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5th International Whey Conference

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New insights into the significance of protein quality in human diets

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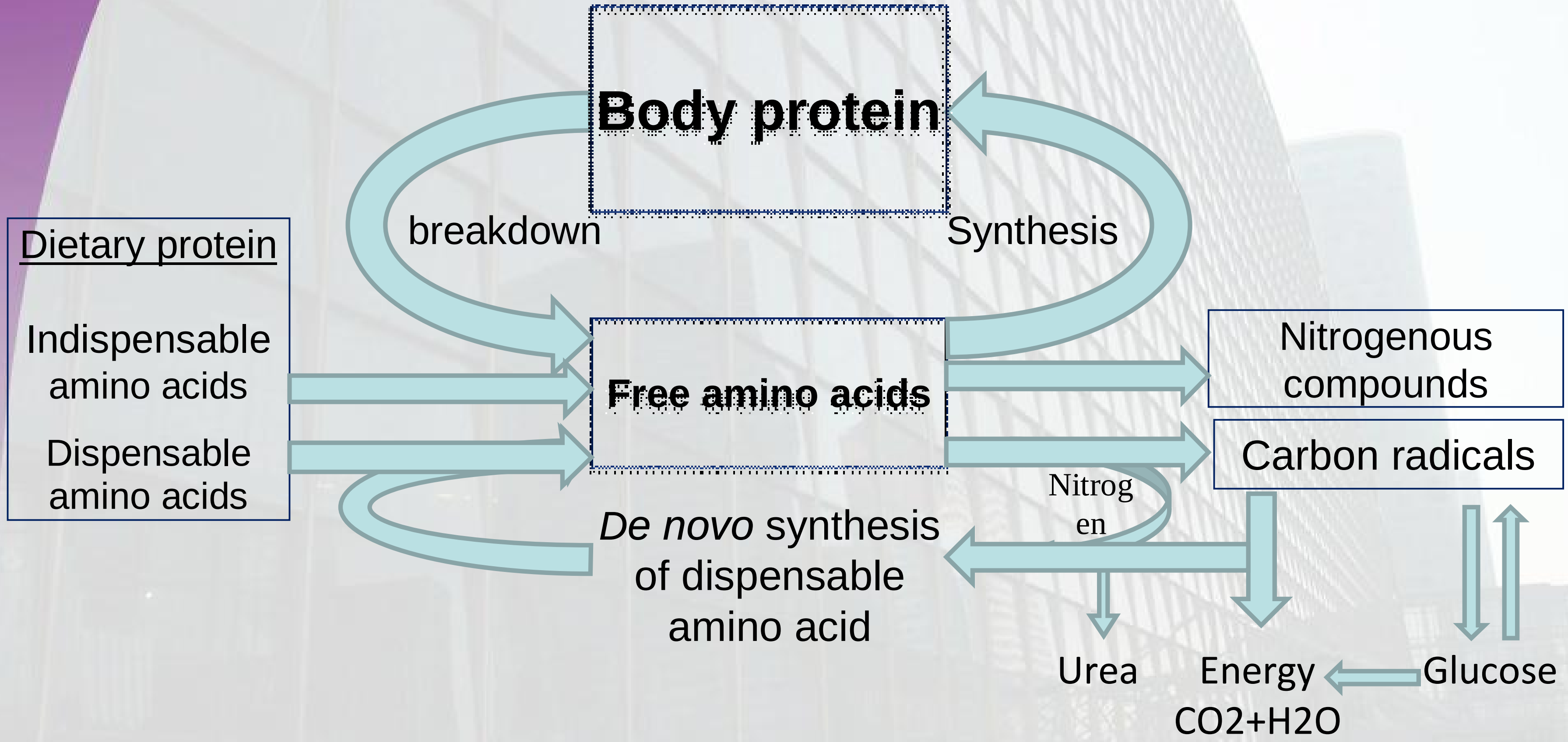
Introduction

- Protein quality describes the ability of a protein source to achieve defined nutritional and metabolic actions related to protein requirement.
- Traditionally, protein requirement is determined as “the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass in persons at energy balance …”.
- During the last decades understanding of protein’s actions expanded and new research indicated increasingly complex roles for protein and amino acids in regulation of various other functions

Protein and amino acid : metabolism and functions

- Support protein turnover and homeostasis as substrate or as signal molecules (leucine)
- participate to energy metabolism either directly or through gluconeogenesis
- some specific amino acid have a regulatory role as precursor of nitrogenous compounds (cysteine, tryptophane, arginine, …)



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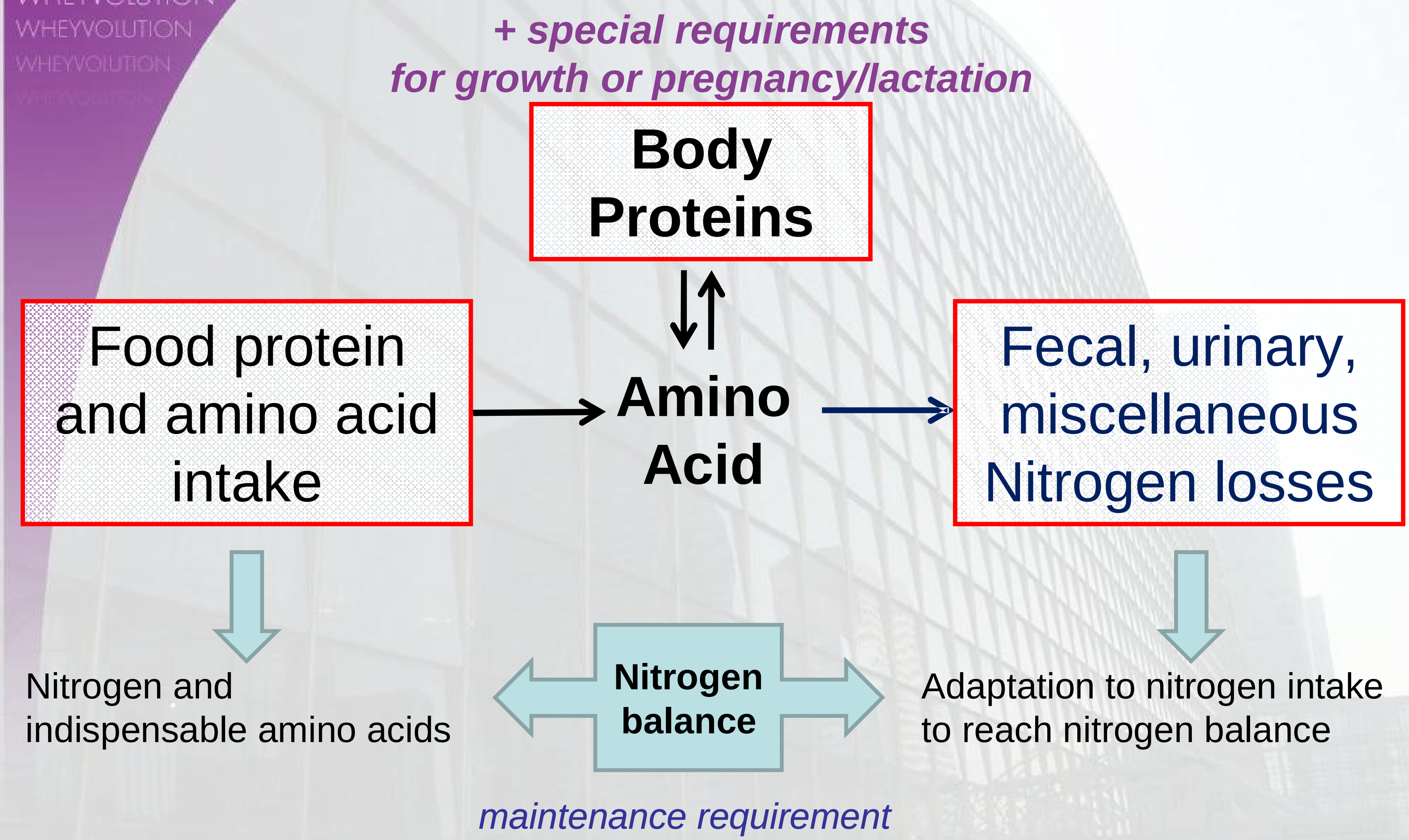
1- Protein quality and nitrogen balance

- The current criterion for protein quality evaluation is to meet maintenance needs plus special needs for growth, pregnancy, or lactation.
- Traditionally, protein requirement is determined as “the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass in persons at energy balance …”.
- This has been discussed in the context of a protein’s ability to provide nitrogen and specific patterns of amino acids to satisfy the requirement for synthesis of protein and its role in maintaining body protein mass.
- This is currently determined from growth measurement, protein and nitrogen digestibility, nitrogen balance, nitrogen retention or protein turnover at whole body level or in specific protein pools.

Protein, amino acid, nitrogen balance and protein requirement



Safe protein requirement and current protein quality

- Providing bioavailable nitrogen and indipensable amino acids to achieve nitrogen balance

- Requirement for Nitrogen : Meta-analysis of N-balance in adults - mean requirement of 0.66 g protein/kg/d and safe protein requirement of 0.83 g protein /kg/d.

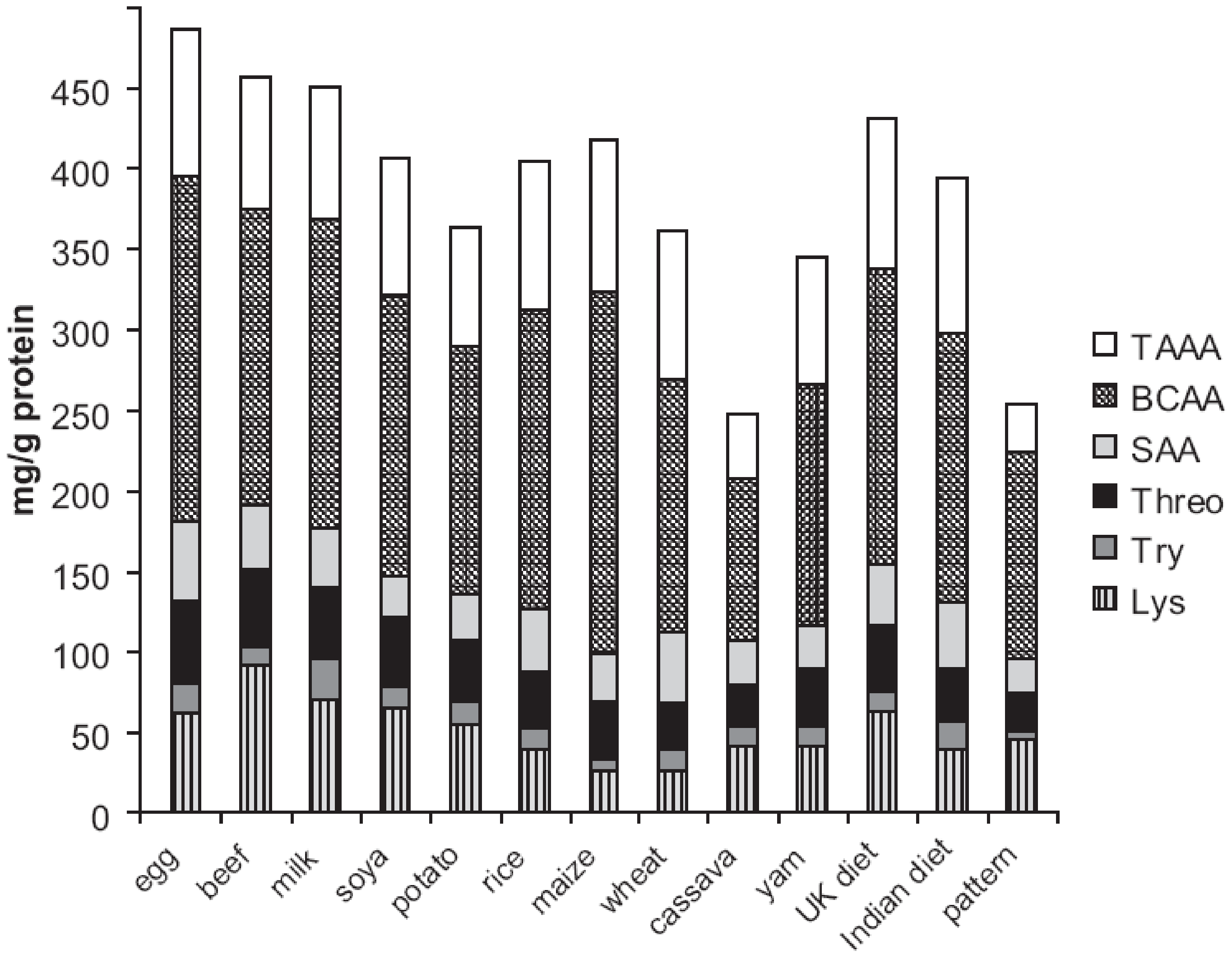
- Requirement for indispensable amino acid : lysine, sulfur (methionine + cysteine), threonine , tryptophan, branched chain (leucine, isoleucine, valine), aromatic (phenylalanine + tyrosine), histidine

Amino acid requirement and reference pattern :

Indispensable amino acid composition of a reference protein reaching amino acid requirement at the safe level of protein intake (0,66 g/kg/d in adult)

	FAO/WHO/UNU 2007	
	mg/kg/j	mg/g protéine*
Histidine	10	15
Isoleucine	20	30
Leucine	39	59
Lysine	30	45
Méthionine + cystéine	15	22
Méthionine	10	16
Cystéine	4	6
Phénylalanine + tyrosine	25	38
Thréonine	15	23
Tryptophane	4	6
Valine	26	39
Total	184	277

Indispensable amino acids in food proteins and diets compared with the requirement pattern
(FAO/WHO/UNU 2007



Distribution of lysine in food proteins and diets for adult

(as % lysine requirement)

Calculated for 0.66 g/kg/d protein intake (= mean protein requirement) and 30 mg/kg/d mean lysine requirement in adult

Wheat	Maize	Rice	Cassava	Yam
57	58	86	92	91
Potato	**Egg**	**Soya**	**Milk**	**Beef**
121	139	144	158	203

Indian diet	Western diet
~ 87	~ 140

(derived from FAO, WHO, 2002)

Indispensable amino acid in animal and plant protein compared with the reference protein amino acid pattern

AA g/100g	Lysine	Methionine + Cysteine
Reference	45	22
Animal	75-95	35-40
Cereals	20-40	30-40
Legumes	55-75	15-30

- Lysine is limiting in cereals
- Sulphur amino acid can be limiting in legumes
- Animal protein have no limiting amino acid

In vivo measurement of nitrogen retention :

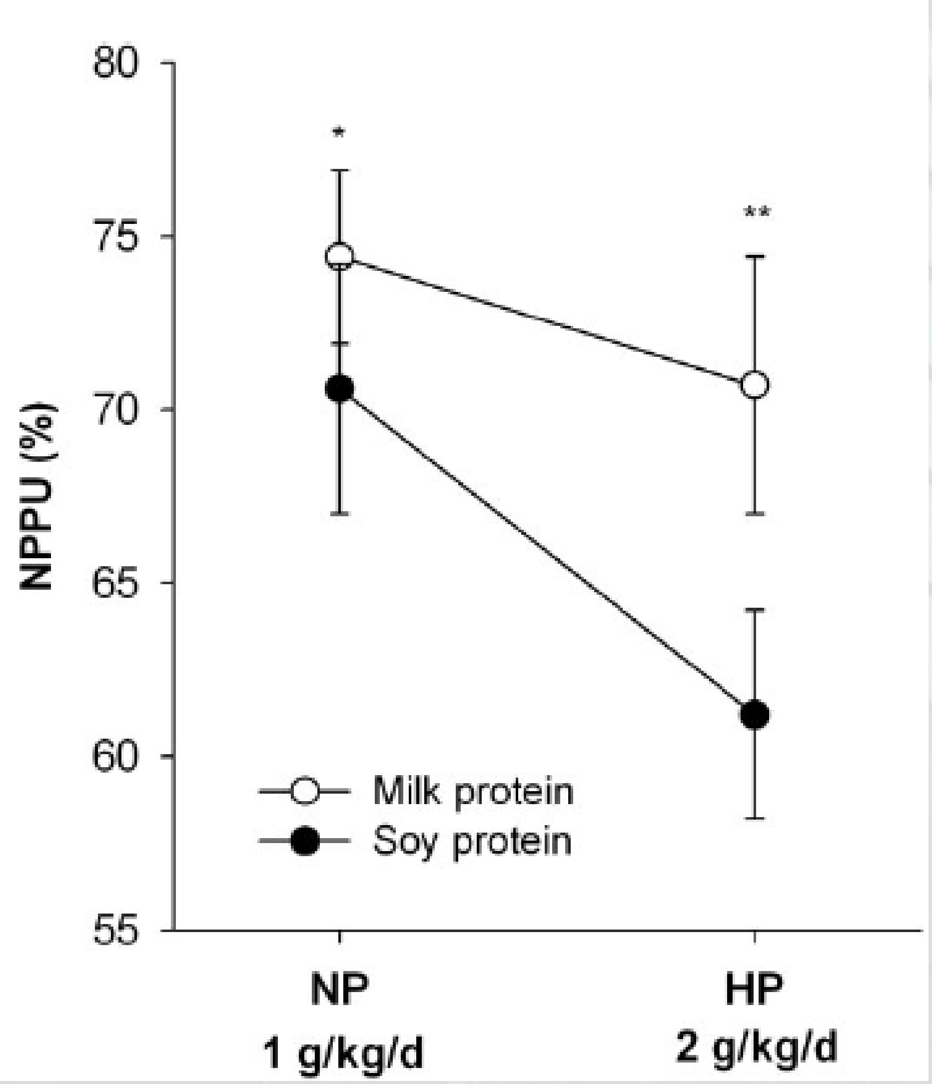
Net postprandial dietary nitrogen utilization (NPPU) of different protein sources in humans

Fouillet et al, J. Nutr. 132: 3208S–3218S, 2002

Protein source	NPPU, %	Postprandial biological value, %
Milk	75	78
Soy	71	77
Wheat	62	69

NPPU is assessed after the ingestion of a single 15N-labeled mixed meal and the measurement of the fraction of dietary N transferred to urea. Postprandial dietary N retention is calculated from the amount of dietary N absorbed that is neither recovered in urine nor in the body urea pool 8 h after the meal ingestion.

Increasing Habitual Protein Intake Accentuates Differences in Postprandial Dietary Nitrogen Utilization between Protein Sources in Humans.



(Morens et al J. Nutr. 133: 2733–2740, 2003).

Net postprandial protein utilization (NPPU) of milk (*n*=12) and soy protein (*n=8) by humans as a function of the prevailing* protein intake (normal protein, NP or high protein, HP) assessed after ingestion of a 15N-labeled protein meal.

There was a significant influence of diet (*P 0.0001), protein source (P 0.0001) and their* interaction (*P 0.001) on NPPU, as assessed by a two-way ANOVA.*

*,**Soy and millk protein groups differ at each protein level, *P 0.02* and *P 0.0001, respectively (post-hoc Tukey's test).*

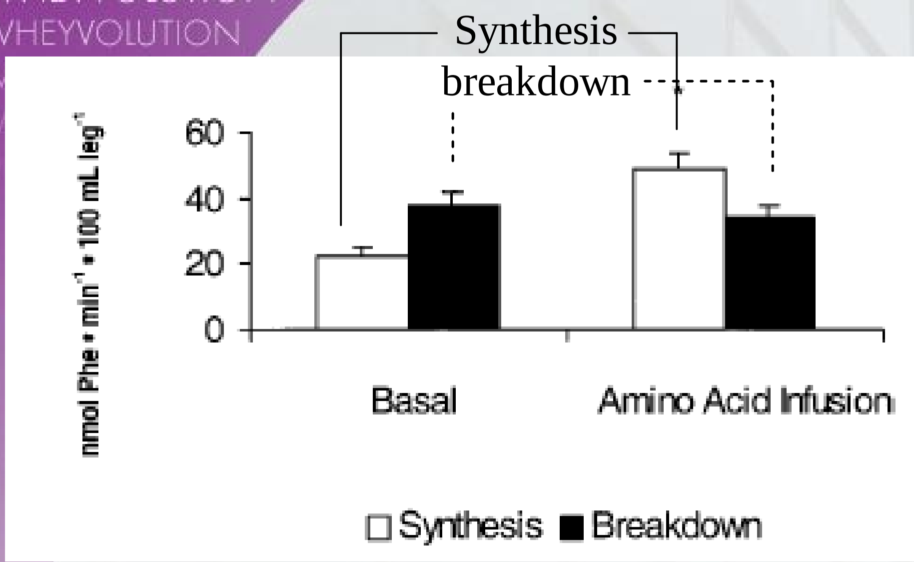
Changes in Quantity Closely Associated with Quality

- Context of current protein intake
  - Most nutritionally complete habitual diets include protein intake higher than these needs
  - Adaptation of protein and amino acid metabolism may modify requirement and quality references
- As protein intake increases, more difficult to generate single reference pattern to predict
  - adaptation of amino acids metabolism
  - consequent fate of the dietary amino acids
  - especially across lifespan and all physiological conditions
- Protein digestibility and colonic health
  - colonic protein degradation and metabolism could generate toxic components

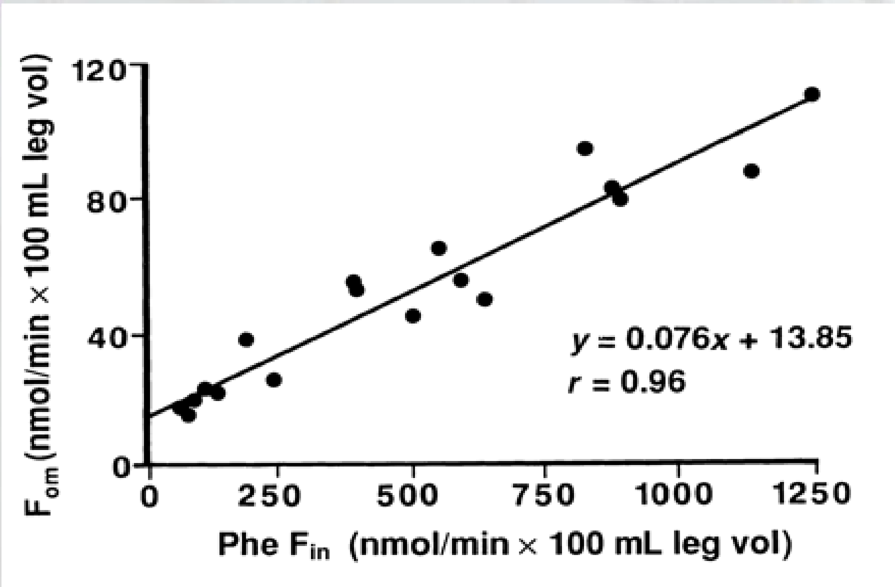
Dietary protein and protein turnover

- AA support protein turnover and homeostasis as substrate and as signal molecules (BCAA, leucine) differently in the different tissues and organs
- Control and limiting factors for protein synthesis
  - Indispensable amino acids are the main limiting factors for body protein synthesis
  - The kinetic of amino acid delivery to tissues influences anabolic response and oxidation in splanchnic and peripheral tissues
  - Leucine is considered as an anabolic signal for protein synthesis (in muscle ?)
- Consequences for protein requirement and quality?

Muscle protein synthesis is stimulated by amino acid and leucine in adult



Effect of balanced amino acid infusion (164 mg/h/kg) on Muscle Protein Synthesis and Breakdown (from Biolo et al, 1997).

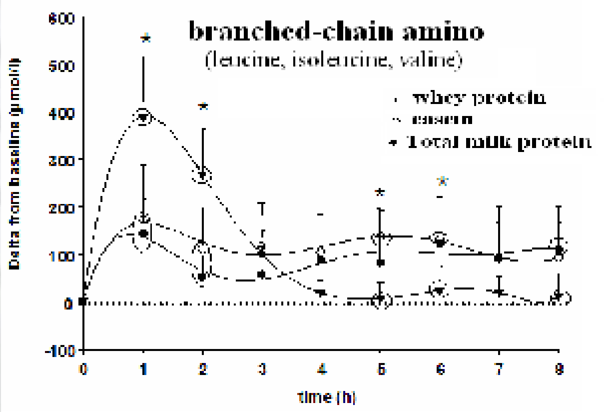


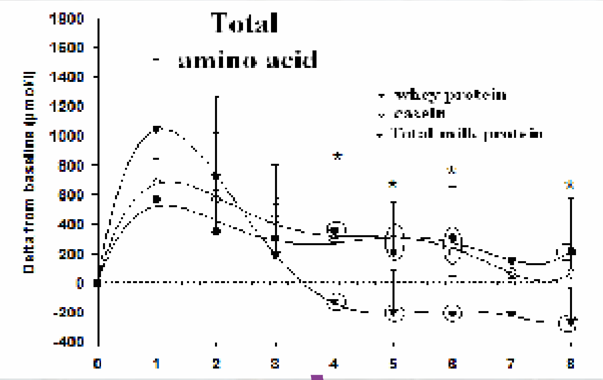
Correlation between muscle protein synthesis and arterial flux of leucine (higher values are obtained with amino acid iv infusion) (Wolfe, 2000).

Consequences of different kinetic and blood amino acid profile induced by whey (W), casein (C) or total milk protein (TMP)

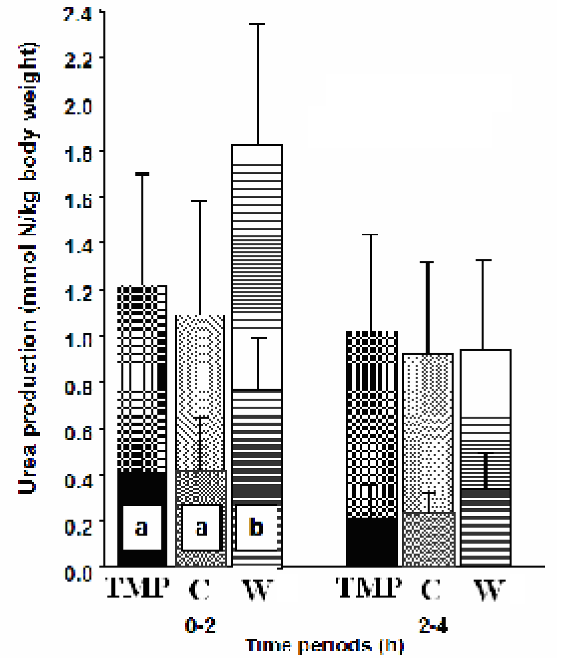
(Lacroix et al, 2006)

Amino acid delivery, protein anabolic effect, signal AA (BAA, …)

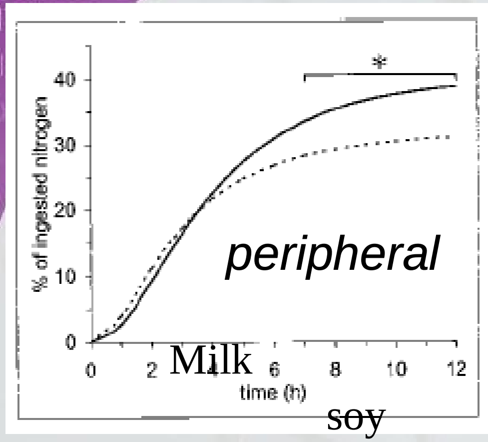


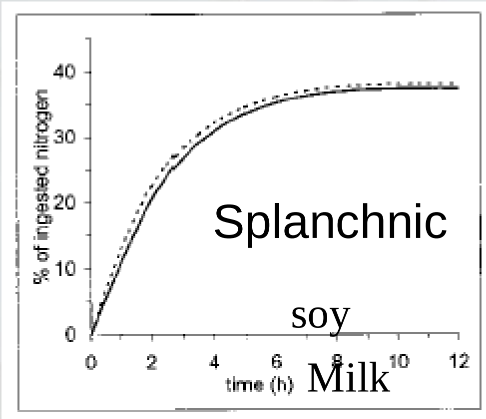


Amino acid deamination, urea production, energy metabolism



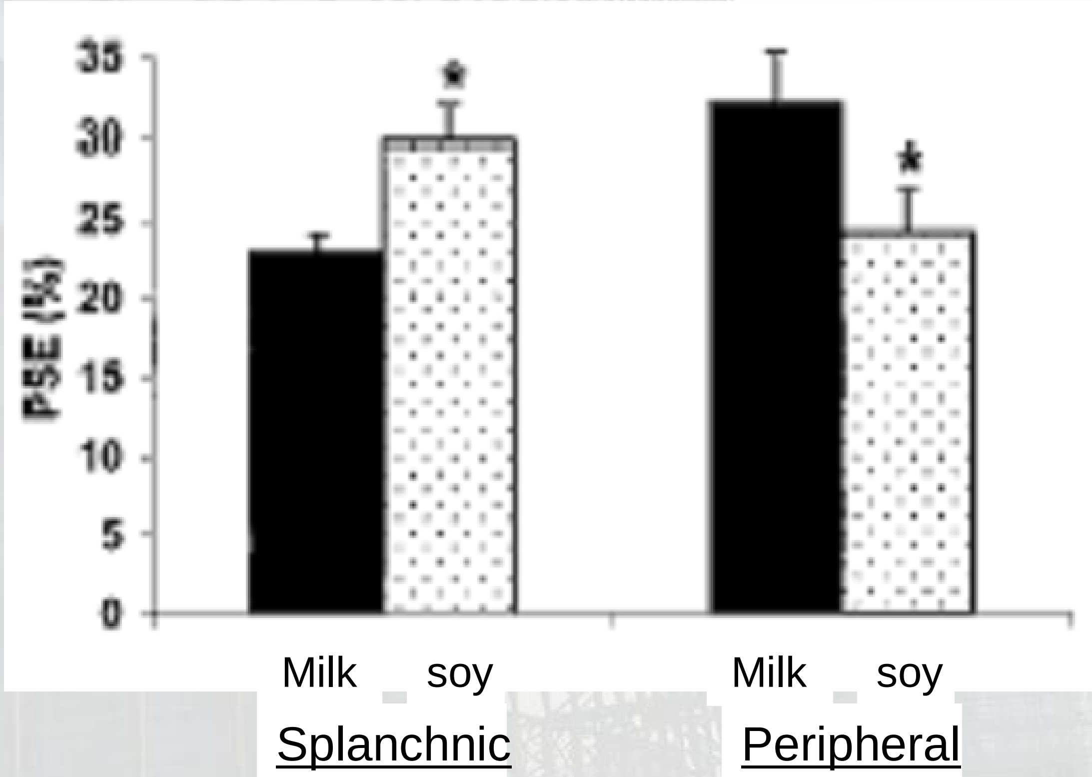
Peripheral and Splanchnic Metabolism of Dietary Nitrogen Are Differently Affected by the Protein Source in Humans (Fouillet el al J. Nutr. 132: 125–133, 2002)





Splanchnic and peripheral retention of dietary amino acids, after the ingestion of a meal made up of milk protein (n 9) or soy protein (n 10) in humans.

Splanchnic and peripheral protein synthesis efficiencies (PSE) of dietary N (8 h) after the ingestion of a meal made up of milk protein (*n 9) or soy protein (n* 10) in humans.



Body Protein Metabolism with advancing age

- Efficiency of indispensable AA use may decrease with advancing age
  - Dose-response relationship between IAA concentrations in the blood and muscle protein synthesis
  - Anabolic resistance of protein synthesis in skeletal muscle can be overcome by increased essential amino acids
  - Need for both nutritional support and resistance exercise
- Specific amino acid pattern of the extra protein involved is unknown
  - Limited studies on relative influence of different protein sources
  - What is the optimal dietary amino acid pattern in terms of specific amino acids, total IAA content, or conditionally indispensable AA?
  - Abundant animal evidence points to potential for leucine

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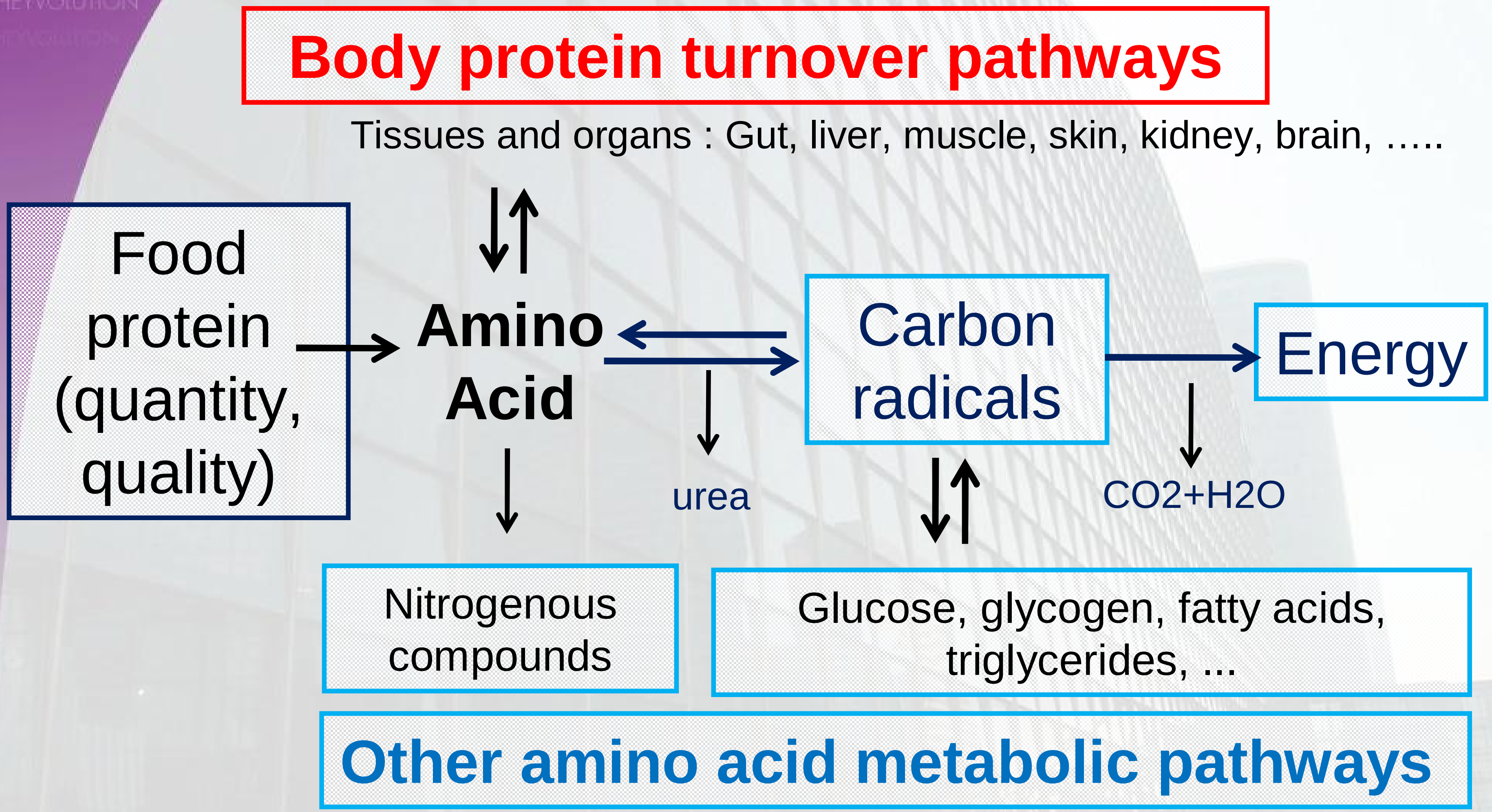
2- Amino acid-dependent modulation of energy and glucose metaolism

- Varying the amount and quality of protein affects energy intake, insulin secretion and action, thereby regulating energy and glucose homeostasis

- High protein diet is accompanied by increased stimulation of insulin and glucagon, high glycogen turnover, and stimulation of gluconeogenesis

- A high protein diet improves glucose, insulin secretion, insulin sensitivity, inhibits liver lipogenesis, and stimulates glycolysis in muscle

Protein and amino acid metabolism pathways



Protein, satiety and food and energy intake

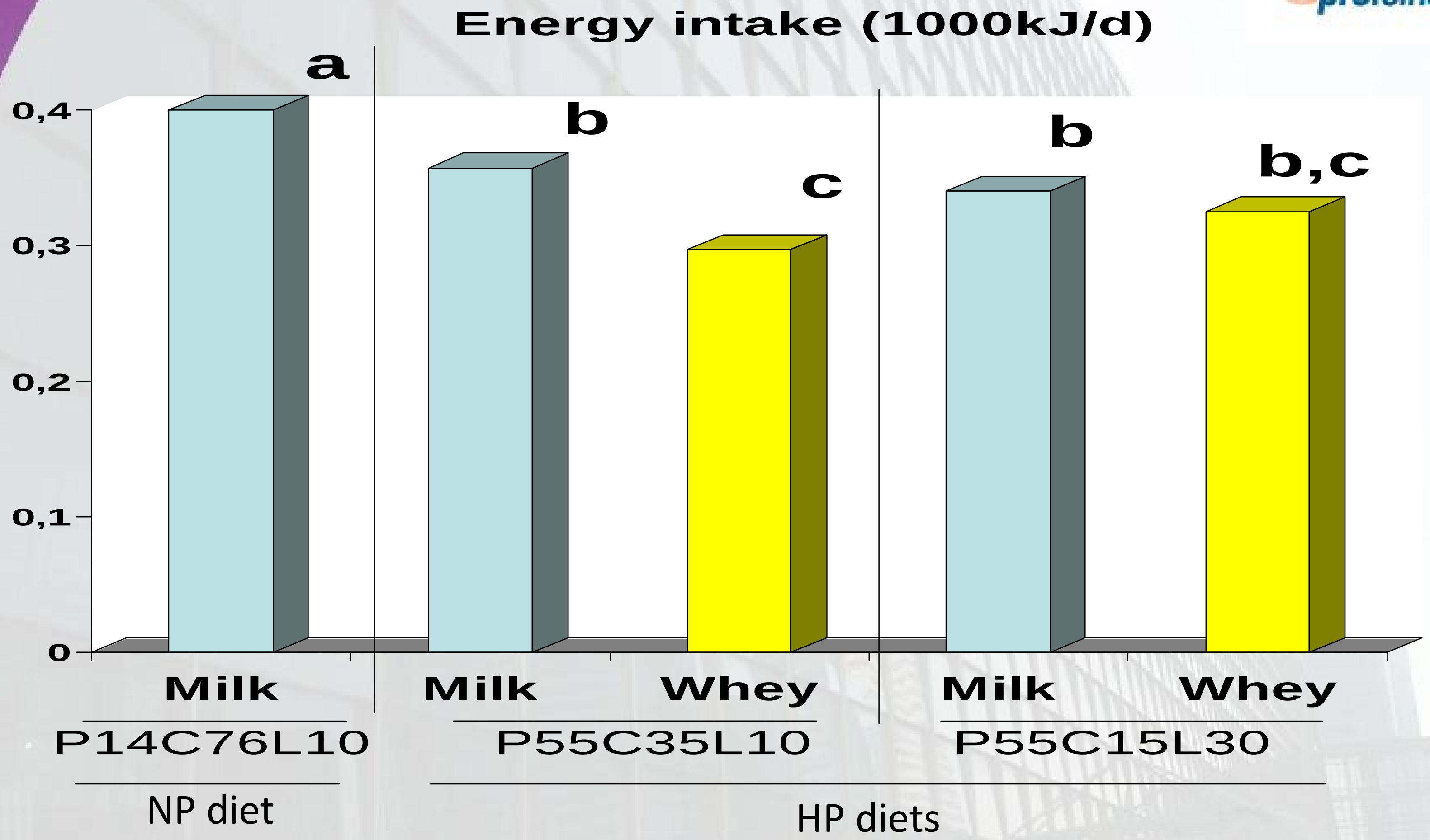
- Dietary protein and amino acids generate signals involved in the induction of satiety and in the control of food intake

- They associate nutrients (amino acids, glucose, …), gut neuropeptides (CCK, PYY, …) and hormones (insulin, …) signals

- They act in the brain either indirectly through vagus-mediated pathways or directly after their release in the peripheral blood

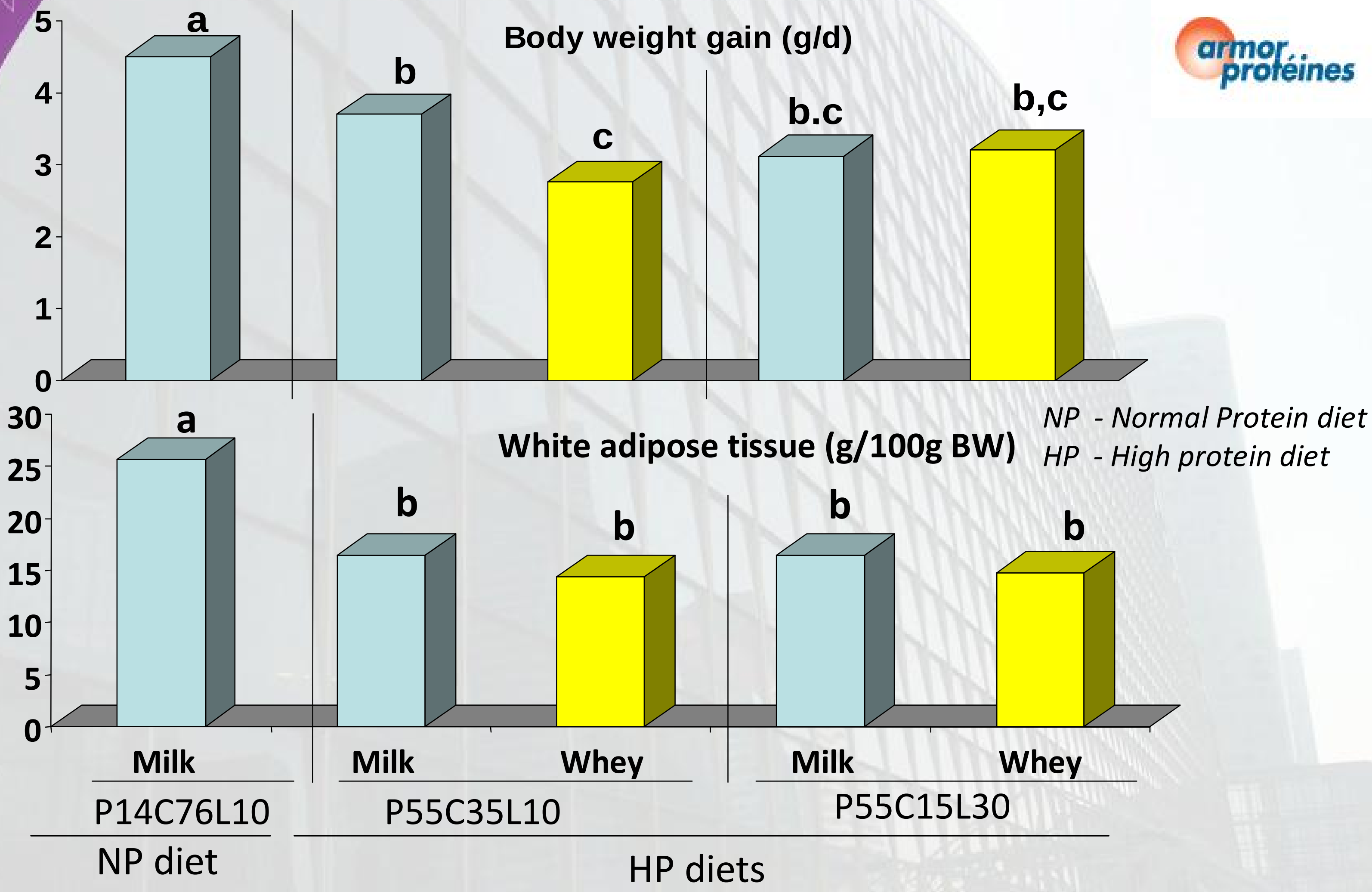
Consequence of a 25 days high milk or whey protein diet on food intake in the rat

(Pichon et al, J Nutr 2006)



Consequence of a 25 days high milk or whey protein diet on food intake and body weight in the rat

(Pichon et al, J Nutr 2006)



A high protein diet reduces food intake, body weight and adipose tissue in rats (8 weeks)

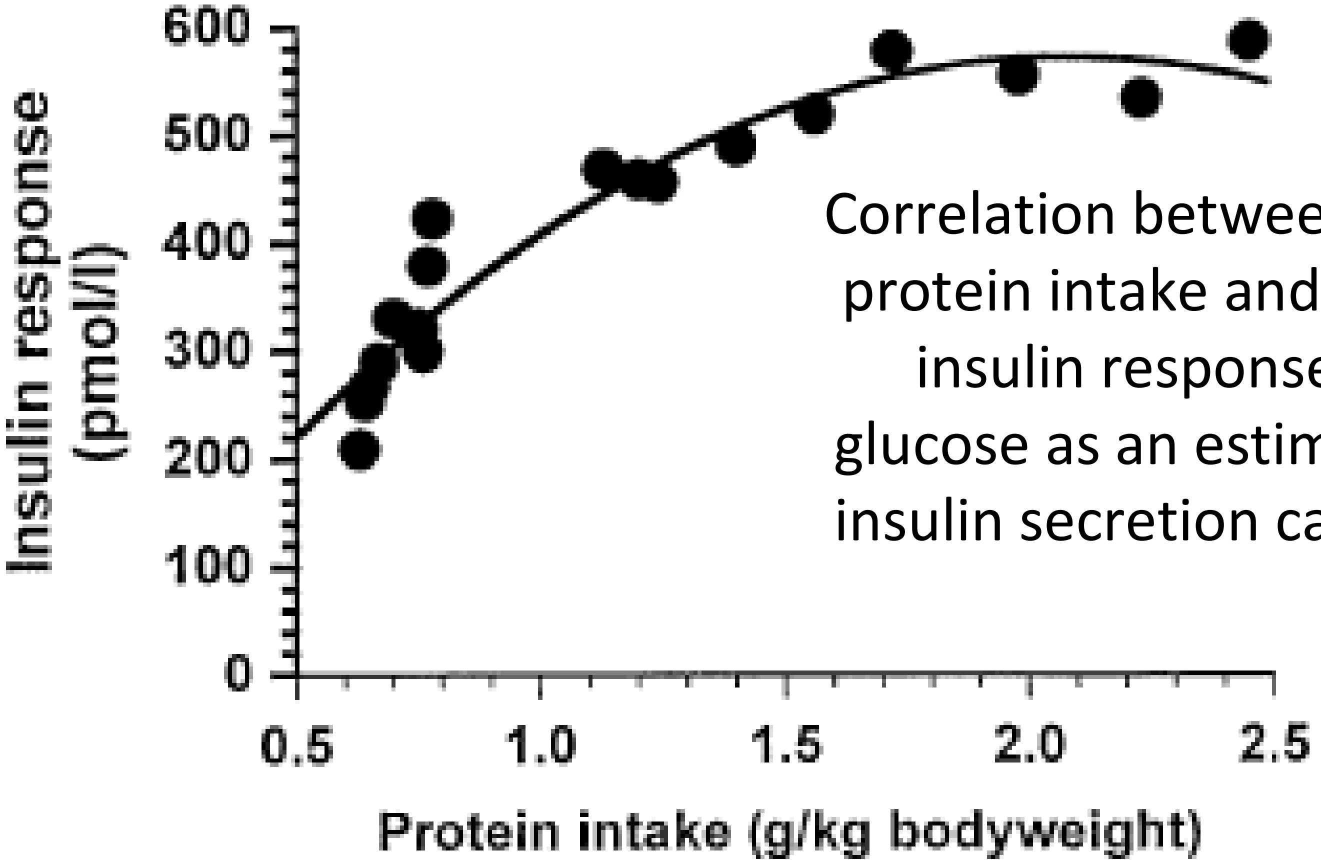
Food intake kJ/d (8th week)	NP	NPr	HP
	341 ± 32^{a}	312 ± 23^{b}	307 ± 23^{b}

	NP	NPr	HP
BW, *g*	431.0 ± 29.7^{a}	392.2 ± 28.7^{b}	388.7 ± 21.1^{b}
Energy efficiency, *kJ feed/g gain*	97.3 ± 14.5	97.9 ± 14.9	97.6 ± 12.6
Stripped carcass, *g*	175.8 ± 11.5^{a}	161.3 ± 14.1^{b}	166.9 ± 12.4^{ab}
White adipose tissue, *g*	70.6 ± 17.5^{a}	60.5 ± 12.4^{ab}	54.1 ± 10.7^{b}

High Carb	HProt
P14C76L10	P55C35L10

Blouet et al, J. Nutr. 136: 1849–1854, 2006.

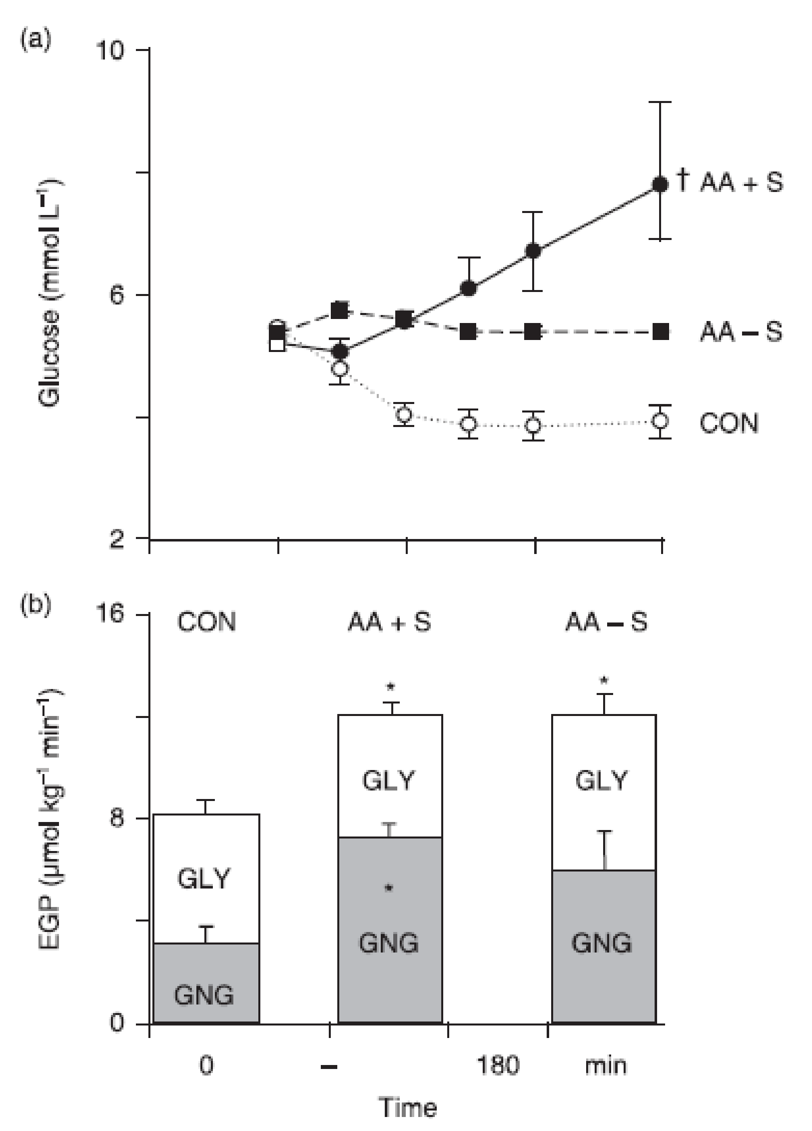
Protein stimulate insulin response



Correlation between daily protein intake and acute insulin response to glucose as an estimate of insulin secretion capacity

Santosa et al, Diabetologia, 2000

Amino acids stimulate liver gluconeogenesis



(a) Plasma concentrations of glucose during an infusion of saline (Con) or amino acids (AA+S) together with somatostatin to inhibit an endogenous release of glucoregulatory hormones including insulin (pancreatic clamps) as well as an infusion of amino acids without somatostatin (AA-S).

(b) Contributions of gluconeogenesis (GNG) and glycogenolysis (GLY) to the tracer determined rates of endogenous glucose production (EGP) in the presence of the above-described conditions; an infusion of saline (CON) or amino acids together with somatostatin (AA + S) as well as an infusion of amino acids without somatostatin (AA − S)

M Krebs, Eur J Clin Invest, 2005

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Increasing protein and lowering carbohydrates decreases de novo fatty acid synthesis (FAS), and enhances adipose tissue lipolysis (LPL) in rats

Pichon et al, J Nutr, 2006

	P14C76L10	P55C35L10	P55L45
Fatty acid synthase activity, *[NADPH,H+] nmol/(mg liver protein·s)*	0.30 ± 0.09[a]	0.21 ± 0.07[b]	0.10 ± 0.04[c]
Lipoprotein lipase activity, *nmol fatty acid formed/(h·10000 cells)*	91.1 ± 50.8	82.8 ± 35.5	137.7 ± 99.3

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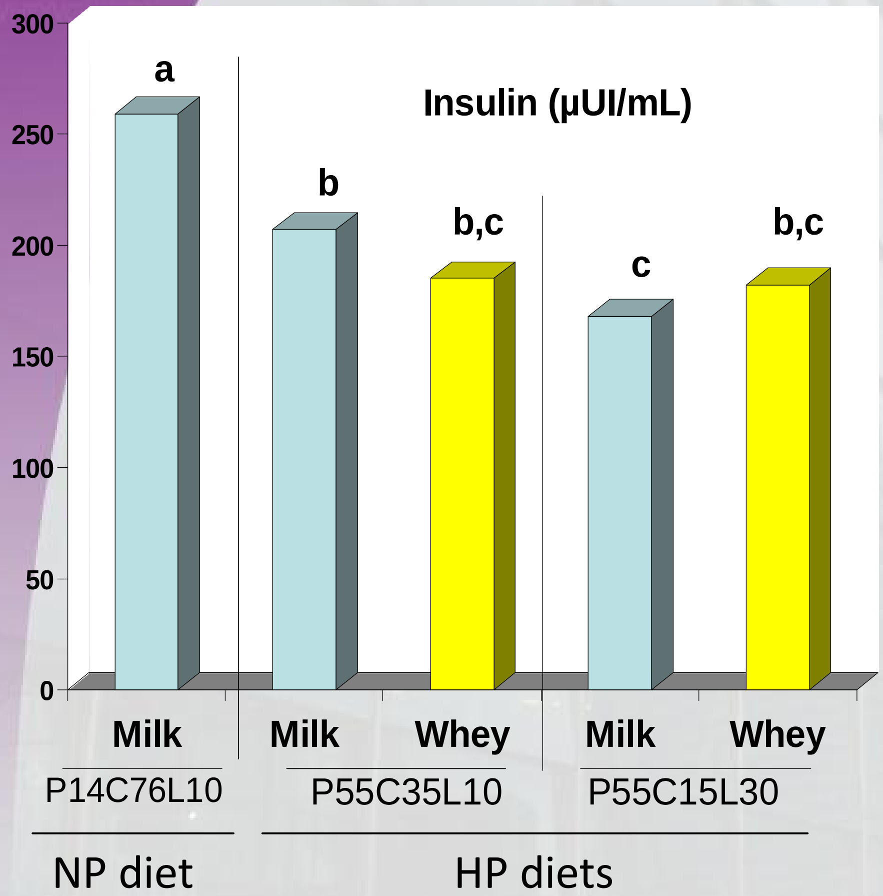
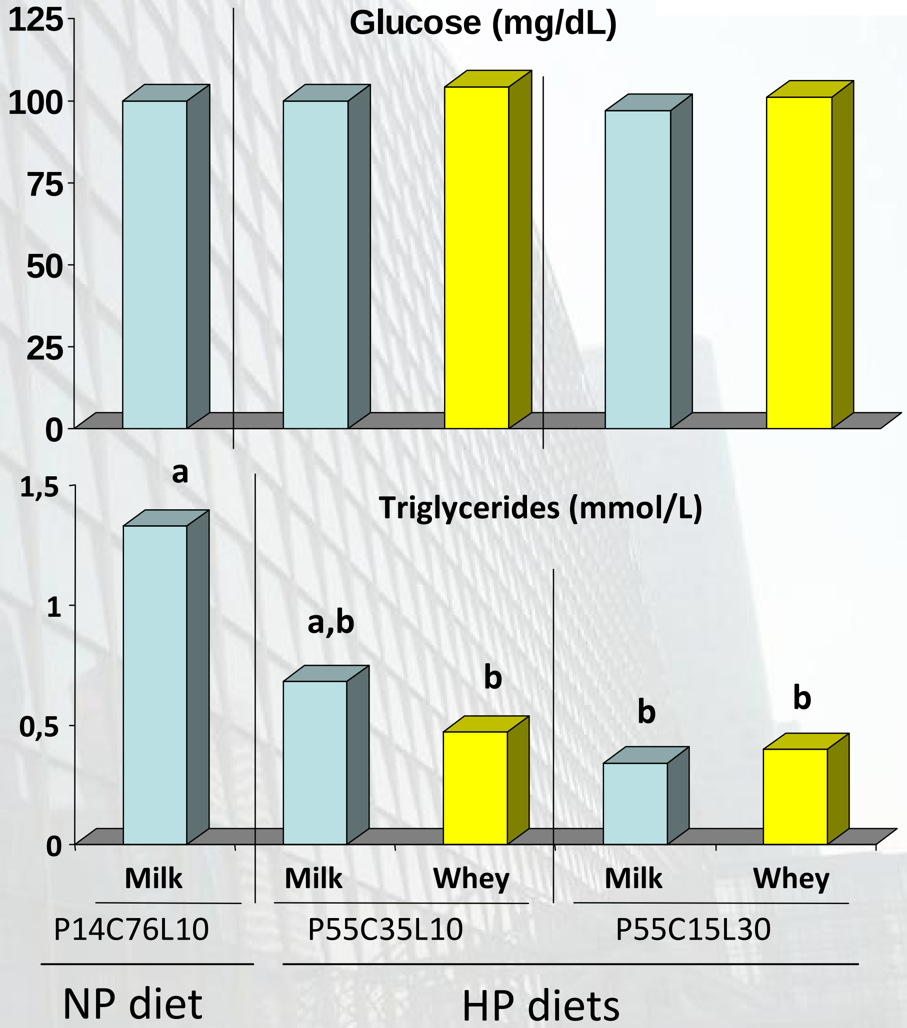
Consequence of a 25 days high milk or whey protein diet on insulin, glucose and triglycerides in the rat

(Pichon et al, J Nutr 2006)





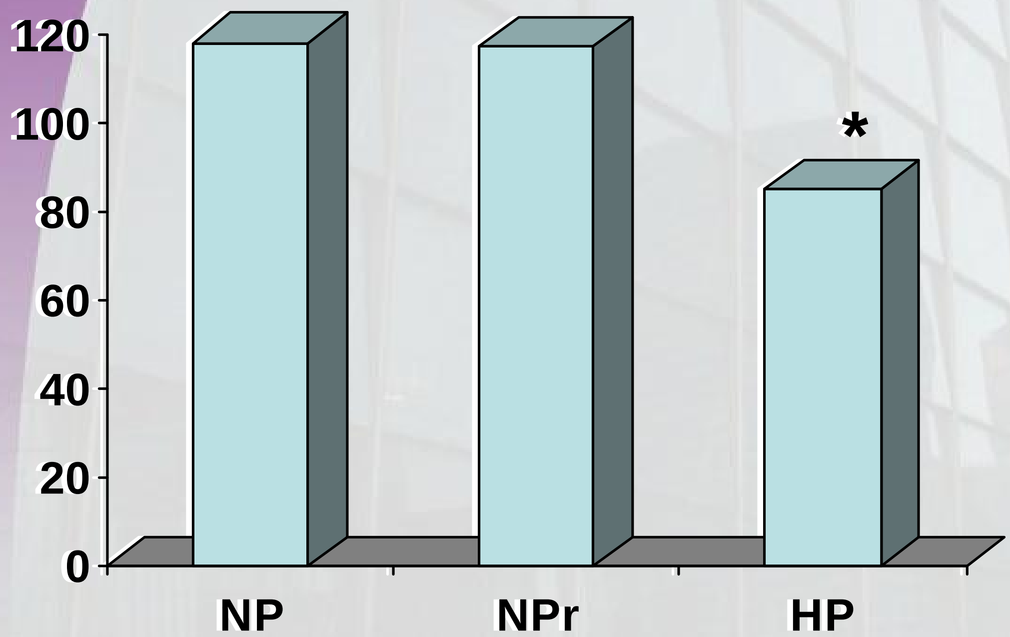
NP - Normal Protein diet
HP - High protein diet

High-Protein feeding improves Glycemic Control in Rats fed ad libitum

Blouet et al, J. Nutr. 136: 1849–1854, 2006.

High Carb	HProt
P14C76L10	P55C35L10

Glucose tolerance (mmol min/L)



Protein improved glucose tolerance in rat fed 7 weeks a HP diet compared to rat fed *ad libitum* a NP diet or rat fed the NP diet pair-fed to the HP diet (NPr)

NP - Normal Protein diet
HP - High protein diet
NPr – NP restricted (pair-fed HP)

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A high protein diet improves glucose, insulin, HOMA index (insulin sensitivity), inhibits liver lipogenesis, and stimulates glycolysis in muscle (7-8 weeks)

Blouet et al, J. Nutr. 136: 1849–1854, 2006.

NP	HP
P14C76L10	P55C35L10

	NP	NPr	HP
Glucose, *mmol/L*	5.5 ± 0.3[a]	5.4 ± 0.2[a]	5.2 ± 0.4[b]
Insulin, *nmol/L*	0.33 ± 0.19	0.27 ± 0.19	0.18 ± 0.08
HOMA	75.4 ± 52.4[a]	66.2 ± 44.1[a]	42.1 ± 20.9[b]

The homeostatic model assessment (HOMA) index was calculated as follows: HOMA = Insulin (pmol/L) x Glucose (mmol/L)/22.5.

	NP	NPr	HP
		Arbitrary units	
Liver SREBP1c	2.1 ± 0.6[a]	1.8 ± 0.7[a]	0.7 ± 0.05[b]
Liver FAS	0.9 ± 0.3[a]	0.8 ± 0.3[a]	0.3 ± 0.1[b]
Muscle SREBP1c	1.1 ± 0.6	1.3 ± 0.4	1.8 ± 0.3
Muscle HKII	1.1 ± 0.3	1.3 ± 0.8	1.9 ± 0.8

- Lower expression of FAS, the rate-limiting enzyme of hepatic lipogenesis
- Lower expression of SREBP-1c, a key mediator of insulin action on FAS expression
- Increased expression of HKII, the first enzyme involved in muscle glucose utilization

3- Amino acid as precursor of nitrogenous compounds

Nitrogenous compounds	glutathione	Cys, gly, glu
	Taurine	Cys
	Carnitine	Lys, Met
	Carnosine	His, b-Ala
	Nucleic acids	Asp, Gln
	Creatine	Arg, Gly
	Nitrogen Monoxyde	Arg
	Polyamines	Arg, Orn
Neuromediators	Serotonin	Trp
	Epinephrine/Norepinephrine	Tyr
	GABA	Glu
	glutamate	Glu

Sensitive pathways in amino acid homeostasis

µmol / kg / j	cystéine	glutamate	Glycine
Flux plamastique	1320	4200	3960
Synthèse de gluthation	550	550	550
Synthèse de taurine	7	-	-

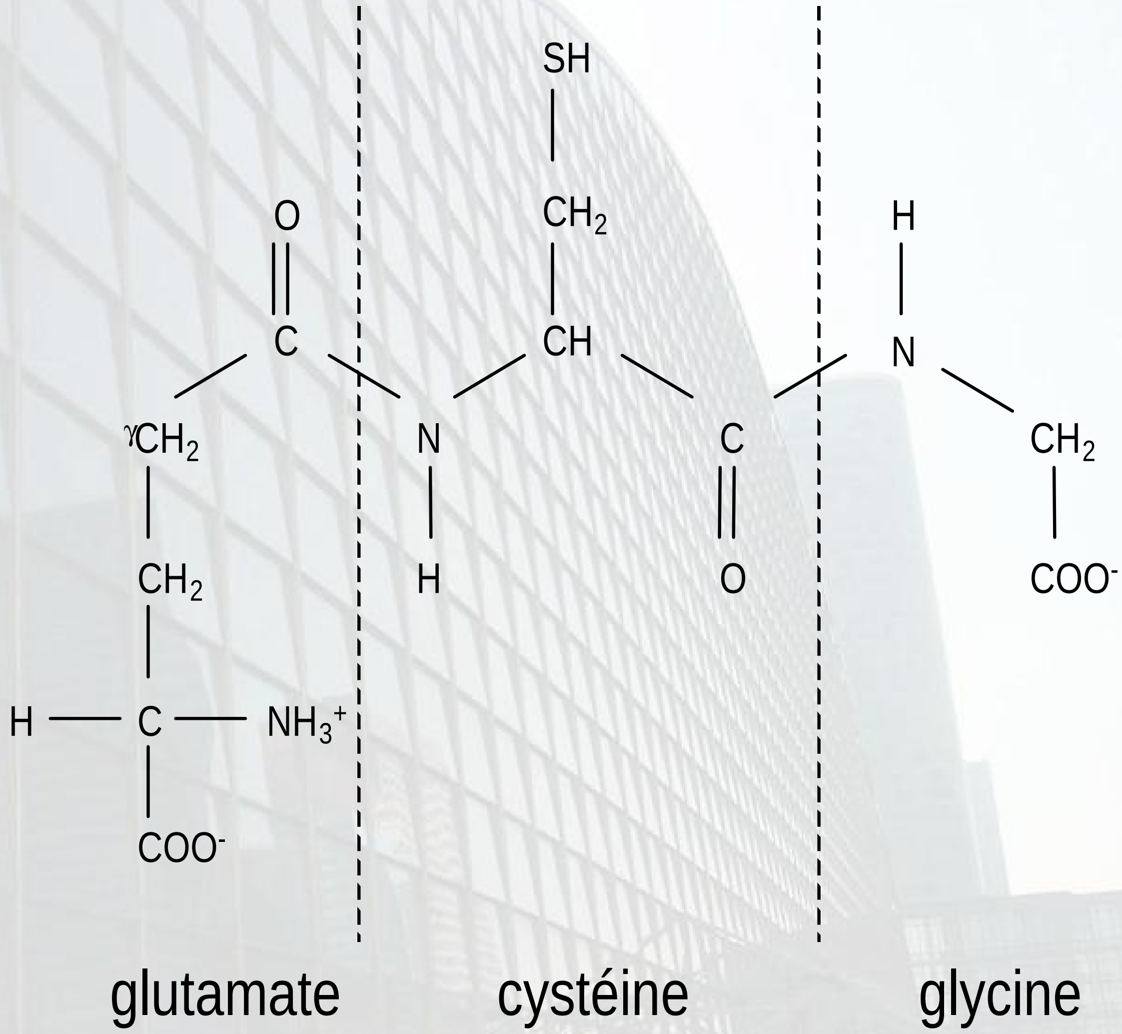
µmol / kg / j	Arginine	Glycine	Méthionine
Flux plamastique	1800	3960	528
Synthèse de créatine	170	170	170
Synthèse de NO	15	-	-

Glutathion synthesis

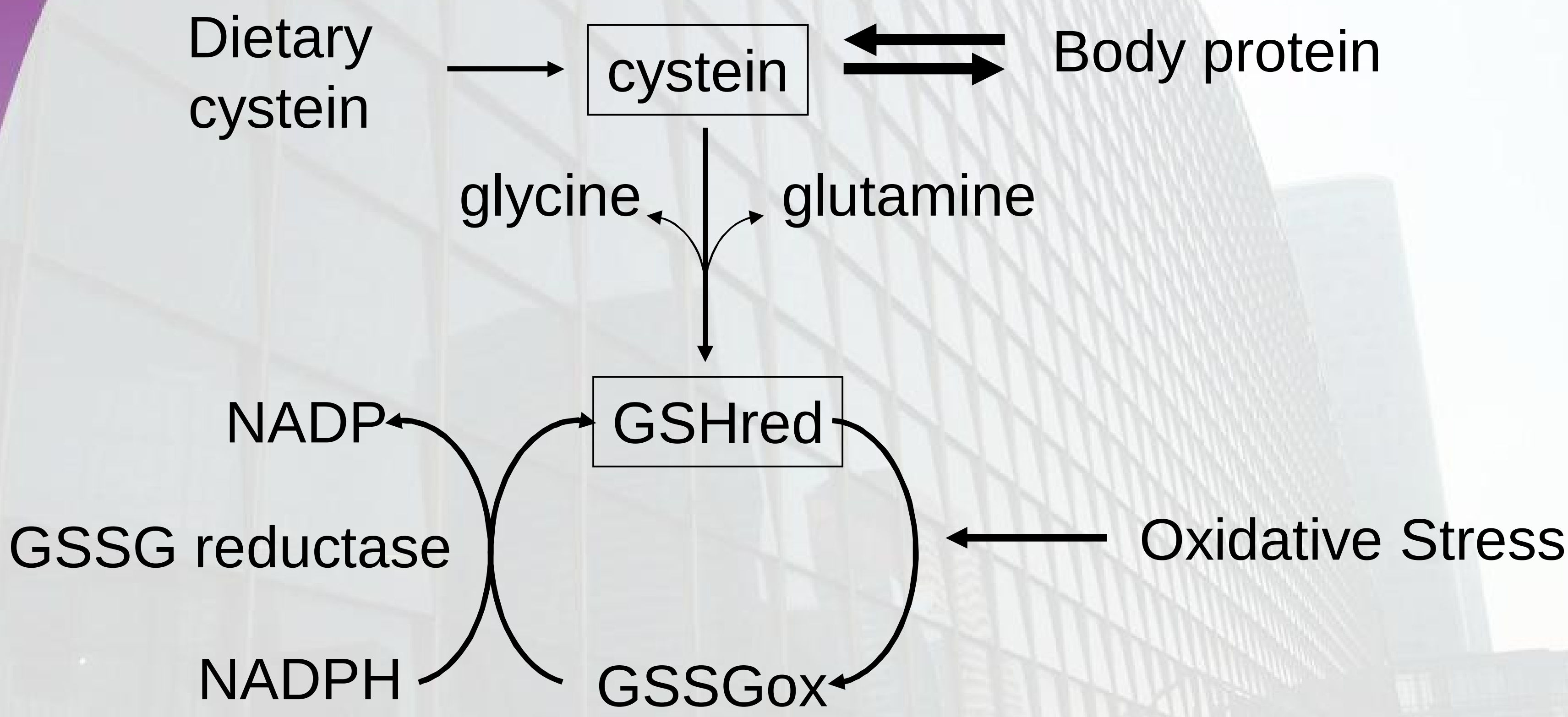
Le glutathion est un tripeptide présent dans les cellules à des concentrations comprises entre 0,5 et 10,0 mM.

Le glutathion est synthétisé dans le cytosol à partir du glutamate, de la cystéine et de la glycine. La proportion de cystéine utilisée par la synthèse de GSH est très importante puisqu'elle représente entre 30 et 50 % de l'utilisation totale de cet acide aminé chez l'homme.

Chez le volontaire sain qu'un apport alimentaire restreint en acides aminés soufrés diminue de manière marquée le taux de synthèse du GSH circulant

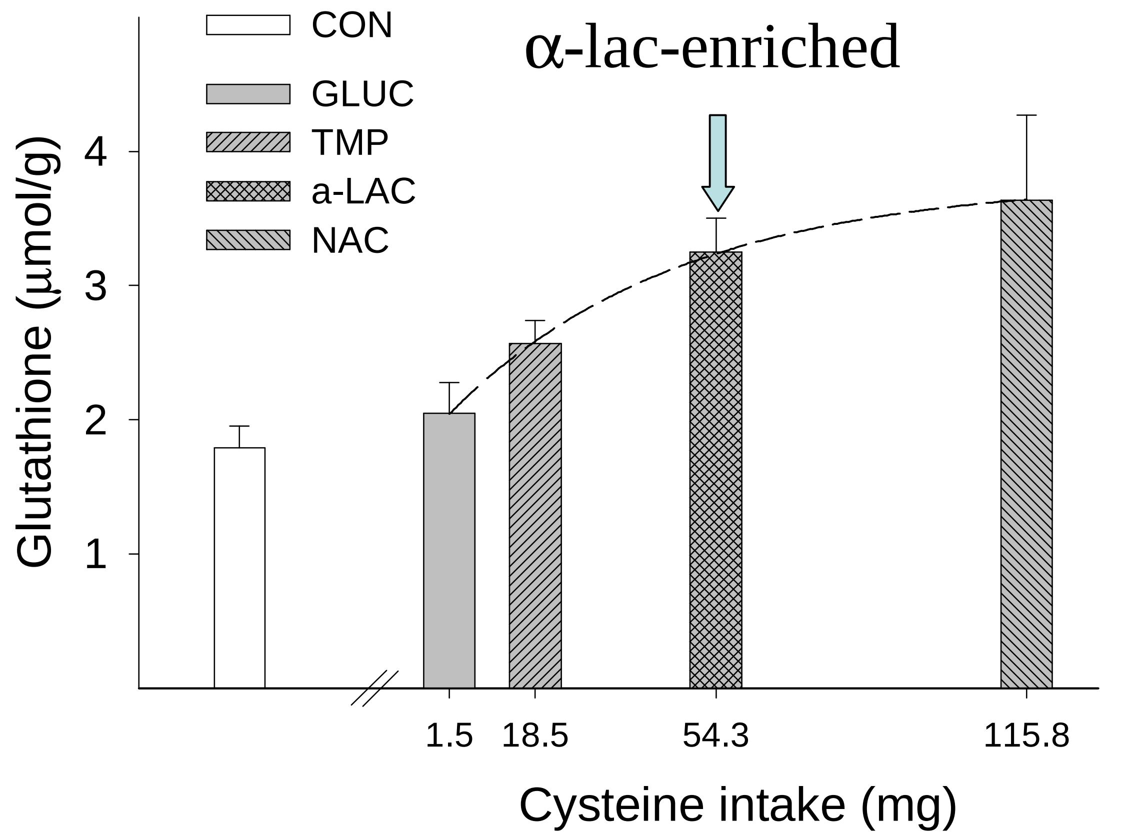


Cystein availability is limiting when glutathion requirement is increased



Whey protein, exercise and stress

A pre-exercice a-lactalbumin-enriched whey preparation improves post-exercise liver glutathione with a dose-dependent effect of the cysteine content in the rat.



Treatment :

- Rats receive the meal complement
- They wait for 1 hours
- They run for 2 hours.
- Liver glutathione is determined after running

(Mariotti et al, 2004)

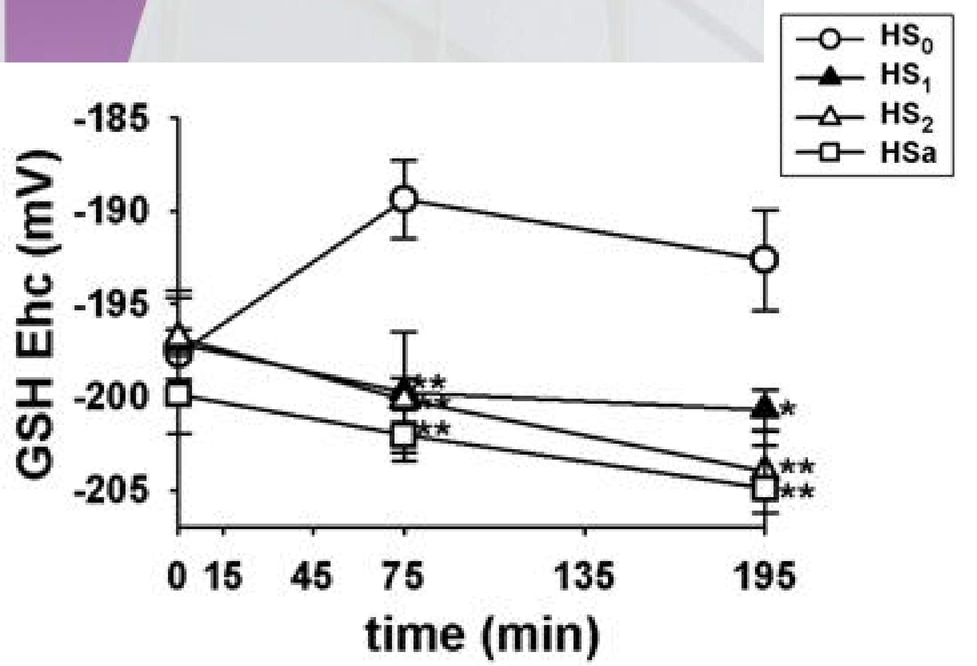
Increasing cysteine dietary intake limits the adverse effects of a high-sucrose diet on oxidative stress and glucose homeostasis in rats.

Blouet et al, 2007

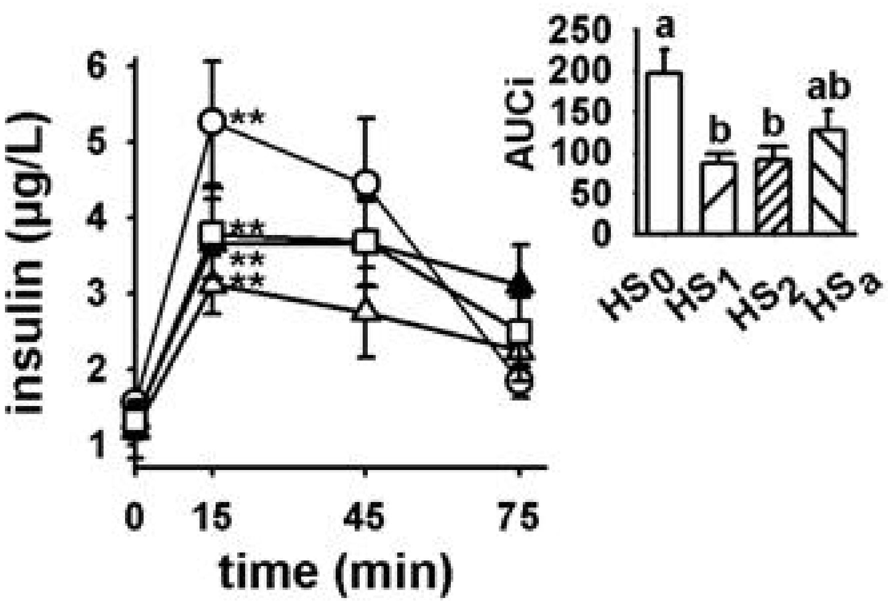
armor protéines

(HS_0) total milk proteins, singly
(HS_1) supplemented with 5.5 g/kg N-acetylcysteine (NAC)
(HS_2) supplemented with16 g/kg N-acetylcysteine (NAC)
(HSa) α-lactalbumin-rich whey concentrate

insulin postprandial AUC values (AUCi, µg.min/L)



Blood GSH Ehc



Plasma insulin concentrations

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Conclusion

- Currently accepted methods for measuring protein quality do not consider the diverse roles of amino acids beyond the first limiting indispensable amino acid for growth or nitrogen balance.

- Research continues to evolve in assessing protein's role in optimal health in relation with the level of protein in the diet and the specific role of individual amino acids.

- The evidence available to date suggests that quality is important not only at the lowest level of dietary protein intake that balances the losses of nitrogen from the body but also at higher intakes.

- The concept of protein quality must expand to incorporate newly emerging actions of protein into the protein quality concept. As a consequence there is need to continue to explore implications for protein and amino acid quality assessment.

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Protein and Health

- Protein quality has been first related to the role of dietary protein in order to avoid deficiency
  - By providing nitrogen and indispensable amino acids
  - to achieve nitrogen balance require 0.8 g/kg/d protein of good quality
- Recent approaches more precisely evaluate protein and amino acid metabolic pathways and their influence on « health markers »
  - Improving body composition, energy homeostasis, glucose homeostasis, insulin sensitivity, lipid metabolism, inflammatory processes, oxidative stress
  - Which quantity and optimal amino acid composition?