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Effects of Consumption of Probiotics and Prebiotics on Serum Lipid Levels in Humans

Dora I. A. Pereira and Glenn R. Gibson*

Food Microbial Sciences Unit, School of Food Biosciences, The Univ. of Reading, England

* Corresponding author. Food Microbial Sciences Unit, School of Food Biosciences, The University of Reading, Earley Gate, Whiteknights Road, Reading RG6 6BZ, England.

Referee: Dr. Marcel Roberfroid, Universite Catholique de Louvain, Faculte de Medicine, Dept. des Sciences Pharmaceutiques, Avenue E. Mournier 73, UCL/BCTC, B-1200 Bruxelles, Belgium

ABSTRACT: The objective of this article is to review existing studies concerning the effects of probiotics and prebiotics on serum cholesterol concentrations, with particular attention on the possible mechanisms of their action. Although not without exception, results from animal and human studies suggest a moderate cholesterol-lowering action of dairy products fermented with appropriate strain(s) of lactic acid bacteria and bifidobacteria. Mechanistically, probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts and therefore affecting the metabolism of cholesterol, or by directly assimilating cholesterol.

For prebiotic substances, the majority of studies have been done with the fructooligosaccharides inulin and oligofructose, and although convincing lipid-lowering effects have been observed in animals, high dose levels had to be used. Reports in humans are few in number. In studies conducted in normal-lipidemic subjects, two reported no effect of inulin or oligofructose on serum lipids, whereas two others reported a significant reduction in serum triglycerides (19 and 27%, respectively) with more modest changes in serum total and LDL cholesterol. At present, data suggest that in hyperlipidemic subjects, any effects that do occur result primarily in reductions in cholesterol, whereas in normal lipidemic subjects, effects on serum triglycerides are the dominant feature.

KEY WORDS: cholesterol, lactobacilli, bifidobacteria, fructooligosaccharides, fermented dairy products.

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I. INTRODUCTION

Coronary heart disease (CHD) is one of the major causes of death and disability in industrialized countries. The results from several epidemiological and clinical studies indicate a positive correlation between elevated total serum cholesterol levels, mainly reflecting the LDL-cholesterol fraction, and risk of emergence of CHD (Lipid Research Clinics Program, 1984). It has been shown that a reduction in total plasma cholesterol levels in populations suffering from primary hypercholesterolemia can lower the incidence of coronary thrombosis in this group (Levine et al., 1995). More recent findings, from large-scale epidemiological surveys, provide evidence that elevated fasting triglyceride levels are associated with a greater risk of CHD, and that this effect is independent of any association with low levels of HDL-cholesterol (Hokanson and Austin, 1996). A significant number of studies have also shown that elevated postprandial triglyceride concentrations are observed in subjects with CHD (Groot et al., 1991) and therefore can be useful as predictors of CHD risk (Patsch, 1992).

Currently, there is extensive interest in the dietary management of serum cholesterol and triglycerides levels. This is largely driven by the expense of drug therapy, the large numbers of individuals affected and unwanted side effects of such treatments. Current dietary strategies for prevention of CHD implicate adherence to a low-fat/low-saturated fat diet (Taylor and Williams, 1998). Although such diets may present an effective therapy, they are difficult to maintain on a long-term basis and their efficacy diminishes over time. As such, new approaches toward the identification of other dietary means of reducing blood cholesterol levels have been evaluated. These include the use of soluble fibers, soy protein, plant sterols, probiotic bacteria, and prebiotic compounds (Taylor and Williams, 1998).

The focus of this review is the effects of probiotics and prebiotics on blood lipid levels in humans, with particular attention given to the mechanisms by which such effects might be exerted.

II. PROBIOTICS

The first record of the influence of certain dairy products on blood lipids dates back more than 30 years. Shaper and colleagues (1963) and later Mann (1974) observed that men from the tribes of Samburu and Maasai warriors in Africa showed a reduced serum cholesterol after consumption of large amounts of milk fermented with a wild *Lactobacillus* strain. Since then, the potential hypocholesterolemic effect of fermented milk products containing lactobacilli and/or bifidobacteria has been investigated in dietary studies using humans and animals.

In recent years the European market for products claiming to lower blood cholesterol, and thereby contributing toward a healthier heart, has grown substantially (Young, 1998). For example, Danone launched Actimel Cholesterol Control® in Belgium, containing the suggested cholesterol-lowering probiotic *Lactobacillus acidophilus* and the branded prebiotic ACTILIGHT® (Beghin-Say), while the Dutch company Mona introduced a cultured dairy-based drink under the Fysiq® brand. Fysiq® contains the probiotic *Lactobacillus acidophilus* and the branded bifidogenic dietary fiber, RAFTILINE® (Orafti). The Danish company MD Foods introduced in Denmark in 1993 a yoghurt-style product Gaio. However, controversy still exists as to the beneficial effects of some of these, and other products on the levels of blood lipids in humans.

A. Human Dietary Intervention Studies

Harrison and Peat (1975) found that serum cholesterol levels in bottle-fed babies decreased as the number of *Lactobacillus acidophilus* in their stools increased. Howard and Marks (1977) observed that the consumption of 2 L of whole-fat milk and skim milk reduced serum cholesterol by 5 and 15%, respectively, while intake of the same amount of milk fat as butter increased serum cholesterol by 7%. Yoghurt has also been shown to decrease serum cholesterol levels in humans (Hepner et al., 1979), although the effect has sometimes been considered to be transient (Rossouw et al., 1981). Several other studies designed to evaluate the potential reduction of serum cholesterol levels by the consumption of certain cultured dairy products have given variable data and no firm conclusions can be drawn (Taylor and Williams, 1998). In most of these studies a decrease in serum cholesterol was only observed in humans during the consumption of very high doses of fermented dairy products. Other investigators using more 'normal' doses of the fermented milk product failed to confirm such findings. It was suggested that the contradictory results obtained could be, at least in part, related to experimental design (Taylor and Williams, 1998). Some of the factors addressed were lack of statistical power, use of inadequate sample sizes, failure to control nutrient intake and energy expenditure during the experiments and variations in the baseline levels of blood lipids. The intraindividual variation in blood cholesterol over a few months ranged from 5 to 15%.

More recent dietary studies using random double-blind placebo procedures and higher ranges of human subjects have reached the same conclusions (Table 1). Some showed the cholesterol-lowering effect of various fermented milk products, while others failed to demonstrate a significant effect on cholesterol or lipoprotein levels by dietary supplementation of these products. Differences in experimental design (type and quantity of the fermented milk product; age and sex distribution, and starting plasma cholesterol levels of the subjects studied; and length of study period), however, make direct comparisons difficult.

Andersson et al. (1995) studied the effect of low-fat milk and fermented low-fat milk, containing strains of *Lactococcus lactis* and *Lactococcus cremoris*, on cholesterol absorption and excretion in ileostomy subjects. In this study, patients consumed 1 L/day of either one of the milk products in addition to their normal diets in a crossover design procedure of 3 weeks with run-in and run-out periods of 2 weeks, each with 1 L of lemonade. No significant change in serum cholesterol was observed after 3 weeks on each milk regimen.

It has been reported that recently a fermented milk product (Gaio®) that is produced through the action of a bacterial culture containing a strain of *Enterococcus faecium* and two strains of *Streptococcus thermophilus* (CAUSIDO® culture) was effective in reducing plasma cholesterol at relatively modest levels of intake. The bacterial strains contained in this product were isolated from the intestinal flora of inhabitants of Abkhasia (Caucasus), a region reputed for the longevity of its people and where fermented milk is a major part of the diet (Agerbaek et al., 1995). In a study performed with Danish middle-aged men a reduction of around 6% of total plasma cholesterol and 10% of LDL-cholesterol was reported after the consumption of 200 ml/day of this fermented milk product over a period of 6 weeks (Agerbaek et al., 1995). No change was observed in HDL-cholesterol or plasma triacylglycerol levels. This

TABLE 1
Details of Recent Human Dietary Studies on the Evaluation of the Cholesterol-Lowering Properties of Fermented Milk Products

Reference	Product type Dose (vol/d) Intake period	Number of Subjects Age (years)	Study Design	Cholesterol levels
Andersson *et al.* (1995)	FLFM 1 l 3 weeks	9 ileostomy patients 29-67	Crossover with 2 weeks washout	= TC = LDL-C
Agerbaek *et al.* (1995)	FM 200 ml 6 weeks	58 M normolipidemic 44	Randomised, DB, and placebo-controlled	-6% TC -10% LDL-C
Richelsen *et al.* (1996)	FM 200 ml 6 months	87 normolipidemic 50-70	Randomised, DB, and placebo-controlled	= TC = LDL-C
Sessions *et al.* (1998)	FM 200 ml 3 months	78 M 76 F Hyperlipidemic	Multicentred, DB and placebo-controlled	= TC = LDL-C
Schaafsma *et al.* (1998)	FM 3x125 ml 2x3 weeks	30 M normolipidemic 33-64	Randomised, DB, and placebo controlled, crossover design with 1 week washout	-4.4% TC -5.4% LDL-C -5.3% LDL/HDL-ratio
Bertolami *et al.* (1999)	FM 200 g 8 weeks	11 M 21 F Hyperlipidemic 36-65	Prospective, randomised, DB, and placebo controlled with a crossover design	-5.3% TC -6.15% LDL-C = HDL-C = TG
De Roos *et al.* (1999)	LA yoghurt 500 ml 6 weeks	78 normolipidemic	Randomised, placebo-controlled and parallel	= TC = LDL-C =HDL-C
Larsen *et al.* (2000)	FM 450 ml 8 weeks	20 M 50 F Overweight and obese 18-55	DB, Randomised, Placebo-controlled	-8.4%LDL-C
Schaarmann *et al.* (2001)	yoghurt 300 g 51 days	29 F 2 groups: normal and hyperlipidemic	Randomised, DB and Placebo controlled	= TC - 50%LDL-C (hyperlipidemic group, NS)

LFM, low fat milk; FLFM, fermented low fat milk; FM, fermented milk; LA, *Lactobacillus acidophilus*; *TC*, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; =, no change; DB, double-blind; M, male; F, female; NS, not significant

study was randomised, double-blind, and placebo-controlled, with a subject group of 58 male volunteers aged 44 years old. In a follow-up study the long-term effect of this product on the level of plasma lipoproteins was evaluated. Richelsen et al. (1996) observed a rapid reduction of LDL-cholesterol after 1 month of consumption of 200 ml/d of the Gaio® product. However, during long-term intake (6 months) the reduction of LDL-cholesterol was similar to that of placebo (chemically fermented) product. The authors concluded that although low-fat milk or fermented milk products may have some hypocholesterolemic effects, the tested product was not superior to the placebo milk product used in the study because cholesterol concentrations had also fallen in the placebo group and attributed this to known seasonal variations in cholesterol levels (Taylor and Williams, 1998). However, the interpretation of the results was made difficult by the fact that during the second half of the study concentrations of *Enterococcus faecium* in the test product dropped from 2×10^8 CFU/ml to about 10^4 to 10^5 CFU/ml. Since *in vitro* studies have shown that *Streptococcus thermophilus* is acid sensitive and does not, to any significant degree, survive passage through the small intestine (Agerbaek et al., 1995), it was suggested that it was the *Enterococcus faecium* that played a significant role in the potential beneficial effect of this fermented milk product. Thus, a reduction in these bacteria may have been responsible for lowered efficacy of the product. In a subsequent study, Sessions et al. (1998) investigated the effects of a similar milk product, fermented with the same bacterial culture, on plasma cholesterol concentrations in men and women with mildly elevated cholesterol levels living in England. The study was multicentred, placebo controlled, and double blind. In this study, no effect on serum cholesterol levels was observed either in the placebo or in the test groups and the bacterial content of the fermented milk remained constant throughout the experiment. The authors speculated that variability in the results obtained with the two studies was related to inherent differences between the populations studied, either in dietary habits or in the intestinal flora.

Two other studies with the Gaio® product have shown evidence for its beneficial effects, but further investigation is needed to clarify some of the findings. Bertolami et al. (1999) studied the effect on the lipid profile of this fermented milk product on patients with mild to moderate primary hypercholesterolemia. The study was prospective, randomized, double-blinded, and placebo controlled, with a crossover design. Thirty-two patients with ages ranging between 36 and 65 years old were included in the trial. Intake of the product lasted for 8 weeks. It was concluded that the fermented milk (Gaio®) produced a small (average 5%) but statistically significant decrease ($P = 0.004$) in total serum cholesterol. However, not all subjects responded to the product, and three of them even showed increased cholesterol levels. A recent study (Larsen et al., 2000) was aimed at evaluating the effects of the Gaio® product and two alternative products on the serum cholesterol levels of overweight and obese subjects. The study was randomized, parallel, double-blind, placebo- and compliance controlled, and performed over a period of 8 weeks. Seventy overweight and obese subjects (20 males and 50 females), 18 to 55 years old, were randomly included and closely matched into five groups. Four of the groups were given 450 ml of fermented milk products daily with similar dietary composition. One of the groups consumed the test yoghurt Gaio®; two groups consumed two new yoghurts with different bacterial cultures (one was fermented with two strains of *S. thermophilus* and two strains of *L. acido-*

philus, and the other with two strains of *S. thermophilus* and one strain of *L. rhamnosus*); the fourth group consumed placebo yoghurt. The last group was given two placebo tablets daily. When comparing all five treatment groups, and after adjusting for small changes in body weight, a significant decrease in LDL-cholesterol was reported in the Gaio® product group only (8.4%, $P<0.05$), after 8 weeks. This decrease would correspond to a reduction in the risk factor for CHD of 20 to 30%, which is of clinical relevance. However, the decrease observed in LDL-cholesterol was significantly different only from groups consuming placebo yoghurt and pills. The reason for the observed hypocholesterolemic effect of the Gaio® product was, once again, proposed to be related to the CAUSIDO® bacterial culture, particularly *E. faecium.* Although the specific *in vivo* action of the strain remains to be investigated, its ability to tolerate bile and assimilate cholesterol were suggested to be responsible for the observed effect. In fact, Rossi et al. (1999) tested the ability of *E. faecium*, *L. acidophilus*, *L. jugurti*, *S. thermophilus*, and *L. delbrueckii* to decrease cholesterol *in vitro* and to grow in the presence of bile salts. The study showed that among the strains tested, *E. faecium* and the mixture of *E. faecium* plus *L. acidophilus* produced the greater cholesterol reduction in the medium after 24 h anaerobic incubation (53 and 65%, respectively).

In a study by Schaafsma et al. (1998), the effect of the Actimel Cholesterol Control® yoghurt (Danone), fermented by *Lactobacillus acidophilus* and with fructo-oligosaccharides added, on blood lipids in adult male volunteers with borderline elevated levels of serum total cholesterol was evaluated. The study was a randomized, placebo-controlled, double-blind, two-way crossover trial with two treatment periods of 3 weeks separated by a washout period of 1 week. During the treatment periods, 30 male subjects consumed 125 ml of either test or reference product three times daily as part of their habitual diet. The test product was milk fermented by yoghurt starters and *Lactobacillus acidophilus*, and containing 2.5% (w/v) fructo-oligosaccharides, 0.5% (w/v) vegetable oil and 0.5% (w/v) milk fat. The reference product was a traditional yoghurt, containing 1% (w/v) milk fat. The authors reported that consumption of the test product resulted in significantly lower values for serum total cholesterol (4.4%, $P< 0.001$) and LDL-cholesterol (5.4%, $P< 0.005$). However, they stated that beneficial effects of the test product on serum cholesterol were largely related to an increase of this parameter during consumption of the reference product. Also, it needs to be further evaluated whether the hypocholesterolemic effect of the test product was attributable to either the *Lactobacillus acidophilus* strain, the fructo-oligosaccharides, or both. The potential dose and time dependency of the observed effect on serum cholesterol level also needs to be assessed.

De Roos et al. (1999), in a randomized, placebo-controlled parallel trial, attempted to evaluate whether the intake of *Lactobacillus acidophilus* strain L-1 lowered serum cholesterol levels in healthy men and women with normal to borderline high-cholesterol levels. The 78 subjects used in this study consumed 500 ml of control yoghurt daily for 2 weeks. They were then randomly allocated to receive 500 ml per day of either control yoghurt or yoghurt enriched with *Lactobacillus acidophilus* L-1 for another 6 weeks. No significant reduction of serum cholesterol levels in the subjects consuming the yoghurt enriched with *L. acidophilus* L-1 was observed after the trial.

A recent human clinical trial was aimed at further studying the interrelationship between the intake of probiotic yoghurt and the concentration of cholesterol fractions (Schaarmann et al., 2001). In this study, 29

healthy women consumed 300 g per day of a probiotic yoghurt (*Lactobacillus acidophilus* and *Bifidobacterium* longum) after a period of eating standard yoghurt (*Streptococcus thermophilus* and *Lactobacillus lactis*). The volunteers were divided in a normocholesterolemic group (total cholesterol <250 mg/dL) and a hypercholesterolemic group (total cholesterol >250 mg/dL). The experiment consisted of three periods (placebo, standard yoghurt, and probiotic yoghurt), each lasting for 51 days. A decrease in the concentration of LDL-cholesterol and triacylglycerides after consumption of standard and probiotic yoghurts was reported. A larger decrease was observed after intake of the probiotic yoghurt when compared with the standard yoghurt in the hypercholesterolemic group, but this, however, was not significant. Inversely, a increase in the HDL cholesterol was observed with the probiotic yoghurt that resulted in a smaller atherogenic ratio (LDL/HDL-cholesterol). No significant differences were observed when comparing the normocholesterolemic and the hypercholesterolemic groups.

B. Mechanism(s) of Action

Existing evidence from human and animal studies suggests a moderate cholesterol-lowering action of some fermented dairy products; however, the potential mechanisms for this claimed effect remain to be clarified.

Several *in vitro* experiments with lactobacilli (Gilliland et al., 1985; Rašić et al., 1992; Noh et al., 1997) and bifidobacteria (Tahri et al., 1995, 1996) showed evidence for the ability of some strains of these bacteria to assimilate cholesterol in the presence of bile acids.

Gilliland and co-workers in an *in vitro* study in 1985 found that certain *Lactobacillus acidophilus* strains could remove cholesterol from a growth medium only in the presence of bile and under anaerobic conditions. Because these conditions are expected to occur in the intestine, the authors concluded that this should enable the organisms to assimilate at least part of the cholesterol ingested in the diet, thus making it unavailable for absorption into the blood. However, the ability to grow in the presence of bile and to remove cholesterol from laboratory medium was found to vary considerably among *L. acidophilus* strains (Gilliland et al., 1985; Gilliland and Walker, 1990; Walker and Gilliland, 1993). It was suggested that an organism must be bile tolerant to manifest cholesterol uptake in the intestinal tract. However, no apparent correlation between bile tolerance and the ability to assimilate cholesterol has been observed. The manifestation of the purported assimilation action *in vivo* was tested using young pigs (Gilliland et al., 1985). Pigs were selected as the animal model because their digestive system, distribution of coronary arteries, and atherosclerotic tendencies resemble those of humans (Ratcliffe and Luginbuhl, 1971). The results of the study showed that feeding of pigs with *L. acidophilus* RP32, selected for its ability to grow in the presence of bile and to remove cholesterol from a laboratory medium, significantly inhibited the increase in serum cholesterol when fed a high-lipid diet ($P<0.05$). The mechanism underlying this effect was referred to as cholesterol assimilation by the *L. acidophilus* strain (Gilliland et al., 1985).

The purported assimilation of cholesterol by *L. acidophilus* was further supported by the work of Rašić et al. (1992). These authors reported that *L. acidophilus* possessed a significantly greater cholesterol uptake ability than *Streptococcus thermophilus* and a commercial yoghurt culture. However, variations in the ability of cul-

tures of yoghurt bacteria and bifidobacteria to assimilate cholesterol were observed.

It has been suggested that the diverse results obtained in different human studies regarding the hypocholesterolemic effect of yoghurt and other fermented milk products can be, at least in part, be due to the different bacterial strains used in fermentation (Agerbaek et al., 1995). Viability of the ingested bacterial strains in the human gut, and the ability to colonize the small intestine (where most of the cholesterol absorption would take place), could ultimately be expected to be of key importance for this effect.

Subsequent work, by Klaver and van der Meer (1993), studied the mechanism of the proposed assimilation of cholesterol by *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. They concluded that removal of cholesterol from the culture medium by *L. acidophilus* RP32 and other species was not due to bacterial uptake of cholesterol, but rather could relate to co-precipitation with deconjugated bile salts in an acidic environment. Deconjugated bile acids are less soluble and less likely to be absorbed from the intestinal lumen than conjugated bile salts (Klaver and van der Meer, 1993; De Rodas et al., 1996). Thus, deconjugation of bile acids in the small intestine could result in a greater excretion of bile acids from the intestinal tract, especially as free bile acids are excreted more rapidly than their conjugated forms. Increased excretion of bile acids should result in lowered serum concentrations, which in turn would decrease the amount of bile acids reaching the liver for secretion back into the intestine through enterohepatic circulation. To replace the excreted bile acids, more would have to be synthesized from cholesterol in the liver. Thus, it has been suggested that in a steady-state situation, deconjugation of bile acids could lead to the reduction of serum cholesterol by increasing the formation of new bile acids or by reducing the absorption of cholesterol throughout the intestinal lumen. However, it remains unclear whether microbial bile deconjugation properties are related to the hypocholesterolemic effects observed *in vivo* (Gilliland et al., 1985), because pH in the intestinal tract of humans is usually neutral to alkaline.

Tahri et al. (1995) studied the hypothesis of the proposed assimilation or coprecipitation of cholesterol by *Bifidobacterium* species. They observed the existence of an intense binding between cell surface and cholesterol, which was considered as an uptake of cholesterol into the cells. This assimilation was dependent on cell growth and the presence of bile salts was shown to be a prerequisite for significant cholesterol removal. The authors concluded that the observed removal of cholesterol from the broth could not be attributed only to the coprecipitation of cholesterol with deconjugated bile salts, but rather to a conjugation of both effects. It still remains necessary to determine whether the requirement for bile salts is related to their detergent effect and if an inhibition of bile salt deconjugation influences the assimilation of cholesterol. Follow-up work (Tahri et al., 1996) further supported this mechanism. The results obtained in this study confirmed the work of Klaver and van der Meer (1993), in that, in the absence of bacterial cells, cholesterol was partially removed from the medium at pH values lower than 5.5 when deconjugated bile salts were added. However, the authors showed that this was a transient phenomenon because the precipitated cholesterol was redissolved when solutions were adjusted to pH 7. Furthermore, they concluded that resting cells of bifidobacteria do not interact with cholesterol, while growing cells of bifidobacteria do seem to assimilate cholesterol in their cell membrane. No significant relationship was, however, noticed between cholesterol

assimilation and the degree of bile salt deconjugation by bifidobacteria, as previously reported for lactobacilli (Gilliland et al., 1985). It has been suggested that bile salts hydrolase activity might have a role in the mechanism of assimilation as nondeconjugating bifidobacteria were not able to assimilate appreciable amounts of cholesterol even if conjugated or deconjugated forms of bile salts were present in the growth medium. The authors concluded that this cholesterol assimilation model could explain the *in vivo* observed hypocholesterolemic effect. Further research is still needed to determine the mechanism of cholesterol assimilation and localisation of the assimilated cholesterol in the cells.

In an animal study designed to evaluate the hypocholesterolemic effect of *L. acidophilus* ATCC43121, 33 pigs were fed a high cholesterol diet for 14 days (De Rodas et al., 1996). Following this, on day 15 and for an additional 15 days, the pigs were assigned to one of four treatment groups, including two levels of calcium (0.7 and 1.4%), with or without the *L. acidophilus* strain added (2.5×10^{11} CFU/ml). It was reported that pigs fed *L. acidophilus* had 11.8% lower total cholesterol than pigs fed a diet without *L. acidophilus*. Similarly, pigs fed 1.4% calcium had significantly lower total cholesterol than pigs fed 0.7% calcium. In addition, during the overall 15-day period, a reduction of 23.9% in serum bile acids by dietary *L. acidophilus* and by 21.4% by 1.4% dietary calcium compared with the controls was observed. Based on these results, the authors concluded that both *L. acidophilus* ATCC43121 and calcium could enhance the reduction of serum cholesterol in pigs that had been fed a high-cholesterol diet. They suggested that with regards to dietary *L. acidophilus,* this is probably done through alterations in the enterohepatic circulation of bile acids as the greater decline in serum cholesterol, coupled with lower serum bile salts could indicate an association between the deconjugation action of the strain and its hypocholesterolemic effect. Similarly, the exact mechanism for the LDL-cholesterol lowering effect of calcium is still unclear. It has been hypothesized that excess dietary calcium can bind bile acids and therefore suppresses reabsorption into the enterohepatic circulation.

Further evidence for cholesterol assimilation by *L. acidophilus* ATCC43121 was given by Noh et al. (1997). These authors reported that cholesterol assimilated by this strain was not metabolically degraded, as most of it was recovered with the cells. They observed that cells grown in the presence of cholesterol micelles and bile salts were more resistant to lysis by sonication than were those grown in their absence. This could suggest a possible alteration of the cell wall or membrane by cholesterol such that lactobacilli were more resistant to sonic disruption. The *in vivo* effect of acidophilus yoghurt on serum cholesterol levels was evaluated using mice as a live model by Akalin et al, (1997). In this study, 60 male mice were assigned to three dietary treatments for 56 days: water (control), yoghurt made from milk inoculated with *S. thermophilus* and *L. delbrueckii*, and yoghurt made from milk inoculated with *S. thermophilus* and *L. acidophilus*. A significant decrease in the mean values for total and LDL-cholesterol concentrations was observed in the group given acidophilus yoghurt on days 28 and 56. The hypocholesterolemic effect of 'standard' yoghurt occurred only at the end of the 56 d period and was lower than that of acidophilus yoghurt. Both HDL-cholesterol and triglyceride concentrations seemed to be unaffected by dietary yoghurt or acidophilus yoghurt.

A recent study (Meei-YN Lin, 2000) investigated the *in vitro* cholesterol reducing abilities of six *L. acidophilus* strains (including Nestle LC1® strain La1). A maxi-

mum cholesterol uptake of 57% was reported when *L. acidophilus* ATCC4356 was grown anaerobically for 24 h in a medium supplemented with bile acids (oxgall). The authors concluded that the *in vivo* hypocholesterolemic effect of *L. acidophilus* cells was due to the direct assimilation by the cells and/or attachment of cholesterol to their surface. As previously mentioned, coprecipitation of cholesterol with decon-jugated bile acids, which occurs *in vitro*, is not likely to take place *in vivo* because pH of the small intestine is higher than 6.0.

Lactic acid bacteria with active BSH, or cultured products containing them, are suggested to lower serum cholesterol levels through an interaction with the host bile salt metabolism (De Smet et al., 1994). The proposed mechanism of cholesterol reduction is comparable to that of a cholestyramine treatment, which, like other bile salt sequestrants such as colestipol, binds bile salts and prevents them from being reabsorbed. Thus, less bile salts would return to the liver, resulting in a loss of feedback inhibition of bile salt synthesis and an increased conversion of cholesterol to bile salts. This led the authors to suggest that the ingestion of lactic acid bacteria containing active BSH might be regarded as a 'biological' alternative to common medical or surgical interventions to treat hypercholesterolemia. Based on these assumptions, De Smet et al. (1998) studied the effects of feeding live *Lactobacillus reuteri* cells containing active bile salt hydrolase (BSH) on plasma cholesterol levels in pigs. The study lasted for 13 weeks. During the treatment period (4 weeks), 20 pigs were given, twice daily, $10^{11,25}$ CFU of the strain. It was reported that after 2 weeks of *Lactobacillus reuteri* feeding, total and LDL-cholesterol in treated pigs were reduced by 11 and 26%, respectively, compared with control animals ($P < 0.05$). After 4 weeks these reductions were 15 and 24%, respectively ($P < 0.05$). No change was observed in HDL-cholesterol concentrations. During the subsequent posttreatment follow-up period, it was reported that total and LDL-cholesterol concentrations in treated pigs increased, but these levels were still significantly lower than values measured in control pigs. A recent animal study involving *L. reuteri* was aimed at an evaluation of the effect of administration of *L. reuteri* CRL1098 (10^4 cfu/day) to mice for 7 days prior to inducing hypercholesterolemia (Taranto et al., 2000). Even at this low dose, *L. reuteri* seemed to be effective in preventing hypercholesterolemia in mice, producing a 17% increase in the ratio of HDL/LDL lipoprotein. Decreases in total cholesterol and triglyceride levels of 22 and 33%, respectively, were observed in the group given milk with *L. reuteri* in comparison with the group given milk alone. Additionally, serum cholesterol in the *L. reuteri* pretreated mice increased by only 38% compared with the 82% increase observed in the group given milk. It was concluded that *L. reuteri* might be effective as a prophylactic agent in preventing hypercholesterolemia.

In a recent *in vitro* study, Usman and Hosono (1999) noticed a significant variation among 28 strains of *Lactobacillus gasseri* in their growth performance in medium containing bile, as well as in their ability to bind cholesterol. They further suggested that no significant correlation existed between bile tolerance and the cholesterol binding ability. In this study, all strains of human origin tested showed greater variation in bile tolerance in agreement with previous work (Gilliland et al., 1985; Klaver and van der Meer, 1993). It was suggested that differences in bile tolerance could be due to growth performance of the individual strains. While in previous studies the action of *L. acidophilus* on cholesterol uptake was evaluated when cells were grown in MRS broth containing a certain amount of bile

salts and cholesterol, the study of Usman and Hosono (1999) was focused on the binding abilities of the intact cells toward cholesterol. The authors found that all 28 strains were able to bind cholesterol, but the binding abilities varied widely. It was suggested that differences were related to chemical and structural properties of the bacterial cell-wall peptidoglycans. However, it remains unclear whether the variation in cholesterol uptake by the strains of *L. gasseri* was due to difference in their cell membranes or to other cell components. In a follow-up to this study, Usman and Hosono (2000) investigated the effect of a nonfermented milk supplemented with *L. gasseri* (10^9 cfu/ml) on serum lipids and fecal steroids in hypercholesterolemic rats. Both strains used in this study (SBT0270 and SBT0274) have previously been shown to exhibit high cholesterol-binding and taurocholate-deconjugating activities *in vitro* (Usman and Hosono, 1999). The total and LDL-cholesterol levels were reported to be 42 and 64%, respectively, lower in the group given *L. gasseri* SBT0270 supplemented milk than in the milk-only group. Triglyceride levels decreased when rats were given milk and nonfermented milk supplemented with the bacterial strains in relation to the control group (water), while milk alone had no observed effect on total and LDL-cholesterol. From the results obtained in this study, the authors attributed the observed hypocholesterolemic effect of *L. gasseri* SBT0270 to its ability to suppress the reabsorption of bile acids into the enterohepatic circulation (by deconjugation) and to enhance the excretion of acidic steroids in feces.

A recent animal study involving *L. casei* strain Shirota (Yakult®) was aimed at determining the effect of skim milk fermented by this strain on plasma lipids in hamsters (Kikuchi-Hayakawa et al., 2000). A 30% decrease in the triglyceride plasma levels was observed in hamsters fed the enriched diet containing the fermented milk product (FMP) compared with the control diet (unfermented milk). However, plasma cholesterol concentration was not affected by the FMP supplement to the diet. In fact in an *in vitro* experiment the same authors observed that *L. casei* strain Shirota, despite growing well in the presence of mixed lipid micelles containing bile acids, did not have the ability to significantly remove cholesterol from the culture broth after 24 h anaerobic incubation (11%), contrary to some other strains tested (*L. acidophilus* [max. 83%], *L. crispatus* [max. 83%], *L. gasseri* [max. 80%]).

Probiotic bacteria once resident in the human gut ferment food-derived indigestible carbohydrates that results in an increased production of short-chain fatty acids (SCFA). This has been suggested to cause a decrease in the systemic levels of blood lipids either by inhibiting hepatic cholesterol synthesis (to an unknown extent), or by redistributing cholesterol from plasma to the liver (Fuller and Gibson, 1998). The SCFA production in the large intestine has been reported to be 100 to 450 mmol/day, with relative proportions of acetate, propionate, and butyrate being about 60:20:15, depending on the substrate (St-Onge et al., 2000). Acetate in the serum seems to increase total cholesterol, while propionate increases blood glucose and tends to lower the hypocholesterolemic response caused by acetate, by reducing its utilization by the liver for fatty acid and cholesterol synthesis. Indeed, a study by Wolever and colleagues (1996) showed that serum acetate and propionate concentrations were related to serum lipid concentrations in both males and females, serum propionate being strongly negatively related to both total and LDL cholesterol ($P < 0.001$). However, sufficient propionate must be produced to offset the effects of acetate generation as a

precursor for lipid synthesis. Therefore, depending on the proportion of each fatty acid produced during bacterial fermentation, plasma cholesterol concentrations thus may be altered through this mechanism.

Additionally, some bacteria may interfere with cholesterol absorption from the gut either by deconjugating bile salts and therefore affecting the metabolism of cholesterol or by directly assimilating cholesterol. A number of bacteria have been reported to hydrolyze conjugated bile acids, such as *Bacteroides* spp., bifidobacteria, fusobacteria, clostridia, lactobacilli, and streptococci (Hylemon and Glass, 1983). There is evidence that the gut flora not only hydrogenates, dehydrogenates, and oxidizes bile acids, but also cleave side chains to yield steroids. *In vivo*, removal of cholesterol would occur because deconjugated bile acids are not well absorbed by the gut mucosa and are excreted through the faeces and urine. The excretion of bile acids results in decreased enterohepatic recirculation and therefore more cholesterol, which is the precursor of bile acids, needs to be utilized for *de novo* bile acid synthesis.

As mentioned previously, *in vitro* studies using different strains of *L. acidophilus* grown on media containing bile have shown that certain strains can modify cholesterol metabolism. Because the amount of bile in the medium did not exceed concentrations normally found in the intestine, it can be expected that this cholesterol assimilation would also occur *in vivo*. The uptake of cholesterol by bacteria would make it unavailable for absorption into the circulation.

However, some work still needs to be undertaken in this field leading toward more conclusive clinical evaluations to understanding of the *in vivo* mechanisms of probiotic effect on blood lipids and to improvement of strain stability characteristics. Also, the *in vitro* ability to reduce cholesterol observed with some strains needs to be confirmed in mixed culture and mixed substrate environments.

III. PREBIOTICS

As previously mentioned, evidence exists that alterations in gut microflora induced by fermentation of nondigestible components of diet (prebiotics) may have the potential to influence systemic lipid metabolism. This has attracted considerable attention, mainly because unlike probiotics, prebiotic substances by definition are not subject to viability problems. Such substances also have greater possibilities for incorporation into a wide range of common foodstuffs.

In contrast with the use of live microorganisms in foods, a prebiotic supplement targets certain components of the indigenous gut microbiota (Gibson and Roberfroid, 1995). The usual target organisms are bifidobacteria and lactobacilli, as they are natural residents of the gut microbiota. The prebiotic approach advocates use of nonviable food components that remain unabsorbed and nondegraded in the upper gastrointestinal tract, but have a selective fermentation in the large gut, exploiting the fact that diet is a major determinant of colonic microflora function (Gibson, 1999). Any food that reaches the colon such as nondigestible carbohydrates, some peptides and proteins, as well as certain lipids, is a potential prebiotic (Gibson, 1999). Nondigestible carbohydrates, in particular fructose oligosaccharides, are the most studied prebiotics. Fructooligosaccharides (FOS) consist of short- and medium-length chains of β-D-fructans in which fructosyl units are bound by a β 2-1 linkage, with the degree of polymerization varying between 2 and 60 (inulin) or 2 and 20 (oligofructose) (Gibson, 1999). FOS such as inulin and oligofructose

are naturally occurring indigestible carbohydrates found in many plant foods like chicory, onions, and asparagus (Roberfroid, 1996). These compounds escape hydrolysis and absorption in the human upper digestive tract and reach the colon where they are fermented by the intestinal bacteria, especially bifidobacteria (Gibson and Roberfroid, 1995; Roberfroid, 1996), to SCFA (mainly acetate) and other metabolites (e.g., lactate; Gibson, 1999).

A. Human and Animal Dietary Studies

Lipid-lowering effects of dietary oligosaccharides have been proposed and specifically a decrease in the plasma triacylglycerol concentration was observed (Levrat et al., 1991; Delzenne et al., 1993; Fiordaliso et al., 1995).

Fiordaliso et al. (1995) showed that the administration of oligofructose decreased total cholesterol levels in the serum of rats. Oligofructose supplementation has also been shown to protect rats against the increase in free cholesterol concentration induced by high-fat diets, without preventing an accumulation of cholesterol in liver tissue (Kok et al., 1996).

Delzenne et al. (1993) studied the influence of dietary FOSs on lipid metabolism in rats. In this experiment the animals were fed oligofructose in the diet for 30 days, at a dose of 20 g/100 g. A large decrease in the concentration of triglycerides, both in the serum and liver, was reported in test animals compared with the levels measured in isocalorically fed control rats. The amount of total cholesterol did not change, but an increase in the ratio of HDL/LDL lipoproteins was observed. The same pattern of seric and hepatic lipid modifications has been observed in rats fed 10% inulin by weight (Levrat et al., 1991). The existence of a putative role of some fermentation products (e.g., SCFA) as mediators of the systemic effects of FOSs was hypothesized (Delzenne et al., 1993). Also, Levrat et al. (1991) showed that high propionic acid fermentations were present in the cecum of rats fed diets containing moderate proportions of inulin. The authors proposed that the hypocholesterolemic effect of inulin corresponded to a metabolic effect, because the oligosaccharide structure of inulin would be unsuitable for adsorption of neutral or acidic steroids in the small intestine.

In a subsequent study, normocholesterolemic rats were fed a bread diet containing either corn starch or 6% (w/v) inulin, and the diets were either cholesterol-free or contained 1% (w/v) cholesterol and 0.1% (w/v) cholic acid (Vanhoof and Schrijver, 1995). A significant reduction in plasma cholesterol concentrations in the cholesterol-free diets with added inulin and a tendency to elevated fecal excretion of neutral steroids were reported. The authors suggested that the increase in cholesterol excretion in animals receiving inulin might be caused by a decrease in cholesterol absorption through a higher viscosity in the upper intestinal tract. This would result in a higher cholesterol catabolism in the liver, which would lead to lower plasma cholesterol concentrations. Indeed, an inverse linear relationship between liver cholesterol concentrations and daily fecal excretion of bile acids was observed in test animals. It was concluded that the cholesterol-lowering effect of inulin in normocholesterolemic rats might be due to increased fecal neutral steroid and bile acid excretion. The suggested mechanism for this effect was a reduction in cecal pH observed in rats consuming inulin (Vanhoof and Schrijver, 1995). At a lower pH the amount of soluble bile acids decreases, as a result lipid absorption decreases and fecal bile acid

excretion increases, despite the fact that inulin itself is unlikely to bind bile acids in the upper digestive tract (Levrat et al., 1994). As previously mentioned, higher fecal bile acid excretion leads to increased utilization of liver cholesterol to resynthesize bile acids (Klaver and van der Meer, 1993; De Smet et al., 1994). In a similar experiment with hypercholesterolemic rats, a tendency to higher bile acid excretion was reported, but no increase in total neutral steroid excretion and no significant effect on plasma and liver cholesterol concentrations was noted (Vanhoof and Schrijver, 1995).

The accurate differences in the results obtained with normo- and hypercholesterolemic rats make conclusions difficult. Further studies are required to elucidate the mechanisms by which inulin may elicit its lipid-lowering effect in humans, because lipid metabolism is somewhat different from the rat models used. In an attempt to overcome this, Trautwein et al. (1998) used Syrian hamsters, which presumably provide a better model for the study of lipid and cholesterol metabolism than rats. This experiment was designed to further evaluate the lipid-lowering potential of inulin and especially its effect on bile acid metabolism. The animals were fed diets containing different amounts of inulin (8, 12, and 16%) for 5 weeks. The authors reported that addition of inulin to the cholesterol-enriched diet of test animals exerted significant hypocholesterolemic and hypotriacylglycerolemic effects, especially at dietary levels of 12 and 16%. They observed that all three levels of dietary inulin caused distinct alterations in the bile acid profile of gallbladder bile. It was concluded that the lipid-lowering action of inulin was possibly due to several mechanisms, including altered hepatic triacylglycerol synthesis and VLDL secretion, and impaired reabsorption of circulating bile acids. In a recent study by Daubioul and colleagues (2000), the effects of oligofructose on lipid metabolism in obese Zucker rats was examined. It was observed that the addition of oligofructose to the diet slowed the increase in body weight without modifying serum triglycerides or glucose concentrations after 7 weeks of treatment. However, after 10 weeks a 57% decrease in the hepatic concentration of triglycerides, relative to controls, was reported. The influence of dietary oligofructose on postprandial triglyceridemia, an important variable associated with development of atherosclerosis in humans, remains to be further clarified.

Because animal studies have shown some evidence for the lipid-lowering effects of FOSs, much attention has been given to their potential effect in humans. The number of human studies is, however, limited and results concerning effects on plasma cholesterol and triacylglycerol are not conclusive (Table 2).

Yamashita et al. (1984) studied the effect of oligofructose intake on blood lipid levels of individuals with noninsulin-dependent diabetes. They reported a 8% reduction in total, and 10% reduction in LDL-cholesterol after the administration of 8 g of a synthetic oligosaccharide for 14 days, compared with a control group given sucrose in the same food vehicles. They also observed that the reduction was greater in hypercholesterolemic subjects. No effect on circulating triacylglycerols was reported. This study was conducted in a parallel design with an intervention and a control group, and consequently had lower statistical power due to the lack of a crossover design where subjects serve as their own control.

More recently, Canzi et al. (1995) studied the effect on lipid metabolism of a prolonged ingestion of 9 g/day of inulin from chicory incorporated into a ready-to-eat breakfast cereal in 12 healthy young male volunteers. A marked effect of inulin in reducing fasting triglycerides concentrations (–27%), and to a lesser extent total cholesterol (–5%), without undue effects on HDL-

TABLE 2
Summary of Human Dietary Studies on the Effect of Fructooligosaccharides (FOS) on Lipid Metabolism

Reference	FOS Dose (g/d) Intake period (d)	Number of Subjects Type Age (years)	Study Design	Fasting serum levels
Yamashita *et al.* (1984)	Oligofructose 8 14	28 (18M,10F) NIDD 47 (std 7)	Parallel, DB (18 test, 10 placebo)	-8% TC -10% LDL-C = TG
Canzi *et al.* (1995)	Inulin 9 28	12 M normolipidemic 23 (std 0.5)	Sequential (4-weeks placebo, 4-weeks inulin)	-5% TC -27% TG
Ellegard *et al.* (1997)	Inulin, oligofructose 17 3	10 ileostomy	DB, crossover with 4- days washout	= TC = LDL-C = HDL-C
Pedersen *et al.* (1997)	Inulin 14 28	64 F normolipidemic 'young'	Randomised, DB, crossover	= TC = LDL-C = HDL-C = TG < LDL:HDL ratio
Davidson *et al.* (1998)	Inulin 18 42	21 Hyperlipidemic 60 (std 5)	Randomised, DB, crossover with 6- week washout	-8.7% TC -14.4% LDL-C = HDL-C = TG
Jackson *et al.* (1999) Williams (1999)	Inulin 10 56	54 Moderate to Hyperlipidemic 35-70	DB, Randomised, placebo-controlled, parallel	= TC = LDL-C = HDL-C -19% TG
Causey *et al.* (2000)	Inulin 20 21	12 M Hypercholeste- rolemic 27-49	Randomised, DB, Crossover with no washout	= TC = LDL-C = HDL-C - 14% TG

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triacylglycerols; =, no change; DB, double-blind; NIDD, non-insulin-dependent-diabetes; M, male; F, female

cholesterol, was reported. From this study, it was concluded that the effects of inulin on plasma cholesterol were not related to intestinal microbial metabolism of bile acids, as has been previously suggested (Yamashita et al., 1984), because they observed that inulin consumption did not affect the counts of bile acids dehydroxylating microorganisms. The authors also observed a large and consistent rise in breath hydrogen, indicating the possibility that some other microbial activities may have been changed in the proximal colon despite the presence of a stable microflora.

In a subsequent study, the effect of inulin and oligofructose on cholesterol absorption and excretion in humans was evaluated further (Ellegard et al., 1997). This study was based on a double blind crossover procedure and included 10 ileostomy subjects. The subjects were given diets, including inulin, oligofructose, or sucrose during three experimental periods of 3 days each. Between the dietary periods there was a washout period of 4 days. No difference was noticed by the subjects between the three diets, which indicates that the doses given (17 g of inulin and oligofructose) were well tolerated. No significant effect of inulin or oligofructose on absorption of cholesterol, or excretion of fat, cholesterol, or bile acids was observed during the study. This led the authors to conclude that the systemic effects of inulin and oligofructose reported previously were not mediated via increases in cholesterol and sterol excretion (the proposed explanation for the hypocholesterolemic effect of other soluble dietary fibers such as pectins; Andersson, 1993).

In another human dietary study, Pedersen et al. (1997) evaluated the effect of a daily intake of 14 g inulin, added to a low-fat spread, on fasting blood lipids in 64 young women in a randomized, double-blind, crossover trial involving two periods of 4 weeks. No significant differences in plasma total cholesterol, HDL- and LDL-cholesterol and triacylglycerol concentrations were observed between the placebo and inulin periods. However, the authors reported a significant decrease in the LDL-cholesterol:HDL-cholesterol ratio at the end of both the control period and the inulin period. These results were in accordance with those reported in a previous study (Luo et al., 1996) involving 12 young healthy males who ingested 20 g FOS/day for 4 weeks in a randomized, crossover experiment.

A follow-up study examined the effect of dietary inulin on serum lipids in hypercholesterolemic subjects (Davidson et al., 1998). This was a randomized, double-blind, crossover study performed using 21 adults with mild-to-moderate hypercholesterolemia, with two 6-week treatment periods separated by a 6-week washout. During the treatment periods the subjects consumed three servings per day of inulin-containing foods, corresponding to a total of 18 g/day. Significant reductions were reported when comparing the response between periods, either in LDL-cholesterol (–14.4%, $P<0.05$) or in total cholesterol (–8.7%, $P<0.05$). However, when comparing the values for any of the lipid variables at the end of the inulin and control periods, no differences were observed. This was mainly attributed to a significant increase in total cholesterol and LDL-cholesterol observed during the control phase, whereas these values did not change appreciably during the inulin phase. Although it was not possible to draw firm conclusions, based on previously reported data showing a lipid-lowering effect of inulin, the authors suggested that inulin consumption prevented the increase in total and LDL-cholesterol observed during the control period.

In a recently conducted study, 54 middle-aged subjects with moderately raised blood lipid concentrations, consumed 10 g per day of inulin or placebo, in a powdered form

(Williams, 1999). No significant changes in concentrations of total, LDL-, or HDL-cholesterol were seen in either group over the 8-week intervention. In contrast, it was reported that fasting serum triglycerides were significantly lower (19%) after 8 weeks in the inulin-treated group, returning to baseline values 4 weeks after treatment. Similar results were reported by Causey et al. (2000) in a dietary study involving hypercholesterolemic men. In this study 12 male subjects were randomly assigned to two controlled diets that differed only in that the test diet contained a daily intake of 20 g of inulin, while in the control diet that was replaced by sucrose, and consumed each diet for 3 weeks. A significant decrease in serum triglycerides levels (40 mg/dL, 14%) was observed with the inulin diet. A trend toward a reduction in serum cholesterol was also observed, although this was not significant.

A recent meta-analysis on the cholesterol-lowering effects of dietary fiber suggested that soluble fibers (pectin, oat bran, guar gum, and psyllium) had a small but significant decreasing effect on total and LDL-cholesterol levels within the practical range of intake (Brown et al., 1999). Inulin appears to have a similar effect on blood lipids when consumed by hyperlipidemic adults. Therefore, preliminary evidence exists for a hypotriglyceridemic effect of FOSs, but, at the present stage of knowledge, it is not possible to conclude a hypocholesterolemic effect.

B. Mechanism(s) of Action

Oligosaccharides are rapidly and completely fermented in the colon (Gibson, 1999) and one hypothesis for a lipid-lowering mechanism is by increasing the synthesis of fermentation byproducts (e.g., propionate) which reach the liver by the portal vein and potentially modulate the hepatic cholesterol synthesis (Chen et al.,1984; Levrat et al., 1994). This hypothesis was supported by a study in rats showing that the decrease in blood lipids after daily administration of an oligofructose-rich diet was associated with a reduction in plasma VLDL particles, and a reduced capacity of isolated hepatocytes to synthesize triacylglycerols (Fiordaliso et al., 1995). Although it was demonstrated that oligosaccharides induce high propionic fermentations in the cecum of rats (Levrat et al., 1994), this fact is considered controversial and does not seem to play a major role in the hypocholesterolemic effect of FOSs. Moreover, several animal studies have reported a reduction in intestinal cholesterol and bile acid absorption, with a consequent increase in bile acid and neutral steroid excretion (Vanhoof et al., 1995; Trautwein et al., 1998). Such findings suggest that interruption of the enterohepatic circulation of bile acids with an enhanced fecal excretion could have a major impact on the observed hypocholesterolemic effect.

Evidence exists that other mechanisms might also be involved in the hypocholesterolemic action of FOSs, but there are still not enough data to draw definitive conclusions.

In contrast, it is commonly accepted that the principal mechanism for the hypotriacylglycerolemic effect of oligofructose and inulin is a reduction in the hepatic *de novo* fatty acid and triacylglycerol synthesis (Fiordaliso et al., 1995; Kok et al., 1996). Data from these studies presented evidence for the hypothesis of a decreased *de novo* lipogenesis in the liver, through a coordinated reduction in the activity of all lipogenic enzymes. This hypothesis was also supported by a reduction in plasma VLDL particles observed in the study by Trautwein et al. (1998), indicating a decreased production and secretion of VLDL-triglyceride. Further evidence for this mechanism was given by recently published work aimed at reviewing the probable biochemical mechanism(s) accounting for the hypolipidemic effect of FOSs

(Delzenne and Kok, 1999). These authors reported a significant postprandial triglyceride lowering effect (27 to 61%, $P < 0.05$) after administrating 10 g/100 g oligofructose to male Wistar rats fed either a standard diet, a fiber-free diet or a high-fat diet. The authors concluded that the triacylglycerol-lowering effect of oligofructose observed in rats was caused by its antilipogenic action in the liver, that is, by reducing the activity and possibly the expression of all lipogenic enzymes.

It has also been observed that FOS intake significantly reduces serum insulin and glucose (Kok et al., 1996), which are both known to be present in the nutritional regulation of lipogenesis. FOS also increases the production of intestinal peptides, namely, GIP and GLP-1. Both of these peptides are known to regulate postprandial insulin release and also to have direct insulin-like actions on lipid metabolism (Morgan, 1996). In conclusion, a relationship seems to exist between the modulation of hormone and intestinal peptide production and the antilipogenic effect of FOSs. However, the exact mechanism of action needs to be clarified.

IV. CONCLUSION

In conclusion, dairy products fermented with the appropriate strain(s) of bacteria can be anticipated to induce a lowering of circulating cholesterol concentrations, thus diminishing the risk of CHD. However, the strains of bacteria used must be bile tolerant, have the ability to deconjugate bile acids, and bind cholesterol. If these criteria are fulfilled, fermented dairy products can be viewed as 'functional' foods in lowering elevated cholesterol concentrations. There are a number of mechanisms suggested for the purported cholesterol-lowering action of probiotics. These include physiological actions of the end products of SCFA fermentation, cholesterol assimilation by the probiotic bacteria, cholesterol binding to the bacterial cell wall, and enzymatic deconjugation of bile acids. The deconjugation of bile acids by gut bacteria increases their rates of excretion and therefore would lead to a higher cholesterol demand by the liver for *de novo* synthesis of bile acids, with a consequent reduction on serum cholesterol. One concern about this mechanism was suggested to be the potential increased risk for colon cancer due to the carcinogenic properties of the deconjugated bile acids (Sanders, 2000). These hypotheses need to be confirmed in animal and human studies and the exact mechanism(s) of action of probiotic bacteria on cholesterol reduction remain unclear.

To date, products that contain live bacteria, such as yoghurt, acidophilus milk, and kefir (fermented dairy products containing several types of bacteria in symbiosis with yeasts) contain strains that do not normally reside in the human intestinal tract. The result being that these bacteria are not able to colonize the intestine and are quickly eliminated in faeces. As such, daily consumption of probiotic products is necessary for any long-term effect on metabolism. This fact could account for some of the conflicting results obtained from several human and animal dietary studies.

In what concerns prebiotics, the data available at present are still rather inconsistent, but seem to indicate that intake of moderate levels of inulin or oligofructose may affect, to some extent, human lipid metabolism. Although convincing lipid-lowering effects of inulin and oligofructose have been observed in animals, the studies have used relatively high dose levels (50 to 200 g/kg). Equivalent doses could not be used in humans because of known adverse gastrointestinal side effects at intake levels above 30 g/day (Delzenne and Kok, 1999). Studies that have investigated the effects of

inulin and oligofructose in humans are few in number, although those that have been conducted are well designed and include relatively large numbers of subjects. Further research is required to confirm the findings. In studies conducted in normal-lipidemic subjects, two reported no effects of inulin or oligofructose on serum lipids, whereas two others reported significant reductions in serum triglycerides (–19 and –27%), with more modest changes in serum total and LDL cholesterol. Present data suggest that in subjects with hyperlipidemia, any effects that do occur result primarily in reductions in cholesterol, whereas in normal subjects effects on serum triglycerides are the dominant feature. This latter response is similar to that observed in animals in which the observed effects on cholesterol are small. This led some authors to conclude that the hypocholesterolemic action of FOSs is modified by dietary cholesterol (Fernandez, 1995), and therefore the hypocholesterolemic response in human subjects could possibly vary depending on whether individuals are normal or hypercholesterolemic.

As mentioned previously, animal studies have identified the inhibition of hepatic lipogenesis as the major site of action for the triglyceride-lowering effects of FOS, and this pathway is known to be relatively inactive in humans unless a high-carbohydrate diet is fed (Delzenne and Kok, 1999). As such, one could suggest that the apparent lack of effect observed in some human studies performed in healthy humans (whose diet consists of much less carbohydrates, and more lipids than rodents) is not surprising and does not demonstrate an absence of effect. Future attempts to demonstrate lipid-lowering effects of FOSs intake must consider the nature of the background diet as a determinant of response.

Therefore, despite the great interest it could constitute in terms of human health, the mechanisms of the effects of nondigestible, fermentable carbohydrates on lipid metabolism in human subjects remains to be elucidated. This is partially because the putative metabolic targets of inulin, relatively well known and described in animal models, are more difficult to study in humans. Several factors should be taken into account in human studies, including dietary intakes of carbohydrates vs. lipids in the background diet, duration of treatment, and serum lipid composition at the beginning of the treatment as important variables that may influence outcome (Jackson et al., 1999).

Further consideration and research must concentrate on (1) defining the mechanisms underlying the putative cholesterol-lowering effects of probiotics, (2) the problems of survivability in the large bowel, (3) the possible role of prebiotics in maintaining adequate survival and colonisation of appropriate cholesterol-lowering species, and (4) identifying the actual effects on human postprandial triglycerides of prebiotics.

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