Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products[1–3]

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ABSTRACT Intake of milk and butter has been clearly associated with higher coronary heart disease rates in different countries and this is likely to be mediated by the hypercholesterolemic effect of dairy fat. Fat-modified dairy products are an innovation involving a technology in which protected unsaturated lipids are fed to ruminants resulting in milk and tissue lipids with reduced saturated fatty acids. We examined the impact of these novel dairy fats on plasma lipids in a human dietary trial. Thirty-three men and women participated in an 8-wk randomized crossover trial comparing fat-modified with conventional dairy products. The trial consisted of a 2-wk low-fat baseline period followed by two 3-wk intervention phases. During the test periods, the fat-modified products resulted in a significant 0.28-mmol/L (4.3%) lowering of total cholesterol ($P < 0.001$). Most of this decrease was in LDL cholesterol, which decreased by 0.24 mmol/L ($P < 0.001$) whereas HDL cholesterol and triacylglycerols remained essentially unchanged. This alteration in the fatty acid profile of dairy products, if applied to populations typical of developed Western countries, represents a potential strategy to lower the risk of coronary heart disease without any appreciable change in customary eating patterns. *Am J Clin Nutr* 1996;63:42–6.

KEY WORDS Dairy fat, dietary fatty acids, saturated fatty acids, plasma cholesterol, low-density lipoproteins, feedlot technology

INTRODUCTION

Intake of milk and butter has been clearly associated with higher coronary heart disease rates in different countries (1–5). The correlation with cardiovascular disease mortality is likely to be mediated by the effect of dairy fats on the plasma low-density-lipoprotein-(LDL) cholesterol concentration, which is a risk factor for coronary heart disease (6).

Dairy products (excluding butter) make a substantial contribution to saturated fat intake (25–29%; 7) in Westernized countries. In Australia, fats from dairy foods currently comprise 25% of the total fat intake and as much as 40% of the saturated fatty acids (8).

The hypercholesterolemic effect of dairy products was demonstrated conclusively in numerous controlled studies. In 1957, Ahrens et al (9) demonstrated that butter was hypercholesterolemic compared with polyunsaturated vegetable oil, but the number of subjects was small. These findings were confirmed by Keys et al (10, 11) and Hegsted et al (12) in the mid-1960s as well as in more recent trials (13–18). With respect to whole milk, two well-controlled trials comparing whole and skim milk within isoenergetic diets demonstrated an elevation in total cholesterol by 7–13% (19, 20). In controlled studies with butter, cheese, or milk, there has been near uniformity of results with decreases in cholesterol of between 5% and 20%, depending on the type and amount of fat that is substituted for dairy fat.

Two of the principal saturated fatty acids in butterfat, myristic and palmitic acids, have been identified as major dietary factors that raise LDL cholesterol (11, 12, 21–26). In particular, myristic acid, of which dairy products are a major source, is reputedly more potent than palmitic acid in its lipid-raising effects (27). The challenge is to partially replace these fatty acids in milk and dairy products with unsaturated fatty acids.

There have been a number of options put forward to modify the cholesterol-raising properties of milk (28–30), including cholesterol removal, milk fat fractionation, and changes in the feeding practices of cows. Normally, the adipose tissue and milk fats of sheep and cattle are consistently high in saturated fatty acids because of the hydrogenation of dietary unsaturated fatty acids to more saturated forms by microorganisms in the rumen, the first compartment of the ruminant stomach. Earlier studies had shown that the fatty acid profile of tissue and milk from ruminants can be favorably altered by a feedlot technology that involves feeding unsaturated fat supplements coated with formaldehyde-treated casein (31–33). Palmitic acid was substantially reduced while linoleic acid was increased, with the result that the consumption of these fat-modified dairy and beef fats led to a substantial lowering of plasma total cholesterol compared with their conventional counterparts. However, these linoleic acid–enriched products had a decreased shelf life, making them nonviable commercially. More recently, this

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[2] We thank Rumentek Industries for supplying the lipid supplement and the low saturated fatty acid milk for use in the trial. This work was conducted under contract to CSIRO from a research and development syndicate arranged by Macquarie Bank (Sydney, Australia).

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Received April 11, 1995.

Accepted for publication August 28, 1995.

problem has been offset through the substitution of oleic acid rather than linoleic acid in the feed (34). Oleic acid, as a monounsaturated fatty acid, is more stable to oxidation than the diunsaturated linoleic acid. This has been achieved by including protected canola seeds in the feed.

We carried out this study to examine whether modifying the fatty acid profile of milk and related dairy products with oleic acid as the major substitute for palmitic and myristic acids will result in a significant reduction in total and LDL cholesterol of subjects consuming the fat-modified dairy products, when compared with conventional dairy products.

SUBJECTS AND METHODS

Subjects

Thirty-three subjects (19 men and 14 women) participated in the study. Their mean (± SD) age was 49 ± 10.3 y, their body mass index was 24 ± 2.5 (in kg/m^2), their initial plasma total cholesterol was 5.96 ± 0.76 mmol/L, and their plasma triacylglycerol was 1.44 ± 0.68 mmol/L at screening.

Informed consent was obtained and the study was approved by the Human Ethics Committee of the CSIRO Division of Human Nutrition.

Experimental design

To produce milk with lower saturated fatty acids, lactating dairy cows were fed a ration that contained a low amount of roughage, which results in reduced acetate production from rumen fermentation and leads to reduced endogenous synthesis of saturated fatty acids. Furthermore, the ration contained a protected lipid supplement that supplied ≈8% fat by weight. This is twice the fat content of conventional rations. The protected lipid supplement was manufactured by Rumentek Industries (Royal Exchange, Sydney, Australia). The lipid is derived from canola and soybean meal and is protected with a protein, resulting in a higher absorption of unsaturated fatty acids and hence higher milk expression of monounsaturated and polyunsaturated fatty acids. Vitamin E was also fed to minimize the effects of oxidation in the ensuing milk because of its altered fatty acid profile. The resulting milk and a conventional milk were used to manufacture a range of dairy products that were matched in fat content. The fatty acid profile of the fat-modified dairy foods was 51% saturated, 10% polyunsaturated, and 39% monounsaturated. In the control dairy foods fatty acids were 70% saturated, 2% polyunsaturated, and 28% monounsaturated. There were minor variations in fatty acid profiles among the test foods and **Table 1** presents the mean fatty acid profile of these products weighted for their relative fat contribution to the test diet. Most of the reduction in saturates was in palmitic and myristic acids, with concomitant increases in oleic and linoleic acids. Unidentified fatty acids were primarily unique lipids and branched-chain fatty acids contributed by ruminal fermentation. Because there was less fermentation of dietary lipid in the cows fed the protected feed supplement, the amount of these unidentified fatty acids was lower. *Trans* fatty acids were also estimated and found to be slightly lower in the fat-modified (2.2%) compared with the conventional milk (3.4%).

The trial consisted of a 2-wk familiarization period (baseline) during which subjects were instructed to follow a low-fat diet in preparation for the two 3-wk intervention phases. During each period subjects consumed a low-fat background diet (15% of energy as fat) supplemented in crossover fashion and in random order, with the test or control products (20% of energy as fat) daily. Subjects consumed four dairy products daily—milk, cheese, butter, and ice cream in controlled amounts. The fat-modified products were slightly paler in color and tended to be softer in texture. However, all products were rated highly in terms of palatability.

TABLE 1
Mean fatty acid profiles of control and fat-modified dairy products

Fatty acid	Control	Fat-modified
	% by wt of total fatty acids	
Butyric (4:0)	5.7	5.5
Caproic (6:0)	2.7	2.5
Caprylic (8:0)	2.9	1.3
Capric (10:0)	2.8	2.3
Lauric (12:0)	3.3	2.3
Myristic (14:0)	10.0	6.7
Palmitic (16:0)	25.9	15.5
Stearic (18:0)	11.7	14.3
Oleic (18:1)	22.8	35.3
Linoleic (18:2)	1.5	6.9
Linolenic (18:3)	0.7	2.2
Unidentified	10.0	4.2

Background diets were prepared from a combination of self-selected foods of known fat content, such as meat and meat products. To facilitate meal planning low-fat frozen meals (< 10 g fat/meal) were provided according to individual needs for approximately three meals per week.

All subjects were instructed in the dietary protocol by a dietitian and interviewed on four occasions during each 4-wk period. They were advised to quantify their fat intake daily and to restrict it to < 15% of energy using simplified food tables.

Intake of foods containing fat was documented daily. Subjects also kept detailed weighed food records of all foods consumed for 3 consecutive days (Sunday, Monday, and Tuesday) in each test period and were provided with electronic scales for this purpose. Food records were reviewed extensively by the research dietitian along with daily records to ensure that they were complete and to ensure that they were consistent with that individual's average fat intake. Specific advice was given to avoid specific foods or nutritional supplements that may have an independent effect on plasma lipids and to maintain a similar pattern of eating throughout the study.

DIARYAN (35) was used to calculate nutrient intakes from the food records. The program was modified to include data on the test supplements and frozen meals from direct food analysis.

Measurements

Blood was drawn from fasting subjects on 2 consecutive days at the end of the baseline phase and three times at the end of the other two periods. The lipid values in each test period were averaged.

Plasma cholesterol and triacylglycerol concentrations were determined by enzymatic methods (36, 37) on an automated analyzer (Cobas Bio; Hoffmann-La Roche, Basel, Switzerland). High-density-lipoprotein (HDL) cholesterol was deter-

mined after precipitating the apolipoprotein B–containing lipoproteins with polyethylene glycol (38). LDL cholesterol was calculated by using the Friedewald (39) equation but modified as follows: LDL cholesterol = total cholesterol − HDL cholesterol − (triacylglycerol × 0.45) to express the data in mmol/L. The fatty acids in the test fats were quantified by gas chromatography with a BP20 column (50 m × 0.32 mm internal diameter; SGE Pty Ltd, Melbourne, Australia) (40). *Trans* fatty acids were quantified on an SGE BPX70 capillary column (25 m × 0.2 mm internal diameter; SGE) by using a temperature program recommended for *cis-trans* separation.

Analyses

The data were analyzed with the SPSS/PC+ (SPSS Inc, Chicago) statistical program (41). Paired *t* tests were used to compare the control with the fat-modified period. The baseline period was not randomized, and hence was not used in the statistical analysis. Pearson's correlation coefficients were calculated for some variables to find out the factors associated with changes in plasma lipids.

RESULTS

Dietary intakes

Both test and control dairy products were highly acceptable. Monitoring of diets indicated apparently excellent compliance although self-recorded food intake is subject to obvious errors. Energy intake was self-controlled by subjects to achieve energy balance so that there was a moderate range of intakes among subjects. **Table 2** summarizes the mean dietary intakes of subjects during each intervention period. Mean body weights did not differ significantly between dietary periods. Except for total energy, nutrient intake data for men and women were not significantly different and were pooled. All macronutrient intakes, particularly the percent of energy from fat, were not significantly different in the control and fat-modified phases. However, there was a significant difference ($P < 0.001$) in fatty acid profile with a decrease in percent of energy from myristic (0.7%) and palmitic (2.2%) acids with an associated increased in stearic (1%), oleic (2.8%), linoleic (1.3%), and linolenic (0.3%) acids.

TABLE 2
Nutrient intakes of subjects during control and fat-modified phases[1]

Nutrient	Control	Fat-modified
Energy		
(MJ/d)	9.4 ± 1.9	9.6 ± 1.8
(kcal/d)	2220 ± 457	2285 ± 418
Protein (% of energy)	16.9 ± 2.3	17.4 ± 3.2
Fat (% of energy)	36.6 ± 4.5	36.9 ± 3.9
Butyric acid (4:0)	1.4 ± 0.2	1.4 ± 0.2
Caproic acid (6:0)	0.7 ± 0.1	0.6 ± 0.1
Caprylic acid (8:0)	0.3 ± 0.1	0.3 ± 0.1
Capric acid (10:0)	0.7 ± 0.1	0.6 ± 0.1
Lauric acid (12:0)	0.8 ± 0.1	0.7 ± 0.1
Myristic acid (14:0)	2.7 ± 0.3	2.0 ± 0.2[2]
Palmitic acid (16:0)	8.1 ± 1.1	5.9 ± 0.8[2]
Stearic acid (18:0)	3.5 ± 0.5	4.5 ± 0.6[2]
Oleic acid (18:1)	9.1 ± 1.7	11.9 ± 1.6[2]
Linoleic acid (18:2)	2.2 ± 0.6	3.5 ± 0.7[2]
Linolenic acid (18:3)	0.4 ± 0.1	0.7 ± 0.1[2]
Carbohydrates (% of energy)	47.2 ± 6.7	47.6 ± 6.8
Alcohol (g/d)	7 ± 12	6 ± 9
Cholesterol (mg/MJ)	32.5 ± 10.6	29.1 ± 9.1
Fiber (g/d)	24 ± 9	26 ± 11

[1] $\bar{x}$ ± SD; n = 33.
[2] Significantly different from control, $P < 0.001$.

Plasma lipoproteins

Table 3 summarizes the mean plasma lipoprotein concentrations during the baseline and intervention phases. During the test periods, the fat-modified products resulted in a 0.28-mmol/L lower total cholesterol concentration ($P < 0.001$). Most of the decrease was in LDL cholesterol, which was 0.24-mmol/L lower ($P < 0.001$) whereas HDL cholesterol and triacylglycerols remained essentially unchanged. The baseline low-fat diet resulted in the lowest total and LDL cholesterol results. However, note that this period was also associated with some degree of energy restriction for several subjects. Furthermore, the baseline period was not included in the randomization process. There were no effects on the magnitude of change in plasma lipoproteins by baseline lipids, sex, waist-hip ratio, or order of products consumed.

DISCUSSION

We conclude that modifying the fat composition of milk and related dairy products through the substitution for palmitic and myristic acids with oleic, linoleic, and linolenic acids will result in a significant improvement in the plasma lipoprotein profile in humans. We observed a decrease of 0.28 mmol/L (4.3%) in total plasma cholesterol by substituting the fat-modified dairy products for conventional products. This occurred without any change in total fat and energy intake or any changes in food group choices or food acceptance. Dietary intakes of palmitic and myristic acids fell relative to the control diet by 37% and 35%, respectively, and this was associated with increases in oleic (31%), linoleic (59%), and linolenic acids (133%). However, there was no change in the ratio of linoleic to linolenic acid. In prior studies with polyunsaturated ruminant fats (31, 32) an 8–10% fall in total cholesterol was reported. However, in these studies nearly all of the dietary fat was provided by the test ruminant fats and the total fat in the diets was 39–50% of energy. By contrast, our study provided only 20% of energy as the test fat. Furthermore, although the percent reductions in palmitic and myristic acids in our test diets were similar to those reported by Nestel et al (32, 33), the greater increase in linoleic acid in the earlier studies would also

TABLE 3
Plasma lipoproteins during baseline, control, and fat-modified phases[1]

Plasma lipids	Baseline	Control	Fat-modified
		mmol/L	
Total cholesterol	5.89 ± 0.89	6.50 ± 0.98	6.22 ± 0.82[2]
LDL cholesterol	3.95 ± 0.75	4.49 ± 0.90	4.25 ± 0.71[2]
HDL cholesterol	1.19 ± 0.30	1.30 ± 0.33	1.28 ± 0.33
Triacylglycerols	1.67 ± 0.85	1.57 ± 0.72	1.54 ± 0.66

[1] $\bar{x}$ ± SD; n = 33.
[2] Significantly different from control, $P < 0.001$.

have contributed to the greater decreases in plasma LDL cholesterol. Although there has been considerable debate over whether linoleic acid or oleic acid is more potent in lowering cholesterol, the consensus appears to favor linoleic acid (10, 12, 42–44). On the other hand, dietary oleic acid leads to less oxidizability of the LDL particle (45).

There was a 10% increase in total plasma cholesterol with the control dairy products relative to the baseline low-fat diet, and the magnitude of this response is consistent with the findings of Roberts et al (19). However, the baseline period was used mainly to familiarize subjects with the background low-fat diet; in addition to the reduction in fat intake the commonly observed initial decrease in plasma cholesterol when subjects begin new diets may have exaggerated the difference.

Of the numerous predictive formulas that calculate changes in plasma total cholesterol from changes in dietary fatty acids, we used the most recent equations of Mensink and Katan (42) and Hegsted et al (44) as a basis for comparison of our results. These were derived from meta-analyses of well-controlled dietary studies in the former case, and in the latter case we used the formula specific for field trials. When applying these formulas to our dietary data, the predicted decreases in total cholesterol are 0.21 and 0.27 mmol/L, respectively, which are similar, particularly for 0.27 mmol/L, to our observation.

Over the past two decades the original technology that was intended to result in milk with an altered fatty acid profile has been substantially refined. Early studies used a ration with protected sunflower seeds that resulted in milk with a high polyunsaturated fat composition (31–34). However, this product was unstable because of oxidation and it was necessary to add butylated hydroxytoluene as an antioxidant to the milk. The change in the nature of the lipids in the ration to a primarily monounsaturated source by using canola seeds and the additional use of vitamin E in the ration, which is also transferred to the secreted milk, has rendered it more stable. Milk production efficiency is enhanced by this commercially viable process.

Objectively, the studies of Roberts et al (19) and Kristi et al (20) suggest that the effects of changing from whole to skim milk consumption will result in a greater reduction in total cholesterol than will the use of fat-modified milks. However, it can also be argued that skim milk is less palatable than a full-fat milk because of the mouthfeel characteristics of fat. Therefore, in terms of general public acceptance, fat-modified milks offer a significant health benefit to full-fat milk consumers, who still make up the majority of milk purchasers. Furthermore, it is technically more difficult to reduce fat in some high-fat dairy products, notably cheese, ice cream, and cream. These items rely much more heavily on their fat content for texture and palatability and lower-fat versions of these products, despite the use of fat substitutes in some cases, have yet to achieve a large market share. It is in this sector that fatty acid–modified dairy products have considerable potential. We noted in this study that subjects rated the fat-modified products highly in terms of taste when compared with their conventional counterparts. Furthermore, under Australian economic conditions, the cost of this technology is mitigated by the increased milk production due to the higher energy density of the feed. Hence, the subsequent cost of fat-modified milk to the consumer is projected to be competitive with both conventional milks and skim milks blended with oil.

The 4.3% reduction in total cholesterol, if applied to the population, represents a 9% reduction in the risk of developing coronary heart disease (46). Although in this trial dairy fats comprised approximately twice the mean contribution of dairy fat to total fat intake, this feedlot technology allows fatty acid modification of meat products, which, in combination with dairy products, would approximate the fat substitution in this study. Such changes in the fat profile of the food supply are a large-scale approach to improving the nation's (Australia) health in a way that is easily acceptable and does not require major shifts in eating patterns.

Thanks are extended to Neil Tomlinson and Brendan Blakely for coordinating the manufacture of dairy products, to Rosemary McArthur and Anne Stevens for their skill in recruiting and managing the subjects during the study, and finally, to all of the members of the Rumentek Dairy Production Scientific Committee for their valuable comments.

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