

Consumption of fermented and nonfermented dairy products: effects on cholesterol concentrations and metabolism¹⁻³

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ABSTRACT The objective of this article was to review existing literature concerning the effects and mechanisms of action of fermented dairy products on serum cholesterol concentrations. Although not without exception, existing evidence from animal and human studies suggests a moderate cholesterol-lowering action of fermented dairy products. Mechanistically, fermented milk has been shown to cause an increase in human gut bacterial content. These bacteria, once resident in the large intestine, are believed to ferment food-derived indigestible carbohydrates. Such fermentation causes increased production of short-chain fatty acids, which decreases circulatory cholesterol concentrations either by inhibiting hepatic cholesterol synthesis or by redistributing cholesterol from plasma to the liver. Furthermore, increased bacterial activity in the large intestine results in enhanced bile acid deconjugation. Deconjugated bile acids are not well absorbed by the gut mucosa and are excreted. Consequently, cholesterol, being a precursor of bile acids, is utilized to a greater extent for de novo bile acid synthesis. These actions combined are proposed as contributing mechanisms to the association of fermented milk consumption with decreased circulating cholesterol concentrations. *Am J Clin Nutr* 2000;71:674-81.

KEY WORDS Cholesterol, fermentation, bacteria, bile acids, fatty acids, dairy products, fermented dairy products

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death in North America. Diet has been identified as a means of controlling serum cholesterol concentrations (1, 2). International research has identified several populations having a high-risk diet but a low incidence of CVD. Among several possible factors in the diets of these populations potentially causing hypocholesterolemia, milk and fermented milk products have been identified. The objective of the present review was to examine available literature supporting the claim that consumption of fermented dairy products modulates cholesterol concentrations and attempt to examine the validity of the mechanisms proposed to explain their effects.

MILK AS A CHOLESTEROL-LOWERING FOOD

Interest in the effect of milk on cholesterol concentrations was first studied in the Maasai people in Africa (3). This population consumes large amounts of meat as well as blood and

milk. Despite this atherogenic diet, the incidence of CVD in this population is quite low. Milk has been proposed as a food in their diet that protects against CVD. Mann (3) determined the effects of a surfactant on serum cholesterol concentrations in Maasai men aged 16-23 y. The men were randomly assigned to 2 groups, one of which acted as a control and drank milk and the other of which drank milk containing Tween 20 (1 mg/L; Sigma Chemical Corp, St Louis), olive oil, and riboflavin. Subjects ate their usual diet of fermented milk for 6 d/wk and consumed meat and blood on the seventh day. The milk was inoculated with a wild strain of *Lactobacillus* and additives were incorporated 2-3 d later. The study was terminated after 3 wk instead of 4 because of the large volume of milk consumed, which rose from 4-5 L/d before the study to 8.3 L/d by the fourth day. In view of such an increase in energy intake, and after failing to reduce milk consumption, the researchers encouraged increased physical activity. However, subjects did not comply with the physical activity component of the study and gained weight. Total cholesterol concentrations decreased significantly in both groups, despite the gain in body weight. It was thus concluded that a factor in milk decreases cholesterol concentrations and that with greater milk consumption, a greater decrease in cholesterol concentrations occurs. Milk was proposed to act as an inhibitor of cholesterol synthesis (3).

This study conducted in the Maasai led several scientists to search for the hypocholesterolemic factor in milk. In a study by Buonopane et al (4), 82 free-living male and female participants were recruited and designated to enter either the treatment group or the seasonal group. Subjects in the treatment group ($n = 64$) were asked to drink ≈ 1.1 L skim milk with 2% solids by weight per day. The seasonal group was included to monitor the effect of season on serum cholesterol concentrations, not to act as a control group, and thus was not required to drink milk. During

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the first week (pretreatment) subjects did not drink the milk supplement. Physiologic measurements and blood samples were collected on 4 occasions throughout the study. Subjects were also required to complete three 5-d food records before physiologic measurements were made and blood was collected. Results showed that there was no change in cholesterol concentrations with seasonal change. There was, however, a significant decrease in serum total cholesterol and triacylglycerol concentrations in a subgroup with a baseline total cholesterol concentration ≥ 4.9 mmol/L, which occurred at 4 wk and again at 8 wk for triacylglycerol. Treatment had no effect on total cholesterol and triacylglycerol concentrations in the subgroup with baseline total cholesterol concentrations < 4.9 mmol/L. Seven of the subjects included in this trial were taking cholesterol-lowering drugs. These subjects did not experience a change in cholesterol concentrations compared with the other subjects. The decrease in plasma cholesterol was concluded to have been caused by milk consumption because there was no change in other hypercholesterolemic factors such as body weight, dietary intake of fiber, cholesterol, and the ratio of unsaturated fat to saturated fat. There was, however, a significant decrease in the percentage of energy coming from fat because fat intake remained stable and energy intake increased. Several agents in milk, such as magnesium, riboflavin, and orotic acid were proposed to be responsible for these cholesterol-lowering effects (4).

Steinmetz et al (5) compared the effects of skim milk with those of whole milk on circulating cholesterol concentrations using a crossover design in which subjects consumed a diet consistent with American Heart Association recommendations. Each subject consumed 660 mL milk/d as part of the 2 experimental diets for 6 wk, each separated by 10–16 wk. Blood samples were taken at baseline, 3 wk, and 6 wk for analysis of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations. When compared with the whole milk group, there was a significantly greater decrease in total cholesterol in the skim milk group at 6 wk. Total cholesterol and LDL-cholesterol concentrations decreased by 0.40 and 0.19 mmol/L, respectively, with skim milk consumption after 6 wk compared with baseline. Both diets resulted in lower HDL-cholesterol concentrations at 6 wk than at baseline. This study did not allow for the conclusion that milk lowers cholesterol concentrations because both diets had a lower fat content than the subjects' habitual diet. Also, the skim milk diet had 28% of energy as fat compared with 33% for the whole milk diet. The percentage of saturated fat in the skim milk diet was 3.8% lower and the cholesterol content was 66 mg lower than in the whole milk diet.

Golay et al (6) compared standard skim milk with skim milk from immunized cows in 11 male subjects with baseline total cholesterol concentrations of 7.01 mmol/L. The authors found that there was a significant decrease in total cholesterol and LDL-cholesterol concentrations when skim milk from immunized cows was consumed in amounts ≤ 0.3 – 0.5 L/d for 8 wk in a controlled dietary environment. It was hypothesized that the higher level of immunoglobulin G (IgG) was responsible for the decline in cholesterol concentrations. Sharpe et al (7) reproduced this study as a 38-wk randomized, controlled, double-blind crossover trial of 5 periods during which 30 subjects were fed the dairy products. Subjects were asked to consume a prudent diet and were followed by a dietitian to encourage maintenance of a consistent dietary fat intake throughout the study. The authors estimated that compliance with milk intake was 97%. Results

again showed significant decreases in total cholesterol and LDL cholesterol with consumption of immunized milk compared with ordinary skim milk. The authors of this study speculated that the effect of consumption of milk from immunized cows on cholesterol concentrations was attributable to changes in gastrointestinal flora, which were thought to enhance bile acid and cholesterol secretion. A decrease in bile acid recycling would ultimately result in a lowering of plasma cholesterol concentrations because cholesterol is used for bile acid synthesis. However, this study did not measure any microbiological variables to support this speculation.

These studies (3, 4, 6, 7) have all concluded that milk, whether whole or skim, lowers serum cholesterol concentrations. Each proposes a different mediator for these effects but none has attempted to show which component of milk—magnesium (4), riboflavin (4), orotic acid (4), IgG (6), an unknown inhibitory factor (3)—or whether the gastrointestinal flora (7) causes this hypocholesterolemic action.

EFFECTS OF FERMENTED DAIRY PRODUCTS ON CHOLESTEROL CONCENTRATIONS

Animal studies

Sharpe et al (7) speculated that the effect of milk from immunized cows acted through the gastrointestinal flora. Following this reasoning, it can be argued that intestinal bacteria could also be effectors in cholesterol metabolism. If the gastrointestinal flora affect circulating cholesterol concentrations, then changes in plasma cholesterol concentrations should be observed during consumption of fermented dairy products.

Several researchers have compared the effects of pasteurized milk, yogurt, and other fermented milks on cholesterol concentrations in animal models. One of these studies, conducted by Beena and Prasad (8), compared, in rats, the effects of a standard yogurt with that of a bifidus-containing yogurt on serum cholesterol concentrations. They studied standard yogurt inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 10 g/L and bifidus yogurt additionally inoculated with *Bifidobacterium bifidum* at 100 g/L. Each yogurt was then fortified with either skim milk powder, condensed whey, or lactose-hydrolyzed whey. Rats were fed a cholesterol-enriched diet supplemented with each of the yogurts ad libitum for 30 d. All yogurts significantly decreased total cholesterol but the lactose-hydrolyzed- and condensed whey-fortified yogurts were the most effective. Whole milk did not have any hypocholesterolemic effect. There was an increase in HDL-cholesterol concentrations in the group fed bifidus yogurt with skim milk powder and a decrease in groups receiving standard yogurt with skim milk powder and lactose-hydrolyzed and condensed whey. All groups fed bifidus yogurt had LDL-cholesterol concentrations 21–27% lower than those given whole milk. Although the bacterial count was not measured, it was concluded that the reduction in total cholesterol in rats given yogurt may have been due to an increased intestinal bacterial content.

Similar results were observed by other groups (9–11). Gilliland et al (9) fed pigs a high-cholesterol, corn-based diet with specific strains of *Lactobacillus acidophilus*. One group acted as a control and was fed 50 mL sterile nonfat milk solids (NFMS), a second group received 50 mL 10% NFMS containing 5×10^{10} cells of *L. acidophilus* P47, and a third was given 50 mL

10% NFMS with 5×10^{10} cells of *L. acidophilus* RP32 once daily. These products were not fermented, bacteria were merely added to the NFMS. Both groups receiving bacteria had a smaller increase in total cholesterol concentrations in plasma than did the control group. It was thus concluded that specific strains of *L. acidophilus* have the ability to modify cholesterol in the gut, making it unavailable for absorption in the blood, therefore causing a lowering of blood cholesterol concentrations (9).

In another study (10), rats were fed rat chow plus either water, skim milk, or milk inoculated with *S. thermophilus* bacteria ad libitum for 29 d. Plasma cholesterol concentrations were significantly lower in the thermophilus milk group than in those given skim milk or water. Although skim milk had no effect on plasma cholesterol concentrations, the lack of effect of skim milk might have been due to the greater weight gain observed in this group. These authors also proposed that orotate metabolites produced in fermentation might have been responsible for the hypocholesterolemic action of *S. thermophilus*-fermented milk (10).

Akalin et al (11) conducted a study in mice to examine the effects of dietary yogurt and acidophilus yogurt on serum total cholesterol, triacylglycerol, and lipoprotein concentrations. Mice were divided into 3 groups: those receiving rodent chow plus either water (control group), yogurt (yogurt group), or acidophilus yogurt (AY group) ad libitum for 56 d. Yogurts were made with 1%-fat milk inoculated with a 3% liquid culture of *S. thermophilus* and *L. delbrueckii* subspecies *bulgaricus* for yogurt and a 0.01% freeze-dried culture of *S. thermophilus* plus *L. acidophilus* for the acidophilus yogurt. The number of live lactobacilli in the freshly fermented milks was $\approx 10^{10}/L$. On day 28, total cholesterol concentrations were 22% lower in the AY group than in the control group. After 56 d, values for the AY group were reduced by 31%, 26%, and 17% when compared with the control group, the yogurt group, and day 28, respectively. For the yogurt group, total cholesterol concentrations were 7% lower on day 56 than on day 28. HDL-cholesterol and triacylglycerol concentrations were not affected by either treatment, but LDL cholesterol on day 56 was 33% lower than on day 28 in the AY group and 11% lower in the yogurt group. LDL-cholesterol concentrations in the control group remained stable. Fecal samples were also collected for fecal lactobacilli and coliform counts. These samples showed an increase in the number of fecal lactobacilli from 8 to 9.5 log colony-forming units (CFU)/g in the AY group and from 8 to 8.5 log CFU/g in the yogurt group throughout the treatment. These findings indicated that *L. acidophilus* established itself more readily in the mouse intestinal tract than did *L. bulgaricus* and, thus, acidophilus yogurt was more effective in reducing serum cholesterol concentrations than was yogurt.

Lactic acid bacteria have also been studied for their hypocholesterolemic properties. Nakajima et al (12) studied the effect of ropy fermented milk, produced with a slime-forming lactococci, on the serum cholesterol concentration of rats fed a high-cholesterol diet. Skim milk was inoculated with either 5% slime-forming or non-slime-forming *L. lactis* subspecies *cremoris*. The control product was made by treating skim milk with heat and acidifying it to pH 4.7 with 10% lactic acid. Rats were fed a high-cholesterol diet containing 10% of the slime-forming *L. cremoris*-fermented milk (ropy), the non-slime-forming *L. cremoris*-fermented milk (nonropy), or the acid milk ad libitum for 7 d. Total cholesterol concentrations were significantly lower and the ratio of HDL cholesterol to total cholesterol was significantly higher in the ropy group than in the acidified-milk group, but HDL-cho-

lesterol and triacylglycerol concentrations were not significantly different between groups.

Because there is evidence that dairy products fermented with one type of bacteria can have an effect on serum cholesterol concentrations, it could be hypothesized that several types of bacteria can also affect cholesterol concentrations and, possibly, to a greater extent. Effects of a single bacteria type compared with those of a mixture of bacteria on serum cholesterol concentrations were examined in rats by Fukushima and Nakano (13). Four groups of rats were fed 30 g rice bran/kg diet fermented with 1) bacilli, lactobacilli, streptococci, *Clostridium butyrium*, *Saccharomyces cerevisiae*, and *Candida utilis*; 2) *L. acidophilus*; 3) *Streptococcus faecalis*; or 4) nonfermented rice bran (control). Fermentation was achieved by adding between 1×10^7 and 1×10^8 CFU/g rice bran. The group receiving the mixture of bacteria showed a significantly greater decrease in cholesterol concentrations than the groups receiving only one type of bacteria. The mixture of organisms was observed to produce greater decreases in VLDL cholesterol, intermediate-density-lipoprotein (IDL) cholesterol, and LDL-cholesterol concentrations; increases in fecal steroid concentrations; and decreases in liver fatty acid composition. β -Hydroxy- β -methylglutaryl coenzyme A reductase activity in the liver decreased significantly as a result of consumption of bacteria and were lower in the mixed-organism group than in the groups given a single bacteria type, whereas fecal cholesterol and bile acid concentrations were higher. This study allows for speculation concerning the mechanism by which bacteria could exert their effects on plasma cholesterol concentrations. Bacteria could cause increased bile acid and cholesterol excretion, as observed in the fecal samples, which would result in the lower cholesterol concentrations seen in the group consuming the mixture of organisms.

Mohan et al (14) reported that broiler chickens fed a diet supplemented per kilogram with 75, 100, or 125 mg dried primary cultures of *L. acidophilus* and *L. casei*, *B. bifidum*, *Aspergillus oryzae*, and *Torulopsis* organisms in tablet form for 8 wk had significantly lower cholesterol concentrations than did a control group not receiving the probiotic. The experimental feed in this study contained a minimum of 0.27×10^9 CFU/g and was fed ad libitum to the broiler chickens. Samples for cholesterol analysis were taken after 6 wk of feeding. It was suggested that mixtures of organisms bind cholesterol and bile acids and may inhibit micelle formation in the gut (13), although there were no data presented to support this claim. Binding of cholesterol with bile acids and inhibition of micelle formation combined with the effect of fermentation on short-chain fatty acid (SCFA) production were mechanisms that had been proposed to explain the potential cholesterol-lowering effects of fermented dairy products (15).

These animal studies all point toward a cholesterol-lowering action of fermented dairy products (7–10, 12–14), which seem to be more effective than milk alone (9, 10). Most evidence indicates that the bacterial content may be responsible for the hypocholesterolemic action. Furthermore, some studies have shown that several types of bacteria in combination exert a greater effect on cholesterol metabolism than does a single type (14).

Human studies

Beliefs about the health benefits of fermented milk products in humans can be traced back to the early 1900s. In 1908, Metchnikov (16) wrote that milks fermented by lactic bacteria



“prevented intestinal putrefaction” and “helped maintain the forces of the body.” Human studies in the area of fermented dairy products and cholesterolemia have been conducted since the 1970s. Hepner et al (17), in 1979, tested the effects of yogurt supplementation on plasma cholesterol concentrations in humans. A first randomized crossover trial involved 17 free-living subjects who supplemented their habitual diet with three 240-mL portions of unpasteurized yogurt or 720 mL 2%-fat milk for 4 wk. After a 4-wk washout period, subjects consumed the alternate treatment. Results showed that in the group receiving unpasteurized yogurt, total cholesterol concentrations decreased by 5% after 1 wk of supplementation, rose during the washout period, and continued to rise slightly during milk supplementation. For the group receiving milk first, plasma total cholesterol concentrations decreased, although not significantly, after 1 wk of milk supplementation, rose during washout, and decreased by 9% after 1 wk of yogurt supplementation. Thirty-six subjects participated in a second experiment in which 4 groups supplemented their diet with three 240-mL daily portions of unpasteurized yogurt, pasteurized yogurt, or 720-mL daily portions of 2%-fat milk, or maintained their regular diet for 12 wk. Both yogurts originally contained live cultures of *L. bulgaricus* and *S. thermophilus*. Serum cholesterol in the group consuming unpasteurized yogurt fell by 9% after the first week of the experiment. In the groups consuming yogurt the decrease was 5%. The authors concluded that milk may have a small hypocholesterolemic effect and that pasteurized and unpasteurized yogurts have similar effects on cholesterol concentrations. The fact that cholesterol concentrations always decreased after 1 wk of supplementation may indicate that the effect is not mediated by the treatment but simply by being involved in the study.

Thompson et al (18) explored the effects of fermented and unfermented milk in an attempt to find the factor in milk that was responsible for its cholesterol-lowering properties. Sixty-eight volunteers participated in a 10-wk study during which they consumed 250 mL of 2%-fat milk. During the first 3 wk of the trial, all subjects were required to consume an additional 1 L of 2% milk per day. For the next 3 wk they resumed consumption of 250 mL milk, and for the last 3 wk were divided into 6 groups. The first 3 groups drank 1 L skim, 2%-fat, and whole milk, respectively; group 4 drank 1 L sweet acidophilus milk unfermented but inoculated with *L. acidophilus*, group 5 drank 1 L of buttermilk fermented with *S. cremoris* and *S. lactis*, and group 6 consumed 1 L yogurt fermented with *L. bulgaricus* and *S. thermophilus*. Plasma total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations remained constant throughout the study. Triacylglycerol concentrations were constant during the first 2 periods and increased in the groups receiving yogurt and acidophilus milk in the last period. Body weight increased in the first and last 3-wk periods, except in the groups receiving 2%-fat and whole milk, in which it increased only in the first 3 wk. A lack of significant effects of the fermented products on cholesterol concentrations was explained by the fact that subjects in this study had cholesterol concentrations <5.0 mmol/L.

Similarly, Rossouw et al (19) conducted a study to compare the effects of skim milk, yogurt, and full-cream milk on human serum lipids. Teenage boys were randomly assigned to consume skim milk (A), yogurt (B), or full-cream milk (C). The study lasted for 5 wk, during the first and last weeks of which no dairy products were provided. For the weeks during which they received 2 L of the assigned dairy product, subjects were asked

to consume the product in 4 aliquots of 500 mL each, once before each meal and again before retiring. Separation of the dairy product into aliquots was done to ease the adjustment of food intake by satiety, and in a manner such that energy intake would remain stable. Two days of food records were used for statistical analysis of dietary intakes. During the baseline week, total cholesterol concentrations decreased significantly in all groups, HDL-cholesterol concentrations decreased significantly in groups A and B, and triacylglycerol also decreased in group B. During the experimental weeks, total cholesterol concentrations fell significantly in group A, rose in groups B and C by the end of the second week, and returned to baseline after the third week. Changes in total cholesterol concentrations correlated with total fat and cholesterol intakes. HDL-cholesterol concentrations increased significantly during the first week of the experiment in all 3 groups and fell to baseline concentrations during the subsequent weeks. On the basis of these findings, the authors concluded that a milk factor might not necessarily be involved in the hypocholesterolemic effect of skim milk. However, they did suggest that the decrease in cholesterol concentrations in the second week of yogurt and full-cream consumption could have been due to a physiologic response to the unusual cholesterol and fat intakes.

Schaafsma et al (20) investigated the effects of milk fermented with traditional yogurt starters, yogurt containing 2 strains of *L. acidophilus*, and yogurt containing 2.5% fructooligosaccharides on blood lipid concentrations. The control product was milk inoculated with traditional yogurt starter cultures. Thirty healthy men with cholesterol concentrations <7.8 mmol/L were randomly assigned to 1 of 2 groups, after being matched for body weight, serum total cholesterol, and apolipoprotein E3 phenotype. The study had a double-blind, randomized, two-way crossover design with two 3-wk treatment periods separated by a 1-wk washout period. During each phase, subjects were instructed to consume three 125-mL portions of the test or control product daily with their habitual meals. The test product decreased total cholesterol and LDL-cholesterol concentrations when compared with the control product. However, the report did not indicate whether changes in these variables were significant when compared with the baseline values.

Another milk product, fermented with *Enterococcus faecium* and 2 strains of *S. thermophilus* (Gaio, Aarhus, Denmark), was studied for possible hypocholesterolemic properties (21). The fresh product, containing 10^{8-12} CFU *E. faecium*/L and $5-20 \times 10^{12}$ CFU *S. thermophilus*/L, was given to healthy men with cholesterol concentrations between 5.0 and 6.5 mmol/L. Subjects were asked to consume 200 mL fermented milk as a supplement to their normal diet for a period of 6 wk. The study was randomized, double-blind, and placebo controlled and the placebo was milk acidified with an organic acid. The groups were similar except that subjects taking the placebo had higher baseline LDL-cholesterol concentrations. There was no change in body weight, HDL-cholesterol, or triacylglycerol concentrations within or between groups during the trial. In the group consuming the fermented milk, significant reductions in total cholesterol concentrations of 3% and 6% were observed after 3 and 6 wk of supplementation, respectively, and LDL-cholesterol concentrations were lowered by 10% after 6 wk of supplementation. No such change was seen in the placebo group. It was thus suggested that the hypocholesterolemic effect of the milk was dependent on the presence of the bacteria in the fermented milk but an explanatory mechanism was not proposed.



A longer-term study (22) using the same fermented milk included women and subjects with cholesterol concentrations <8.0 mmol/L (5.78 ± 0.20 mmol/L). Subjects were randomly assigned to either the test product (Gaiò) or acidified milk. During the first 4 wk, subjects maintained their habitual diet without any supplement. For the next 24 wk, subjects consumed either product at 200 mL/d. Subjects were instructed not to change their eating habits and not to consume any fermented dairy product other than that supplied for the study. Baseline measures were similar in both treatment groups. In women, LDL-cholesterol concentrations were significantly reduced by 8% after 1 mo of fermented milk consumption and remained stable for the rest of the experiment. There was no change in LDL cholesterol in the placebo group. In men, a linear 10% reduction in LDL cholesterol was seen for the first 3 mo of fermented milk consumption. However, LDL-cholesterol concentrations were also significantly lower in the placebo group after 3 mo, but after 6 mo, reductions were similar in both groups. It was concluded that the fermented milk could significantly reduce LDL-cholesterol concentrations within 1 mo of consumption. However, it was also recognized that the placebo may have also exerted a hypocholesterolemic effect.

de Roos et al (23) conducted a placebo-controlled, double-blind trial in which 78 healthy normocholesterolemic subjects supplemented their diet with 500 mL yogurt/d. All subjects consumed the control yogurt made with a starter culture of *S. thermophilus* for the first 2 wk and were randomly assigned to either the control or test yogurt for the next 6 wk. The test yogurt contained from 4.8×10^9 to 2.7×10^{10} CFU of *L. acidophilus* strain L-1 in each 500-mL sample. Groups were similar with respect to sex distribution, age, body mass index, and total cholesterol concentrations at baseline. There was no change in total cholesterol, HDL-cholesterol, LDL-cholesterol, or triacylglycerol concentrations over the 6-wk period in either group. It was concluded that yogurts containing *L. acidophilus* strain L-1 do not lower blood lipids in men and women with normal-to-borderline-high cholesterol concentrations.

Studies conducted in human subjects are thus ambiguous regarding the effects of fermented dairy products on serum cholesterol concentrations (Table 1). The presence of confounders, such as changes in the fat content of the diets (18, 19), prevents direct conclusions from being drawn from the results. In addition, subjects tended to have low serum cholesterol concentrations at the beginning of some studies (18, 19). Also, the absence of a proper placebo is problematic because milk has also been shown to have hypocholesterolemic properties. Furthermore, some authors failed to report the bacterial composition (19) and concentration (17–20) of the product studied, making comparisons between studies difficult.

GUT COLONIZATION

Because the pH of the stomach is a major barrier to bacteria, it normally contains only small numbers of Gram-positive bacteria, such as lactobacilli and streptococci. These bacteria are believed to be transient microorganisms entering the body with food or saliva (24). Charteris et al (25), in 1998, studied the viability of different strains of lactobacilli and bifidobacteria under conditions found throughout the gastrointestinal tract and found that 14 of the 15 strains studied lost $>90\%$ viability during simulated gastric transit. The only intrinsically resistant strain was *L. fermentum* KLD. However, addition of milk proteins improved gastric transit

tolerance, allowing 2 strains, *L. casei* 212.3 and *Bifidobacterium infantis* 25962, to be completely pH resistant. Addition of mucin did not alter tolerance of lactobacilli but increased that of bifidobacteria. It was thus concluded that some strains of lactobacilli and bifidobacteria species may survive passage through the human stomach, and to a greater extent when ingested with milk products or foods containing milk proteins (25).

Once in the large intestine, bacterial retention can result from specific adherence of the bacteria to epithelial cells, from non-specific adherence to other intestinal bacteria, or from penetration and entanglement in the mucous coat (26). Administration of 125 g bifidobacteria-fermented milk/d, containing 1×10^9 bacteria/g, 3 times/d for 12 d was found to increase the proportion of exogenous bifidobacteria in the colonic flora (27). This was studied indirectly by Marteau et al (28), who looked at the effects of regular consumption of foods containing live *L. acidophilus* (1×10^7 cells/g) and *B. bifidus* (1×10^8 cells/g) bacteria for 3 wk on fecal enzyme activities. The fermented dairy product, which also contained a mesophilic lactic culture of *S. lactis* and *S. cremoris* (1×10^8 cells/g), was consumed at 100 g, 3 times/d for 3 wk. The supplementation period was preceded and followed by 3-wk periods during which subjects were not permitted to consume fermented dairy products. Nitroreductase, an enzyme involved in the conversion of nitrated aromatic compounds into potentially harmful amines in the intestine, showed a decrease in its activity during the supplementation period and remained low in the follow-up phase. Enzymatic activity of β -glucosidase, a digestive enzyme found predominantly in the small intestine, increased significantly during the supplementation phase and returned to baseline in the follow-up phase. However, there was no change in breath hydrogen and methane excretion with supplementation of fermented milk. These results led the investigators to speculate that chronic ingestion of a fermented dairy product containing *L. acidophilus* and *B. bifidum* leads to metabolic modifications of the colonic flora (28). It may thus be concluded that the gut bacterial content can be modified through chronic ingestion of bacteria-containing foods.

EFFECT OF GUT BACTERIA ON SHORT-CHAIN FATTY ACID AND CHOLESTEROL METABOLISM

Bacteria in the large intestine ferment unabsorbed carbohydrates and endogenous polysaccharides, including mucus, to produce ≈ 100 –450 mmol SCFAs/d (29). The relative proportions of acetate, propionate, and butyrate produced have been reported to be $\approx 60:20:15$, depending on the substrates being fermented (30). All substrates produce acetate as their major end product, but the amount of propionate and butyrate produced varies. Therefore, observation of altered patterns of SCFAs in the human colon is evidence of fermentation (31).

When SCFAs are present in the gut, they are quickly absorbed by the large intestine and then metabolized by the liver (32). Wolever et al (33) examined the interaction between colonic and serum SCFAs to see how much of the acetate produced in the colon is transferred to the blood. They found that rectal infusions of sodium acetate or sodium propionate increased serum concentrations of each of these substrates. Findings from this study also allowed the authors to conclude that acetate increases total cholesterol and decreases fatty acids and that propionate increases blood glucose and lowers the hypercholesterolemic response caused by acetate, a precursor of cholesterol. This



TABLE 1

Summary of findings on the effect of fermented dairy products on plasma cholesterol concentrations¹

Reference and year	Study population	Products tested	Results
Beena and Prasad (8), 1997	Rats	Standard and bifidus yogurt	Both yogurts reduced TC concentrations
Gilliland et al (9), 1985	Pigs	NFMS and AY	Lesser increase in TC concentrations with AY
Rao et al (10), 1981	Rats	Water, milk, and thermophilus milk	TC concentrations were lower in groups fed thermophilus milk than milk or water
Akalin et al (11), 1997	Mice	Standard and AY	AY decreased TC concentrations and fecal bacteria compared with water and yogurt consumption; AY reduced LDL cholesterol by 33% and yogurt did so by 11%
Nakajima et al (12), 1992	Rats	Ropy fermented milk and acidified milk	Fermented milk reduced TC concentrations compared with acidified milk
Fukushima and Nekaano (13), 1996	Rats	Mixture of organisms, single type organisms, and none	Mixture of organisms resulted in a greater decrease in TC concentrations than did single type organisms
Mohan et al (14), 1996	Chickens	Mixture of organisms, and none	TC concentrations were lower with consumption of a mixture of organisms than with none
Hepner et al (17), 1979	Men and women	Yogurt, milk, and pasteurized and unpasteurized yogurt	Yogurt reduced TC by 5%, both yogurts reduced TC concentrations to a similar extent
Thomson et al (18), 1982	Men and women	Milk and acidophilus milk, fermented buttermilk, and yogurt	No effects on blood lipid concentrations
Rossouw et al (19), 1981	Teenage boys	Yogurt, milk, and cream	No effects on blood lipid concentrations
Schaafsma et al (20), 1998	Men	Fermented yogurt and milk	Yogurt reduced TC and LDL-cholesterol concentrations compared with milk
Agerbaeck et al (21), 1995	Men	Fermented milk and acidified milk	Fermented milk reduced TC and LDL-cholesterol concentrations
Richelsen et al (22), 1996	Men and women	Fermented milk and acidified milk	Women: fermented milk reduced LDL-cholesterol concentrations; men: both products reduced LDL-cholesterol concentrations
de Roos et al (22), 1998	Men	Yogurt and AY	No effects on blood lipid concentrations

¹NFMS, nonfat milk solids; TC, total cholesterol; AY, acidophilus yogurt.

effect was again observed when the same authors showed that propionate inhibited acetate incorporation into plasma triacylglycerol and also tended to inhibit ¹³C incorporation from labeled acetate into plasma cholesterol (34).

In a study in which women were supplemented daily for 7 wk with capsules containing 7.5 g propionate (30), plasma HDL-cholesterol concentrations increased whereas LDL cholesterol decreased compared with a control, dibasic calcium phosphate capsules, and with baseline values when body mass index was held constant. Control subjects were matched for body mass index, area under the baseline glucose-tolerance-test curve, and percentage of total cholesterol as HDL cholesterol. The authors cautioned that the gradual absorption of propionate derived from bacterial fermentation may not result in the same effects as those seen in this study because dietary intake results in rapid intestinal absorption of propionate.

For gut bacterial fermentation to play a role in the hypolipidemic effect of a food, sufficient propionate must be produced to offset the effects of acetate generation as a precursor for lipid synthesis (15). An increase in bacterial count or a change in the composition of the bacterial population in the large intestine would result in increased fermentation and SCFA production. Depending on the proportion of each fatty acid produced, plasma cholesterol concentrations may thus be altered through this mechanism.

EFFECT OF BACTERIA ON BILE ACIDS AND CHOLESTEROL METABOLISM


Another mechanism through which bacteria associated with fermented dairy products may exert their hypocholesterolemic action is via bile acids. Cholic and deoxycholic acids, bile acids produced from cholesterol by hepatocytes, are conjugated with glycine and taurine, respectively. These acids enter the small intestine, where they are absorbed and directed to the liver. However, during reabsorption, conjugated bile acids are exposed to intestinal microflora. Bacteria found in fermented foods, such as *Bacteroides* species, bifidobacteria, fusobacteria, clostridia, peptostreptococci, lactobacilli, and streptococci hydrolyze conjugated bile acids (24). Animal studies have also shown that labeled bile acid is eliminated faster in normally nourished rats than in germ-free rats (35). Furthermore, pigs kept in a nonsterile environment have lower serum cholesterol concentrations and a 4-fold higher excretion of neutral steroids than do pigs kept in a sterile environment (36). These results agree with those obtained by Goddard and Hill (37), who found that rats and guinea pigs excreted [¹⁴C]cholesterol as steroids in the urine and feces (94% and 90% for rats and guinea pigs, respectively) when injected with 2–10 μCi (74–370 kBq) labeled cholesterol in the cecum. Excretion was maximal after 72 h but ≈90% of the neutral steroids were recovered in the first

24 h. These findings provide evidence that gut flora not only hydrogenate, dehydrogenate, and oxidize bile acids, but they also cleave side chains to yield steroids.

It has been suggested that if a strain of *Lactobacillus* exhibiting a high degree of bile salt hydrolase activity could be incorporated in the intestinal tract, the amount of bile hydrolysis would increase (38). This ultimately leads to a faster rate of cholesterol conversion to produce more bile acids (39). *L. acidophilus* is one of the lactobacilli that can, when grown anaerobically in a bile-containing environment, remove cholesterol from laboratory media and convert it to bile acids (38). In vivo, removal of cholesterol occurs because deconjugated bile acids are not reabsorbed in the large intestine and are excreted through the feces and urine. Excretion of bile acids results in decreased enterohepatic recycling of bile acids and thus increases de novo bile synthesis.

In vitro studies using different strains of *L. acidophilus* grown on media containing bile have indeed shown that certain strains of the bacteria modify cholesterol metabolism (9). Because the amount of bile in the medium did not exceed the concentrations normally seen in the intestine, it can be expected that cholesterol binding to bile acids would also occur in vivo. This assimilation of cholesterol by bacteria would make cholesterol unavailable for absorption into the circulation.

CONCLUSIONS

In light of these findings, several fermented dairy products available on the market today have the potential of being classified as useful cholesterol-lowering agents. These foods include fermented vegetables, bifidus- and acidophilus-containing yogurt and milk beverages, and kefir, a fermented dairy product containing several types of bacteria in symbiosis with yeasts. High numbers of bacteria ($>1 \times 10^8$ CFU/g) in the food consumed will ensure passage of sufficient numbers of bacteria into the intestine to exert effects on metabolism. Because the bacteria contained in these foods are absorbed with other macronutrients that alter the stomach's pH, bacterial survival is increased as they pass in the large intestine. Once in the large intestine, these bacteria ferment indigestible carbohydrates and produce SCFAs. The relative proportions of the SCFAs produced will likely alter cholesterol synthesis. Also, the intestinal bacteria can bind bile acids to cholesterol, resulting in the excretion of bile acid-cholesterol complexes in the feces. Decreased bile acid recycling through the enterohepatic circulation would result in cholesterol uptake from the circulation into the liver for de novo synthesis of bile acids. Therefore, dairy products fermented with the proper strain of bacteria can be anticipated to result in a lowering of the circulating cholesterol concentrations, thus diminishing the risk of CVD in the population. These bacteria must be bile resistant and 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 the lowering of elevated cholesterol concentrations, and hence, in the prevention of CVD. To date, products that contain live bacteria, such as yogurt, acidophilus milk, and kefir, contain strains that normally do not reside in the human gastrointestinal tract. The result is that these "foreign" bacteria do not colonize the intestine and are quickly eliminated in the feces. Daily consumption of probiotic products is therefore necessary for any long-term effect on metabolism. 

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