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Supplement: Guidance for Assessing Probiotics Beneficial Effects—How to Fill the GAP

Guidance for Substantiating the Evidence for Beneficial Effects of Probiotics: Impact of Probiotics on Digestive System Metabolism[1–3]

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Abstract

Probiotic bacteria have been studied for their potential impact on the metabolism of dietary components in the small intestine lumen including lactose digestion, metabolism of lipids such as cholesterol, and oxalate metabolism. In the large intestine, they contribute to the metabolism of otherwise indigestible dietary carbohydrates (e.g., prebiotics) and have a favorable effect on colonic protein and ammonia metabolism, although their effect on the digestive fate of phytochemicals and xenobiotics is still uncertain. Probiotics also influence metabolism in the host tissues, in particular the gastrointestinal mucosa and the liver. Underlying mechanisms include supply of additional enzymatic activities in the gut lumen and alterations of the composition or metabolic pattern of the gut resident microbiota. For future studies, selection of probiotic strains should include assessment of their metabolic activities, and the outcome of the intervention studies should also take into account the composition of the probiotic matrix and the background diet of the target population. New technologies such as metabolomics hold great promise for assessment of probiotics functionality. J. Nutr. 140: 677S–689S, 2010.

Impact of probiotics on digestive system metabolism

Probiotics can affect metabolic processes relevant for human physiology by passive adhesion of substrates, by providing their own specific enzymatic capacity, by modulating the functioning of the autochthonous microbiota, or by modulating the metabolic and enzymatic functioning of the host intestinal cells, liver, or other tissues.

Conclusions on effects of probiotics on metabolism

Modulation of human health via metabolic effects of probiotics holds great promise as various examples demonstrate that specific strains with particular metabolic traits can deliver concrete health benefits.

In particular:

There is good evidence in humans that consumption of *Lactobacillus* probiotic strains increases the gut populations of lactobacilli.

There is convincing evidence that specific probiotic strains improve lactose digestibility in lactase-deficient individuals.

There is some evidence in humans suggesting that fermentation processes in the mouth and the gut and metabolic activities in the gut mucosa can be altered by certain probiotic strains.

There is not yet convincing evidence in humans that phytochemical bioactivation in the gut can be enhanced by probiotics.

There is promising evidence in animal models suggesting that probiotic strains with particular metabolic traits can

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enhance gut bioactivation of phytoestrogens; this warrants further testing in human intervention studies.
There is emerging evidence on other metabolic effects: halitosis, oxalate metabolism.

However, in most cases the health benefit of modulated metabolism by probiotics remains to be established.

Ongoing basic research on microbiota–host crosstalk will help to identify novel metabolic targets for probiotic intervention. For metabolic effects, probiotics should be standardized not only for the number of colony-forming units (CFU) but also on physiological state and metabolic activity.

Examples from existing studies assessing effects of probiotics on metabolism are provided in **Table 1** (1–84). Below some of the effects of probiotics on metabolism are described in detail to illustrate the main points.

Metabolism of dietary components in the gut lumen

Small intestine

Effects of probiotics on lactose digestion

Lactose (galactose-β-1,4-glucose) is the predominant carbohydrate in milk. It requires a specific intestinal enzyme, lactase, to be split into the 2 constitutive monosaccharides, which are absorbed in the small intestine. In individuals with lactase deficiency, the unabsorbed lactose is fermented by autochthonous microbiota in the large intestine, producing SCFA and gas (hydrogen and/or methane and carbon dioxide). The increased osmotic load resulting from unabsorbed lactose in the ileum and colon and the colonic gas production contributes to the symptoms of lactose intolerance. The kinetics of hydrogen production after ingestion of lactose is an easy indirect method to assess lactose maldigestion in hydrogen producers (85). The phenomenon of lactose maldigestion is genetically determined and occurs in ~70% (2–100%) of the adult population in the world (86).

Lactose digestion and probiotics. Probiotics with lactase activity may contribute to the digestion of lactose when added to lactose-containing foods. Despite a large variability in lactase activities (strain-specific lactase activities may vary over 100-fold) (87), only specific probiotic strains are effective: many fermented milks have been found to be without any significant effect on lactose digestion, but yogurt and kéfir do provide lactase activity (Table 1).

Lactose digestion and yogurt. The first double-blind randomized study in humans (2) reported a significant effect of yogurt containing live *L. bulgaricus* and *S. thermophilus*. Milk fermented by *L. acidophilus* was without an effect, as was the control consisting of heat-treated fermented milk without living bacteria. The requirement for specific living yogurt cultures has been confirmed by many studies (Table 1) and in different ethnic groups. Various yogurt symbioses, identified either by strain number or brand name of products, have been tested with similar results in 23 studies (**Fig. 1**). Eight studies confirmed the benefits of living cultures, and 6 studies reported the specificity of yogurt cultures. The mechanism of the effect is not yet fully understood. The first hypothesis was that the effect is directly related to the lactase activity of the strain. The current hypothesis is that both lactase capacity and permease activity are required to allow the lactose to get into the bacteria to be digested. One strain (S 85) has been tested by 2 different laboratories with similar results, and 1 study compared the association of 2 *L. bulgaricus* with 2 *S. thermophilus* (4 products) and found no significant differences among products. Most of the studies focusing on lactose absorption measured by breath test used similar protocols and similar threshold criteria. On average, breath test studies enrolled a median of 11 participants (from 7 to 30) after an overnight fast excluding fermentable sugars from the dinner. Breath hydrogen concentrations are measured every 30 min for 4 to 12 h (median 8 h) on 1 single day and expressed either as the area under the curve or peak concentration. Yogurt reduced hydrogen excretion by 76 ± 11% (mean ± SEM). Lerebours et al. (5) reported a similar effect of yogurt on lactose digestion on the first day of yogurt ingestion and after 2 wk of yogurt ingestion.

Impact of different probiotics on lactose intolerance. The phenomenon of lactose maldigestion is widespread throughout the world, and the adverse symptoms associated with it are called lactose intolerance. Symptoms of lactose intolerance may result from different mechanisms originating in the small intestine (reaction to osmotic load) or in the large intestine (reaction to fermentation rate). Improvement of lactose digestion, therefore, should mitigate the lactose intolerance symptoms. A recent metaanalysis, however, concluded that lactose is not a major cause of intestinal symptoms for lactose maldigesters following usual intakes of dairy foods (88). A recent intervention study with defined probiotics also concluded that, in lactose-intolerant participants, symptoms of lactose intolerance were improved without an effect on lactose digestion (89). This observation is in line with the outcome of a systematic review that concluded that probiotic supplementation in combination with nonfermented dairy products does not mitigate the symptoms of lactose intolerance in adults (90). However, the authors also reported that certain strains, concentrations, and preparations of probiotics might be effective.

Yogurt. Convincing evidence suggests that the intake of yogurt causes fewer symptoms of lactose intolerance than does the consumption of milk. This may have several reasons including high lactase activity (β-galactosidase) of bacteria used in the yogurt production, partial hydrolysis of lactose in fermented products, and slower intestinal transit because of the digestion of lactose by yogurt. The combined result is a slower delivery of lactose to the intestine, thus optimizing the action of residual β-galactosidase in the small bowel. Yogurt symbiosis provides similar lactose digestibility, and this looks like a specificity of a symbiosis beyond the specificity of strains. One study analyzed the role of separate yogurt cultures, *L. bulgaricus* and *S. thermophilus*, and reported that *L. bulgaricus* alone was able to provide part of the lactose digestibility, and this was enhanced by fermentation. All studies using "yogurt-like products" without living bacteria confirmed the importance of the living state of the symbiosis.

In conclusion, fermentation by lactic acid probiotics may decrease lactose content by ~20–40% (21,89), but only specific yogurt symbiosis and kéfir support lactose digestion. The improvement of lactose digestion by some probiotics may not alleviate the symptoms of lactose intolerance in every subject. A metaanalysis of existing data will be welcome.

Effects of probiotics on lipid metabolism

The digestion and absorption of lipids are complex metabolic phenomena occurring mainly in the small intestine. Some

TABLE 1 Probiotics and metabolism in the digestive system

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Lactose digestion/intolerance					
Gilliland and Kim 1984 (1)	6 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R[1]	Yogurt with live bacteria was more active than heat-treated yogurt in reducing breath H_2.
Savaiano et al. 1984 (2)	9 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R	Yogurt with live bacteria reduced breath H_2. Frozen yogurt was less efficient.
Martini et al. 1987 (3)	9 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R	Yogurt with live bacteria reduced breath H_2. Frozen yogurt was less efficient.
Rizkalla et al. 2000 (4)	24 adults (with lactose maldigestion)	Yogurt fresh or heat-treated	1 d	R	Less breath H_2 with heated yogurt
Lerebours et al. 1989 (5)	16 adults (with lactose maldigestion)	Yogurt with starter bacteria	2 wk	R	Long-term intake of lactose did not improve lactose digestion compared with short-term.
Hertzler et al. 2003 (6)	15 adults (with lactose maldigestion)	Kefir or yogurt with defined starter cultures	1 d	R	Kefir and yogurt equally reduced breath H_2.
Martini et al. 1987 (7)	4 adults (with lactose maldigestion)	Milk with yogurt with starter bacteria	1 d	R	Intact yogurt starter bacteria more efficiently reduced breath H_2 than disrupted bacteria.
Pelletier et al. 2001 (8)	24 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R, PC[2]	Less breath H_2 with live yogurt bacteria
Vesa et al. 1996 (9)	14 adults (with lactose maldigestion)	Yogurt or fermented milk with defined bacteria	1 d	R	No differences between fermented products in breath H_2 production
He et al. 2004 (10)	45 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R	Heat-treatment eliminated beneficial effect on breath H_2.
Martini et al. 1991 (11)	7 adults (with lactose maldigestion)	Yogurt with starter bacteria and milk with *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *B. bifidus* with different microbial β-galactosidase activity	1 d	R	All yogurts improved lactose digestion regardless of β-galactosidase activity.
Martini et al. 1991 (12)	12 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R	Yogurt aided in the digestion of lactose resulting in less breath H_2. However, yogurt did not aid in digestion of additional lactose
Kotz et al. 1994 (13)	10 adults (with lactose maldigestion)	Yogurt with increased β-galactosidase activity	1 d	R	High lactase activity was associated with reduced breath H_2. This lactase was much less acid-resistant.
Lin et al. 1991 (14)	10 adults (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R, PC	Isolated yogurt starter bacteria itself reduced breath H_2.
Shermak et al. 1995 (15)	14 children (with lactose maldigestion)	Yogurt with starter bacteria	1 d	R	Yogurt with viable bacteria induced the same AUC for breath H_2 as milk, however, with a delayed time to rise and a lower rate of rise.
Lin et al. 1998 (16)	20 adults (with lactose maldigestion)	Milk containing *L. acidophilus* or *L. bulgaricus* at 10e8 and 10e9	1 d	R, PC	While *L. bulgaricus* significantly reduced breath H_2 and symptoms, *L. acidophilus* only reduced symptoms at 10^9 but not at 10^8.
Jiang et al. 1996 (17)	15 adults (with lactose maldigestion)	Milk containing different strains of *B. longum*	1 d	R, PC	Only when *B. longum* was grown in medium containing lactose it significantly reduced breath H_2.
Dehkordi et al. 1995 (18)	16 adults (with lactose maldigestion)	Milk containing *L. acidophilus*	1 d	R	*L. acidophilus* did not reduce breath H_2.

(*Continued*)

TABLE 1 Continued

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Mustapha et al. 1997 (19)	11 adults (with lactose maldigestion)	Milk containing different strains of *L. acidophilus*	1 d	R, PC	While some *L. acidophilus* strains reduced breath H_2, others were inactive.
Newcomer et al. 1983 (20)	18 adults (with lactose maldigestion)	Milk containing *L. acidophilus*	1 d	R, PC	No effect of *L. acidophilus* on lactase deficiency symptoms
McDonough et al. 1987 (21)	14 adults (with lactose maldigestion)	Yogurt and heated yogurt with/without added lactase; acidophilus milk	1 d	R	Yogurt with live bacteria reduced breath H_2 more efficiently than heated yogurt. Sonication of acidophilus milk reduced breath H_2.
Kim and Gilliland 1983 (22)	6 adults (with lactose maldigestion)	Milk with *L. acidophilus*	1 d	R, PC	*L. acidophilus* reduced breath H_2.
Saltzman et al. 1999 (23)	18 adults with lactose maldigestion)	*L. acidophilus* BG2FO4	1 d	R	*L. acidophilus* BG2FO4 failed to change breath H_2 excretion after lactose ingestion.
Yesovitch et al. 2004 (24)	10 adults (with lactose maldigestion)	Mixture of 8 strains at 2 doses	1 d		Mixture of 8 strains did not improve lactose digestion.
Zhong et al. 2006 (25)	11 adults (with lactose maldigestion)	Yogurt with *S. thermophilus*, *L. bulgaricus* and *B. animalis* or a capsule containing *B.longum*	2 wk		Yogurt as well as *B. longum* alone improved symptoms of lactose intolerance.
Lipid metabolism					
Naruszewickz et al. 2002 (26)	36 adults (hypercholesterolemic)	*L. plantarum* 299V	6 wk	R, PC	Decrease of blood pressure Decrease of blood fibrinogen No change in blood lipids
Simons et al. 2006 (27)	46 adults (hypercholesterolemic)	1 *L. fermentum* strain	10 wk	R, PC	No change in blood lipids
Lewis et al. 2005 (28)	80 adults (hypercholesterolemic)	*L. acidophilus* strain B	6 wk	R, PC	No change in blood lipids
Anderson et al. 1999 (29)	40 adults (hypercholesterolemic)	*L. acidophilus* L-1	4 wk	R, PC, cross over	Inconclusive: the 2 arms differ.
Lin et al. 1989	354 adults (hypercholesterolemic)	1 *L. acidophilus* strain + 1 *L. bulgaricus* strain	6 wk	R, PC	No change in blood lipids
Greany et al. 2004 (31)	37 adults (hypercholesterolemic)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	6 wk	R, PC	No change in blood lipids
Kiessling et al. 2002 (32)	29 adults (hypercholesterolemic)	*L. acidophilus* 145 + *B. longum* 913	7 wk	R, PC	Decrease of blood total and HDL cholesterol
Anderson et al. 1999 (29)	29 adults (hypercholesterolemic)	*L. acidophilus* L-1 + *S. thermophilus* MUH 341	3 wk	R, PC	Decrease of blood total cholesterol No change in other lipids
Larsen et al. 2006 (33)	15 adults (hypercholesterolemic)	*L. paracasei* + *B. animalis* subsp. *lactis* BB-12	3 wk	R, PC	No change in blood lipids No change in bowel habits
Kawase et al. 2000 (34)	20 adults (hypercholesterolemic)	*L. casei* TMC 0409 + *S. thermophilus* TMC 1542	8 wk	R, PC	Decrease of blood pressure
Agerholm-Larsen et al. 2000a (35)	14 adults (hypercholesterolemic)	2 *S. thermophilus* strains + 2 *L. acidophilus* strains	8 wk	R, PC	Decrease of blood pressure No change in blood lipids
Agerholm-Larsen et al. 2000b (35)	4 adults (hypercholesterolemic)	2 *S. thermophilus* strains + 1 *L. rhamnosus* strain	8 wk	R, PC	Decrease of blood pressure No change in blood lipids
Kullisaar et al. 2003 (37)	21 adults (hypercholesterolemic)	*L. fermentum* ME-3 + *L. plantarum* LB-4 + *L. buchneri* S 15	3 wk	R, PC	Increase of serum antioxydant activity
Songisepp et al. 2005 (38)	26 adults (hypercholesterolemic)	*L. fermentum* ME-3 + *L. plantarum* LB-4 + *L. buchneri* S 15	3 wk	PC	Increase of serum antioxydant activity
St Onge et al. 2002 (39)	13 adults (hypercholesterolemic)	KÕfir	4 wk	R, PC	No change in blood lipids
Fabian and Elmadfa 2006 (40)	33 adults (hypercholesterolemic)	Yogurt Yogurt + *L. casei*	2 wk	R, PC	Decrease of blood total and LDL cholesterol Increase of blood HDL cholesterol
Xiao et al. 2003 (41)	16 adults (hypercholesterolemic)	Yogurt + *B. longum* BL1	4 wk	R, PC	No change in blood lipids
De Roos et al. 1999 (42)	78 adults (hypercholesterolemic)	Yogurt + *L. acidophilus* L-1	6 wk	R, PC	No change in blood lipids
Bertolami et al. 1999 (43)	32 adults (hypercholesterolemic)	1 *E. faecium* strain + 2 *S. thermophilus* strains	8 wk	R, PC	Decrease of blood total cholesterol
Richelsen et al. 1996 (44)	90 elderly (hypercholesterolemic)	1 *E. faecium* strain + 2 *S. thermophilus* strains	6 mo	R, PC	Decrease of blood lipids

(*Continued*)

TABLE 1 Continued

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Schaafsma et al. 1998 (45)	30 elderly (hypercholesterolemic)	Yogurt + 1 *L. acidophilus* strain + Prebiotics	3 wk	R, PC	Decrease of blood total and LDL cholesterol
Hlivak et al. 2005 (46)	43 elderly (hypercholesterolemic)	*E. faecium* M-74	1 y	R, PC	Decrease of blood total and LDL cholesterol
Rossouw et al. 1981 (47)	10 children (hypercholesterolemic)	Yogurt (2 liters)	3 wk	R, PC	No change in blood lipids
Protein metabolism					
De Preter et al. 2006 (48)	3x15 adults (healthy)	*Saccharomyces boulardii*	4 wk	R, PC	*No* effect on *p*-cresol excretion
De Preter et al. 2006 (49)	43 adults (healthy)	*Saccharomyces boulardii*	Single dose or 4 wk	R, PC	*No* effect on *p*-cresol and N-excretion
De Preter et al. 2007 (50)	20 adults (healthy)	*L. casei Shirota* + *B. breve*	Single dose or 4 wk	R, PC	Long-term intake tended to decrease urinary N-excretion
De Preter et al. 2004 (51)	20 adults (healthy)	*L. casei Shirota*	2 wk	R, PC	Reduced *p*-cresol excretion
Takayama et al. 2003 (52)	11 adults: capsule 11 adults: powder (with hemodialysis)	*B. longum* (as powder or in gastroresistant seamless capsule)	5 wk		*B. longum* provided as capsule reduced serum levels of indoxyl sulfate; powder was not active
Phytoestrogen metabolism					
Bonorden et al. 2004 (53)	37 adults (healthy premenopausal women)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	2 mo	R, PC	No influence on the effects of soy on estrogen metabolism No change in equol excretion
Nettleton et al. 2004 (54)	40 adults (healthy postmenopausal women)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	6 wk	R, PC	No change in plasma isoflavone concentration No change in equol excretion
Nettleton et al. 2005 (55)	40 adults (healthy postmenopausal women)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	6 wk	R, PC	No influence on the effects of soy on estrogen metabolism
Nettleton et al. 2005 (56)	40 adults (healthy postmenopausal women)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	6 wk	R, PC	No influence on the effects of soy on plasma reproductive hormone concentrations
McMullen et al. 2006 (57)	39 adults (healthy men)	*L. acidophilus* DDS + *B. longum* (DDS Plus)	2 mo	R, PC	No change in equol excretion
Tsangalis et al. 2005 (58)	16 adults(healthy postmenopausal women)	*B. animalis* subsp. *lactis* BB-12	2 wk	R, PC	No change in urinary isoflavone concentration
Tsangalis et al. 2007 (59)	16 adults (healthy postmenopausal women)	*B. animalis* subsp. *lactis* BB-12	2 wk	R, PC	No change in equol excretion
Cohen et al. 2007 (60)	36 adults (healthy premenopausal women)	*L. rhamnosus GG*	1 mo	R	No influence on the effects of soy on estrogen metabolism
Larkin et al. 2007 (61)	18 adults (mildly hypercholesterolemic, postmenopausal women and men > 45 y.old)	*L. rhamnosus GG* + 1 *L. acidophilus* strain + 1 *B. bifidus* strain	5 wk	R, PC	No change in plasma and urinary isoflavone concentrations No change in equol excretion
Mycotoxins (Xenobiotic metabolism)					
El-Nezami et al. 2006 (62)	90 adults (healthy)	*L. rhamnosus* LC 705 + 1 *P. freudenreichii* Shermani strain	5 wk	PC	Decrease of urinary excretion of AFB-N7-guanine
Mutagens (Xenobiotic metabolism)					
Matsumoto et al. 2004 (63)	7 adults (healthy)	*B. animalis* subsp. *lactis* LKM 512	2 wk	PC	Increase of fecal spermidine Decrease of fecal mutagenicity
Genotoxicity of fecal water (Xenobiotic metabolism)					
Oberreuther-Moschner et al. 2004 (64)	9 adults (healthy)	*L. acidophilus* 145 + *B. longum* 913	7 wk	R	Decrease in fecal water genotoxicity
Gut microbiota composition					
Guerin-Danan et al. 1998 (65)	39 toddlers (healthy)	*L. casei* DN-114001	1 mo	R, PC	Increase of the number of participants with > 10^6 CFU lactobacilli/g feces

(*Continued*)

TABLE 1 Continued

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Spanhaak et al. 1998 (66)	20 adults (healthy)	*L. casei* Shirota	1 mo	R, PC	Increase of fecal lactobacilli and bifidobacteria
Tuohy et al. 2007 (67)	20 adults (healthy)	*L. casei* Shirota	3 wk	R, PC	Increase of fecal lactobacilli and enterococci
Goossens et al. 2003 (68)	22 adults (healthy)	*L. plantarum* 299V	1 mo	R, PC	Increase of fecal lactobacilli
Fujiwara et al. 2001 (69)	34 adults (healthy)	*L. gasseri* SBT2055	1 wk		Increase of fecal lactobacilli Decrease of fecal staphylococci
Tannock et al. 2000 (70)	10 adults (healthy)	*L. rhamnosus* DR20	6 mo		Increase of fecal lactobacilli and enterococci Alteration of the profile of fecal lactobacilli
Satokari et al. 2001 (71)	10 adults (healthy)	*B. animalis* subsp. *lactis* BB-12	2 wk		No change in the profile of fecal bifidobacteria
Mohan et al. 2006 (72)	69 preterm infants	*B. animalis* subsp. *lactis* BB-12	3 wk	R, PC	Increase of fecal bifidobacteria Decrease of fecal clostridia and enterobacteria
Ahmed et al. 2007 (73)	80 elderly (healthy)	*B. animalis* subsp. *lactis* HN019	1 mo	R, PC	Increase of fecal bifidobacteria, lactobacilli and enterococci Decrease of fecal enterobacteria
Li et al. 2004 (74)	30 preterm infants	*B. breve*	7 wk	R	Earlier gut colonization with bifidobacteria
Zhao et al. 2004 (75)	50 adults (with liver corrhosis)	*Bifidobacterium* + *L. acidophilus* + *Enterococcus Bacillus subtilis* + *E. faecium*	2 wk		Increase of fecal bifidobacteria
Gut microbiota metabolism					
Guerin-Danan et al. 1998 (65)	39 toddlers (healthy)	*L. casei* DN-114001	1 mo	R, PC	Decrease of fecal β-glucosidase and β-glucuronidase activities
Pawlowska et al. 2007 (76)	25 children (liver-transplanted)	*L. casei* DN-114001	2 mo	R, PC	Decrease of fecal β-glucosidase and β-glucuronidase activities
Spanhaak et al. 1998 (66)	20 adults (healthy)	*L. casei* Shirota	1 mo	R, PC	Decrease of fecal β-glucosidase and β-glucuronidase activities Decrease of fecal acetic and propionic acids Increase of fecal moisture content
De Preter et al. 2008 (77)	42 adults (healthy)	*Saccharomyces boulardii L. casei* Shirota *B. breve*	1 mo	R, PC	Increase of fecal β-glucosidase activity (*B. breve*)
Goossens et al. 2003 (68)	22 adults (healthy)	*L. plantarum* 299V	1 mo	R, PC	No change in fecal SCFA,β-glucosidase and β-glucuronidase activities
Johansson et al. 1998 (78)	48 adults (healthy)	*L. plantarum* 299V	3 wk	R, PC	Increase of fecal total carboxylic acids Increase of fecal acetic and propionic acids Increase of stool volume Decrease of flatulence
Fujiwara et al. 2001 (69)	34 adults (healthy)	*L. gasseri* SBT2055	1 wk		Decrease of fecal *p*-cresol Decrease of stool odor
Tannock et al. 2000 (70)	10 adults (healthy)	*L. rhamnosus* DR20	6 mo		No change in fecal SCFA, azoreductase and β-glucuronidase activities
Zhao et al. 2004 (75)	50 adults (with liver cirrhosis)	*Bifidobacterium* + *L. acidophilus* + *Enterococcus Bacillus subtilis* + *E. faecium*	2 wk		Decrease of fecal pH and ammonia
Wang et al. 2007 (79)	66 preterm infants	*B. breve* M-16V	1 mo	R	Decrease of fecal propionic and butyric acids
Kang et al. 2006 (80)	46 adults (healthy)	*Weissella cibaria* CMU	1 d		Decrease of volatile sulfur compounds in mouth air
Burton et al. 2006 (81)	23 adults (with halitosis)	*Streptococcus salivarius* K12	2 wk	R, PC	Decrease of volatile sulfur compounds in mouth air
Henker et al. 2001 (82)	1 children case report (with halitosis)	*E. coli* Nissle 1917	3 mo		Decrease of ketones in mouth air

(*Continued*)

TABLE 1 Continued

Disease/marker and reference	Participants	Type of probiotic(s)	Duration	Type of study	Outcome
Metabolism in gut mucosa and liver					
Linsalata et al. 2004 (83)	22 adults (*H. pylori* positive and dyspeptic)	*L. brevis* CD2	3 wk	R, PC	Decrease of gastric ornithine decarboxylase activity and polyamine levels
Zhao et al. 2004 (75)	50 adults (with liver cirrhosis)	*Bifidobacterium* + *L. acidophilus* + *Enterococcus Bacillus subtilis* + *E. faecium*	2 wk		Decrease of blood ammonia Decrease of plasma endotoxin
Loguercio et al. 2005 (84)	78 adults (with liver disease)	Mixture of 8 strains	3 mo		Improvement of routine liver damage tests Decrease of plasma lipid peroxidation markers and *S*-nitrosothiol

[1] Randomized.
[2] Placebo control

probiotics may interfere with 1 of the steps of the digestion/absorption of lipids, although the underlying mechanisms are as yet poorly understood. Twenty-two human studies published in the last 10 y were selected from the literature (Table 1). This is the area where most studies have been published on effects of probiotics on metabolism. In 2000, a metaanalysis on the effects of probiotics on plasma cholesterol levels was published, but the more recent data warrant a new metaanalysis (36).

The main markers used to explore effects on the cardiovascular system and lipid metabolism were circulating total, HDL-, and LDL-cholesterol concentrations. Triglycerides were also often measured, and 3 studies focused on systolic blood pressure. Different randomized controlled protocol designs have been set up (either parallel or crossover), with a main duration of 5 wk, enrolling an average of 25 participants, giving an average of 250 mL of milk fermented by different strains (in 14 studies) at an average concentration of 10^8 CFU/g, or with capsules (in 7 studies) providing from 10^6 to 10^{10} CFU per serving. Controls were placebo capsules for freeze-dried cultures given in capsule forms. For fermented milks, the controls consisted of nonfermented milk, chemically coagulated milks, milk fermented by a different culture, or yogurt. Volunteers were mostly adults, either healthy participants (12 studies) or mildly hypercholesterolemic participants (13 studies).

Eight of the 16 different strains tested were able to reduce blood cholesterol significantly by a mean value of 5.6% (from 2.3 to 13%, P between 0.05 and 0.005). A significant reduction of blood pressure (–10%) was also reported. Four of 5 studies using probiotics in capsules were negative, whereas 6 of 13 using fermented milks were positive. There is no report comparing the same strain in 2 different products: capsule versus fermented milk.

In conclusion, some specific probiotics can have a beneficial impact on lipid metabolism, which links to the established risk markers for cardiovascular disease as demonstrated by well-designed human studies. Some others are not active, and there is not yet a good understanding of the reason for these differences. Fermented milks were more often active than capsules, but there is no comparative study to assess the difference of efficacy of the same strain in the 2 processes, if any.

Effects of probiotics on oxalate metabolism

Oxalate is a component of the human diet that is absorbed in the small intestine and in the upper large intestine. Hyperoxaluria is a common risk factor for urolithiasis, and if there is some dietary management able to reduce oxalate intake, there is still a need to reduce this risk factor for those suffering oxalate urolithiasis. Following research in cats and dogs suffering from oxalate urolithiasis, a few recent human studies reported the efficacy of some strains on hyperoxaluria. The first study on 10 patients suffering from fat malabsorption after jejunoileal bypass reported a significant 19% reduction ($P < 0.05$) in 24 h urinary oxalate excretion after 1 mo of ingestion of a mixture of strains including *Oxalobacter* (91). A review (92) reported that *Oxalobacter formigenes* is a normal inhabitant of the human colon. Hoppe et al. (93) reported a significant 20% reduction of oxaluria in 9 patients during a 4-wk administration of *Oxalobacter* in a frozen paste or with enteric coating. Goldfarb et al. (94), in a randomized double control trial, tested a mix of 4 lactic acid bacteria (*L. acidophilus*, *L. brevis*, *B. infantis*, *S. thermophilus*) in 20 patients suffering of idiopathic hyperoxaluria for 8 wk and reported no effect, supporting a specificity of *Oxalobacter* versus the other probiotics. Duncan et al. (95) reported the reduction (from 45 to 27 mg/g of creatinine) of

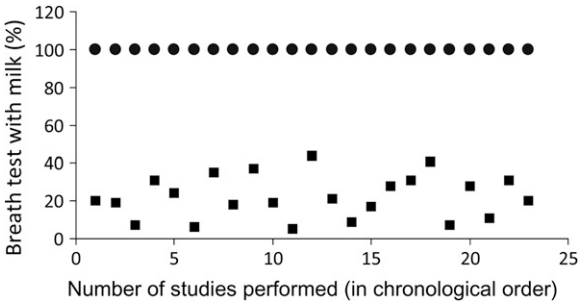

FIGURE 1 Impact of different yogurts on lactose digestion. A total of 23 studies have been performed that compare breath hydrogen excretion after ingestion of milk (*circles*) or ingestion of different yogurt symbiosis (*squares*). Yogurt breath test values are expressed as a percentage of milk value to allow interstudy comparison.

oxaluria after ingestion of 100 mM oxalate solution by 4 human volunteers lacking oxalate-degrading activity in their feces, when ingesting 500 mg of *O. formigenes* HC1.

Large Intestine

Effects of probiotics on composition and metabolic markers of the gut microbiota

The intestinal microbiota is involved in a wide variety of metabolic processes that can play a role in health and disease of the host. As transient microorganisms in the gastrointestinal tract, probiotics are likely to provide additional enzymatic activities and to interact with the resident microbial community, thereby leading to modifications of the ecosystem balance and/or its metabolic characteristics.

Several trials published in the last decade have addressed the effects of different species of lactic acid bacteria on the composition of the intestinal microbiota in healthy participants, using randomized, and most of the time placebo-controlled (PC), study designs. The probiotic strain was given either as part of a fermented drink (*L. casei* Shirota and DN-114001, *L. plantarum* 299v) or as freeze-dried bacteria added to milk or yogurt (*L. gasseri* SBT2055, *L. rhamnosus* DR20, and *B. animalis* subsp. *lactis* BB-12 and HN019), at a dose ranging from 10^7 to 10^{11} CFU/d, for a duration usually ranging from 2 to 4 wk. In these studies, consumption of *Lactobacillus* strains consistently led to an increase of the fecal populations of lactobacilli (66–70) or to an increase of the number of participants with a high level ($>10^6$ CFU/g) of lactobacilli in their stools (65). In parallel, the probiotic strains each induced several other alterations such as an increase of fecal bifidobacteria [*L. casei* Shirota (66)] or enterococci [*L. casei* Shirota (67); *L. rhamnosus* DR20 (70)] and a decrease of fecal staphylococci [*L. gasseri* SBT2055 (69)].

The limited number of trials assessing the effect of *Bifidobacterium* strains does not allow generic conclusions. Using a culture-dependent approach, Ahmed et al. (73) observed an increase of fecal bifidobacteria, lactobacilli, and enterococci accompanied by a decrease of fecal enterobacteria in healthy elderly consuming *B. animalis* subsp. *lactis* HN019. On the other hand, Satokari et al. (71), using a culture-independent approach, did not see any change in the fecal bifidobacteria profile following consumption of *B. animalis* subsp. *lactis* BB-12. Most of these studies have also examined the effect of the probiotic strains on basic metabolic activities of the gut microbiota. Guérin-Danan et al. (65) and Spanhaak et al. (66) both observed a decrease of fecal β-glucosidase and β-glucuronidase activities in participants consuming *L. casei* DN-114001 or Shirota. A recent study confirmed the effect of the strain DN-114001 (76), but De Preter et al. (77) reported no effect of the strain Shirota on the same enzyme activities using different experimental conditions (66) with the strain Shirota. As for the fecal concentration and profile of SCFAs, the different *Lactobacillus* strains that were tested induced inconsistent modifications, ranging from no change (65,68–70) to a decrease (66) or an increase (78) of acetic and propionic acids. In conclusion, studies using *Lactobacillus* strains as probiotics consistently reported an increase of fecal *Lactobacillus* populations. Other alterations seemed to depend on the probiotic strain and/or the experimental design. In any case, the overall limited number of studies does not allow firm conclusions to be drawn on the effects of the tested probiotic strains.

In addition to these studies performed in healthy participants, others have been focused on specific populations with the aim of using probiotic strains therapeutically, i.e., improvement of microbiota composition or metabolic activities. The gut microbiota of preterm infants in neonatal intensive care units (gestational age <37 wk) differs from that of term infants, with a delayed colonization by bifidobacteria as a main feature. This has prompted studies on early administration of bifidobacteria, starting within 24 h after birth, on gut colonization in preterm infants. Mohan et al. (72) showed that the fecal counts of bifidobacteria and of enterobacteria and clostridia were increased and decreased, respectively, in preterm infants consuming *B. animalis* subsp. *lactis* Bb-12, compared with controls receiving a placebo. In another study, consumption of a *B. breve* strain induced earlier gut colonization with bifidobacteria, from the age of 2 to 4 wk onwards, compared with the control group in which no bifidobacteria could be detected during the 7-wk observation period (74). *B. breve* was also able to reduce butyric acid production within the gut of preterm infants, an interesting effect considering that butyric acid is involved in the etiology of necrotizing enterocolitis in premature babies (79,96).

Halitosis, more commonly known as oral malodor, is caused by low-molecular-weight fatty acids, ammonia, and volatile sulfur compounds in the exhaled air. Such products arise mainly from the metabolism of oral bacteria located on the dorsum of the tongue; alternatively, they can result from bacterial processes in the gut, followed by resorption into the blood and exhalation via the lungs. Henker et al. (82) reported the effect of *E. coli* Nissle 1917 in a child suffering from gut-caused halitosis. Breath gas analysis after a 3-mo treatment indicated a dramatic reduction of the number and concentration of volatile chemicals (mainly ketones); the breath gas profile after treatment was nearly normalized. Following this case report, a randomized PC study showed that another bacterial strain, *Streptococcus salivarius* K12, was able to reduce substantially (>100 ppb) oral volatile sulfur compound levels after only 1 wk of treatment in people with halitosis (81). Simultaneously, Kang et al. (80) identified the species *Weissella cibaria* as another potential candidate for reducing volatile sulfur compounds in exhaled air. Further studies should be undertaken to confirm these promising data.

Effects of probiotics on the metabolism of indigestible dietary components

Carbohydrates. Most carbohydrates are enzymatically hydrolyzed and absorbed in the small intestine. The microorganisms of the large intestine degrade undigested complex carbohydrates. Probiotics could potentially modify the degradation of complex carbohydrates and dietary fiber in the large intestine, but this has

not yet been addressed properly in clinical studies. Although experimental data from in vitro studies and animal models suggest that probiotics extend carbohydrate utilization including prebiotics (97), no information is available about the effects of probiotics on the metabolism of prebiotics in humans.

Proteins. De Preter et al. have studied the impact of probiotics on the proteolytic activity in the colon (48–51). Their studies show that *S. boulardii* had no effect on fecal and urinary N-excretion, whereas *L. casei* Shirota and *B. breve* significantly reduced urinary *p*-cresol excretion, indicating a favorable effect of these probiotics on colonic protein and ammonia metabolism. Oral administration of *B. longum* in a gastroresistant capsule, but not in a powder formation, decreased the serum level of indoxyl sulfate (52). Putrefactive substances such as indoxyl sulfate are produced from tryptophan in the large intestine because of excessive production by overgrowth of aerobic bacteria. These studies suggest that specific probiotics beneficially modulate protein metabolism in the colon.

Phytochemicals. Unlike in ruminants, which have specialized stomachs to ferment plant material, many phytochemicals are poorly digested and absorbed in the human small intestine. In this case, they end up in the large intestine, where they are metabolized by the resident microbiota. However, to date, assessment of the effects of probiotics on metabolism of phytochemicals in human gut has been limited to a few studies with isoflavones, which are phytoestrogens abundant in soy foods. Consumption of isoflavones has been associated with changes in sex steroid metabolism and may decrease the risk of breast cancer (98).

Intestinal bacteria play an important role in the metabolism of isoflavones as they release the bioavailable and bioactive aglycone configurations from the food-borne inert glycoside conjugates. In addition, they are able to chemically change the aglycone forms into more potent derivatives such as equol, which binds to estrogen receptors with higher affinity than the parent molecule daidzein. In Western populations, only one-third of people excrete large quantities of equol following dietary exposure to daidzein. It has been hypothesized that probiotics could metabolize isoflavones or alter intestinal bacteria and enzymes involved in isoflavone metabolism, thereby increasing isoflavone bioactivity. In vitro, Tsangalis et al. (99) isolated 4 different β-glucosidase-producing bifidobacteria able to hydrolyze isoflavone glycoside conjugates, 3 of them also converting daidzein to equol. These findings prompted the same authors to investigate the potential of 1 of these strains, *B. animalis* subsp. *lactis* Bb-12, to promote the conversion of daidzein to equol in human gut, using a randomized, double-blind, PC, crossover design in which postmenopausal women consumed soymilk or soymilk fermented with Bb-12 (10^{10} CFU/d) for 2-wk periods. However, the bioavailability of soymilk isoflavones, as assessed by urinary isoflavone and equol excretion, was the same in both dietary periods (58,59).

Kurzer's group also investigated extensively the ability of probiotics to increase isoflavone bioavailability and bioactivity, using the commercial product DDS Plus (*L. acidophilus* DDS+1 and *B. longum,* 10^9 CFU/d of each strain) in a series of randomized, PC trials. In postmenopausal women, urinary 2-hydroxyestrogens and the ratio of 2:16-hydroxyestrone, which is inversely associated with breast cancer risk, increased after 6 wk of soy consumption in those participants who produced equol. But this, as well as plasma isoflavone concentration and equol urinary excretion, was not influenced by probiotics consumption (54–56). Likewise, 2 mo of consumption of the DDS Plus probiotic capsules did not alter equol-producing status or plasma reproductive hormone concentrations in men and premenopausal women (53,57). Other trials with *L. rhamnosus* GG (4×10^{12} CFU/d), alone or in combination with *L. acidophilus* and *B. bifidus* (10^8 CFU/d of each strain), were similarly unsuccessful (60,61). In all studies, the intestinal microbiota and enzyme activities likely to be involved in isoflavone metabolism were not analyzed. The initial hypothesis was thus not directly addressed, and it cannot be determined whether the tested probiotic bacteria failed to alter intestinal microbiota or whether putative modifications failed to influence isoflavone bioavailability and bioactivity.

A recently published animal study could revive interest in designing new human trials with probiotics carefully selected for their ability to metabolize phytoestrogens. In this study (100), the authors used a *Eubacterium limosum* strain shown to produce in vitro the potent phytoestrogen 8-prenylnaringenin from isoxanthohumol, its inactive precursor contained in hops. In humans, activation of isoxanthohumol to 8-prenylnaringenin depends on intestinal microbial metabolism, but this occurs only in one-third of individuals. Oral administration of *E. limosum* to rats triggered 8-prenylnaringenin production in those that were germ-free and increased 8-prenylnaringenin production in those that were colonized with a low-level activity of human fecal microbiota. This type of study supports the idea that probiotic consumption can increase intestinal production of active forms of phytoestrogens, paving the way for balancing exposure to these compounds in an initially heterogeneous human population.

Xenobiotics. There is considerable evidence that the intestinal microbiota play a role in the metabolism of xenobiotics and drugs, either directly or via indirect effects on the liver. There is also evidence that probiotics can influence this metabolic capacity. However, this evidence comes almost exclusively from studies with animal models. Key findings include effects of probiotics on genotoxic load in the colon and are based on the assumption that the metabolic activities of the microbiota are causal in this genotoxic load. Indeed, mechanisms hypothesized to explain the cancer protective effects of probiotics in animal models include alterations in the metabolic capacity of the gut microbiota. At present, there is very sparse indirect evidence that probiotic bacteria may influence xenobiotic metabolism in humans as well (62–64). End points monitored include urinary DNA adducts and fecal mutagenicity, the assumption being that these parameters are related to metabolic activity of the microbiota. This is definitely an area that requires further study. Finally, to target the gut microbiota with tailor-made pre- and probiotics to modify drug-metabolizing capacity in humans is a challenge for the future.

Metabolism in the gastrointestinal mucosa and the liver

The intestinal microbiota closely interacts not only with the intestinal mucosa but also with the liver. Indeed, the liver continuously receives blood and hence metabolites and molecular signals from the gut through the portal system. In this context, the question arises of the potential effect of probiotics on intestinal wall and liver functions, especially metabolic functions. This question was addressed in a few intervention studies involving participants suffering from various types of chronic liver or gut dysfunctions.

In an elegant example of selection of a probiotic strain based on a metabolic trait, Linsalata et al. (83) used *L. brevis* for studies on *H. pylori* because it has a specific arginine deiminase activity. This was expected to reduce the bioavailability of arginine, an amino acid essential for *H. pylori* viability and tissular polyamine synthesis. Using a randomized, double-blind, PC design, they showed that high oral doses of *L. brevis* CD2 (1 tablet containing 2×10^{10} lyophilized cells 9 times/d) for 3 wk reduced the polyamine concentration and the activity of ornithine decarboxylase, an enzyme involved in polyamine biosynthesis, in the gastric mucosa of dyspeptic *H. pylori*-positive patients. The gastric load of *H. pylori* was concurrently reduced.

Two studies investigated the effect of commercially available capsules containing mixtures of probiotic bacterial strains (24,75) on parameters of liver dysfunction in patients suffering from chronic liver diseases. In cirrhotic patients (75), blood ammonia concentration, a risk factor for hepatic encephalopathy, was decreased after daily consumption of capsules for 2 wk. This modification was accompanied by an acidification of the stools and a lowered fecal ammonia concentration. Composition of the intestinal microbiota, assessed by culture techniques, was slightly altered, with an increase of *Bifidobacterium* populations. This study, however, was not placebo controlled. The mixture of 8 strains was consumed for 3 mo by patients with 4 types of chronic liver diseases (nonalcoholic fatty liver disease, alcoholic cirrhosis, hepatitis C virus–related chronic hepatitis, hepatitus C virus–related cirrhosis) (84). In all groups, a significant decrease of the plasma concentrations of liver enzymes indicative of liver damage (alanine aminotransferase, aspartate aminotransferase) and of S-nitrosothiols, which are NO-related free radicals, occurred. These effects still persisted after a 1-mo washout period. Here, too, no placebo group was included in the study.

Recommendations

Specific recommendations can be made for future human intervention studies assessing impact of probiotics on metabolism.

Strain selection

There is a need for proper reporting including physiological state and metabolic activity as it may impact on the efficacy of the strain on metabolism of the host, or on the generation or degradation of a component in the matrix during fermentation or the shelf life of a product.

Background diet

Background diet (possibly containing substrates for the metabolic activity) should be reported and standardized when needed to avoid side bias (e.g., cholesterol or phytochemical or xenobiotic metabolism) and to provide similar amounts of the tested compound in control and tested groups.

Markers and models

There are markers exploring functions sensitive to some probiotics: Lactose metabolism—Hydrogen Breath test, ^{13}C glucose; Cholesterol metabolism—Total, LDL-, HDL-cholesterol, oxaluria.

Alternative models are needed to test the efficacy of probiotics on toxic compounds and/or carcinogenic process.

The use of stable isotopes needs to be more widely applied to follow the fate of a substrate, as exemplified with ^{13}C lactose.

New technologies hold great promise to study effect on metabolism: metabolomics is a potential tool that deserves further investments (101).

Conclusions

There are reported benefits of some specific probiotics, on lactose digestibility, on lactose intolerance, and on blood lipids. They are supported by clinical designs powerful enough to discriminate between strains active and nonactive on some functions.

There are reported effects on gut microbiota and some recent findings on the effect of some probiotics, on halitosis and on oxalate metabolism opening new potential areas of benefits.

There are more exploratory modifications of protein or xenobiotic metabolism, gut mucosa, and liver metabolic activities that deserve further intervention studies.

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