNED	40% Beef
BEEFBURGERS WITH ONION FROZEN RAW	77% Beef
BEEFBURGER & ONION FRIED NOT 100% MEAT	60% Beef
BEEFBURGERS ECONOMY FROZEN RAW	60% Beef
BEEFBURGER ECONOMY FRIED	60% Beef
SAUSAGE IN BATTER FRY COMM OIL	54% Sausage
BRIDIES SCOTCH PIES	14% Veal
MINCED BEEF PIE PURCHASED TWO CRUSTS	18% Beef
CORNISH PASTIE PURCHASED	14% Beef
MINCED BEEF PIE PURCHASED	18% Beef
MINCED BEEF PIE PASTRY TOP&BOT	18% Beef
MINCED BEEF PIE TOP PASTRY	35% Beef
PORK AND EGG PIE	26% Pork
PORK PIE INDIVIDUAL	26% Pork
PORK PIE SLICED	30% Pork
SAUSAGE ROLL SHORTCRUST PASTRY PURCHASED	28% Pork
STEAK & KID PIE 2 CRUSTS S/C PASTRY NOT IND.	3% Beef, 11% Kidney
BACON AND EGG IN A BUN	33% Bacon
BEEF CURRY NO RICE RETAIL	11% Beef

ROAST BEEF DINNER WITH YORKSHIRE PUD POTATOES VEG	8% Beef
ROAST BEEF IN GRAVY PURCHASED READY MEAL	54% Beef
CANNELLONI	15% Beef
CHINESE MEAT BUNS	30% Pork
HAMBURGER IN BUN TAKEAWAY	43% Beef burger
SPARE RIBS IN BARBECUE SAUCE NO BONES	27% Pork
CHEESEBURGER TAKEAWAY NOT QTER POUNDER	39% Beef burger
CHINESE LUNCHEON MEAT STEAMED	50% Beef
HAMBURGER QUARTER POUNDER TAKEAWAY	67% Beef burger
HAMBURGER QUARTER POUNDER WITH CHEESE TAKEAWAY	58% Beef burger
HAMBURGER BIG MAC MC DONALDS	75% Beef
DONER KEBAB	50% Lamb
KOFTE KEBAB	42% Lamb
SHISH KEBAB	37% Lamb
LASAGNE FROZEN	16% Beef
LASAGNE FROZEN	3% Bacon
PORK ROAST DINNER FROZEN READY MEAL	16% Pork
ROAST PORK IN GRAVY FROZEN READY MEAL NO POTS/VEG	54% Pork
SAMOSA-MEAT FILLED	28% Lamb
SPARE RIBS, BARBECUE STYLE, EG TAKEAWAY, WITH BONES	27% Pork
SHEPHERDS PIE FROZEN PURCHASED READY MEAL	26% Lamb
SWEET AND SOUR PORK, BATTERED WITH/WITHOUT SAUCE	50% Pork
STEAK & KIDNEY PIE 2 CRUST S/C	8.5% Kidney, 21% Beef
INDIVIDUAL STEAK PIE FLAKY PUR	30% Beef
INDIV STEAK & KIDNEY PIE FLAKY	8.5% Kidney, 21% Beef
OXTAIL SOUP CANNED	4% Beef, 1% Other offal
BEEF CURRY AS SERVED WITH RICE RETAIL	6% Beef
TAGLIATELLE CARBONARA, REDUCED FAT, READY MEAL, RETAIL	12% Ham
CHICKEN AND BACON PASTA GRATIN RETAIL	3% Bacon
SAUSAGE HOTPOT WITH BAKED BEANS CANNED RETAIL	16% Sausage
BACON & TURKEY IN BREADCUMBS RETAIL	55% Bacon
BBQ SIZZLERS RETAIL	58% Pork
PORK STEAKS WITH HONEY & MUSTARD RETAIL	76% Pork
LASAGNE, REDUCED FAT RETAIL	14% Beef
PORK SAUSAGE SNACK BAR MEAL	62% Sausage
CHICKEN, BACON, MUSHROOM & CREAM PIE	5% Bacon
LAMB SHEPHERDS PIE RETAIL	15% Lamb
LOW-FAT LIVER PATE	100% Pate
SPINACH AND HAM QUICHE RETAIL	8% Ham
BEEF WELLINGTON	31% Beef
CHICKEN & BACON PANCAKES RETAIL	2% Bacon
POLLACK, CHICKEN, PRAWN AND SALAMI PAELLA RETAIL	1.3% Salami
BEEF ENCHILLADAS READY MEAL RETAIL	21% Beef
CHICKEN WITH BACON, FROMAGE FRAIS, MASH, REDUCED FAT RETAIL	2.7% Bacon
POTATO, BEAN AND BACON MELT RETAIL	13% Bacon
QUICHE WITH BEEF, PORK, HAM AND PEPPERS	2% Ham, 5% Beef, 5% Salami
BEEF COBBLER WITH BAKED BEANS AND CARROTS RETAIL	12% Beef
SHEPHERDS PIE WITH BAKED BEANS RETAIL	12% Lamb
BEEF IN RED WINE SAUCE WITH MASHED POTATO RETAIL	22% Beef
BLACK PUDDING IN BATTER TAKEAWAY	17% Blood
CORNISH PASTY REDUCED FAT RETAIL	14% Beef
HAM PATE LOW FAT PURCHASED	100% Pate
STEAK & KIDNEY PIE FILLING CAN	60% Beef, 15% Kidney
HAM MUSHROOM AND CHEESE LATTICE RETAIL	16% Ham
SLICED LAMB ROAST DINNER FROZEN RETAIL	14% Lamb

BURGER KING WHOPPER ONLY	30% Beef
BURGER KING WHOPPER WITH CHEESE ONLY	27% Beef
BURGER KING DOUBLE WHOPPER ONLY	46% Beef
BURGER KING DOUBLE WHOPPER WITH CHEESE ONLY	43% Beef
FRANKFURTER IN A BUN WITH KETCHUP ONIONS & MUSTARD	18% Frankfurter
BEEF CASSEROLE READY MEAL IN GRAVY AND VEG	22% Beef
BEEF CURRY FROZEN/CHILLED READY MEAL NO RICE	24% Beef
BEEF HOT POT WITH POTS READY MEAL RETAIL	24% Beef
CHILLI CON CARNE.NO RICE READY MEAL	35% Beef
LAMB HOT POT WITH POTS READY MEAL	31% Lamb
MOUSSAKA READY MEAL CHILL/FROZEN/LONG LIFE	24% Lamb
SHEPHERDS PIE FROZEN/CHILLED LAMB READY MEAL	26% Lamb
STEWING STEAK CANNED WITH POTATOES CARROTS & DUMP.	26% Beef
HAM CHEESE AND LEEK PIE RETAIL	7% Ham
DONER KEBAB WITH PITTA READY PURCHASED	27% Lamb
CHICKEN & BACON LASAGNE PURCHASED READY MEAL	3% Bacon
SAUSAGES IN BATTER GRILLED RETAIL	54% Sausage
FORTIFIED PASTA SHAPES WITH MINI SAUSAGES	14% Sausage
FORTIFIED PASTA SHAPES WITH MINI SAUSAGES	14% Bacon
BURGER KING DOUBLE SUPREME	63% Beef
CUMBERLAND PIE POTATO & CHEESE TOPPING RETAIL	19% Beef
STEAK PIE CANNED STEAK LARD GOLDEN CHURN IN PASTRY	50% Beef
HAM LEEK AND POTATO PIE RETAIL	12% Ham
CORNED BEEF CRISPBAKE RETAIL	38% Corned Beef
CHICAGO RIBS PORK & TVP IN MARINADE RETAIL	27% Pork
LAMB CURRY, TAKEAWAY EG ROJAN JOSH, NO RICE	32% Lamb
SCOTCH EGG MINI	28% Pork
CHILLI CON CARNE CANNED	23% Beef
BOLOGNESE SAUCE CANNED	28% Beef
BEANS BAKED WITH ADDITIONS (BURGERS) NOT SAUSAGES	18% Beef
MEAT BALLS AND PASTA/BAKED BEANS	7% Beef
MICROWAVE SAUSAGES PORK AND Beef	100% Sausage
PORK PIE BUFFET	22% Pork
BAKED BEANS LOW FAT SAUSAGE	22% Sausage
MEAT BALLS IN BARBECUE SAUCE	53% Beef
LAMB ROAST ROLL COOKED	78% Lamb
PORK ROAST ROLL COOKED RETAIL	80% Pork
SAUSAGE ROLL FLAKY PASTRY PURCHASED	28% Pork
BEEFBURGERS LOW-FAT FRIED	80% Beef
BEEFBURGER AND ONION GRILLED	77% Beef
BEEFBURGER ECONOMY GRILLED	60% Beef
RAVIOLI NOT CANNED	16% Beef
MEAT BASED PIZZA THIN CRISPY BASE	10% Beef, 8% Salami, 8% Ham
MEAT BASED PIZZA DEEP PAN BASE	3.5% Beef, 3% Salami
MEAT BASED PIZZA FRENCH BREAD BASED	2.6% Beef, 2.6% Ham
COMBINATION PIZZA THIN CRISPY BASE	3.4% Salami
COMBINATION PIZZA FRENCH BREAD BASE	3.4% Salami
COMBINATION PIZZA DEEP PAN BASE	2.8% Salami
QUICHE LORRAINE S/C PASTRY PURCHASED	14% bacon
BEEF CHICKEN AND PORK SATAY	22% Pork, 22% Beef
MINCED BEEF PANCAKES GRILLED RETAIL	12% Beef
TAGLITELLE CARBONARA READY MEAL RETAIL	7% Bacon
TURKEY AND PORK LUNCHEON MEAT RETAIL	29% Pork
TURKEY AND BACON LOAF RETAIL	40% Bacon,
BACON CRUNCHIES COATED BREADCRUMBS PURCHASED	21% Bacon, 8.6% Pork
CORNED BEEF PASTY PURCHASED	20% Corned Beef
MINCED BEEF CRISPBAKES OVEN BAKED PURCHASED	12% Beef

KOSHER SALAMI CHICKEN AND BEEF	40% Beef, 20% Salami
POTATO & CORNED BEEF PASTY PURCHASED	20% Corned Beef
XTRA LEAN STEWING STEAK IN GRAVY CANNED	77% Beef
MEXICAN CHILLI SLICE RETAIL	11% Beef
CHILLI CON CARNE WITH RICE READY MEAL	19% Beef
SPAGHETTI BOLOGNAISE READY MEAL	14% Beef
SPAGHETTI BOLOGNAISE LOW FAT	11% Beef
BEEF CURRY WITH RICE READY MEAL RETAIL	12% Beef
MEATBALLS IN GRAVY WITH MASHED POTATO READY MEAL	10% Beef, 3.5% Sausage
BEEF STEW & DUMPLINGS FROZEN OR CHILLED READY MEAL	10% Beef
BACON AND CHEESE GRILLS RETAIL	48% Bacon
CHICKEN IN WHITE SCE HAM MUSH & RICE READY MADE	7% Ham
MINCED BEEF & VEGE (POT PEAS CARROTS) READY MEAL	12% Beef
BEEF STEWED MADE W CANNED STEWING STEAK + PULSES	12% Beef
READY MEAL-STEAK IN RED WINE + VEG	18% Beef
SWEET AND SOUR PORK WITH RICE FROZEN READY MEAL	11% Pork
SWEET AND SOUR PORK FROZEN READY MEAL NO RICE	36% Pork
SAUSAGE BURGER RETAIL	44% Sausage
MACARONI CHICKEN AND BACON READY MEAL RETAIL	3% Bacon
FRESH EGG PASTA RAVIOLI RETAIL	6% Pork, 2.4% Beef
STEWED MEAT (CANNED) & POTATO PIE	14% Beef
TURKEY & HAM CRISPBAKES RETAIL	4% Ham
GAMMON STEAKS IN HONEY MUSTARD & GINGER RETAIL	71% Ham
CORNED BEEF AND ONION PIE PURCHASED	20% Corned Beef
BEEF ORIENTAL WITH RICE RETAIL	10% Beef