Modified milk fat reduces plasma triacylglycerol concentrations in normolipidemic men compared with regular milk fat and nonhydrogenated margarine[1–3]

Hélène Jacques, Annie Gascon, Joseph Arul, Armand Boudreau, Charles Lavigne, and Jean Bergeron

ABSTRACT

Background: A modified milk fat with reduced cholesterol was developed by fractionation technology.

Objective: The effect of this modified milk fat on the lipoprotein profile of 21 normolipidemic men was compared with that of regular milk fat and nonhydrogenated margarine.

Design: A crossover design was used for the administration of the 3 experimental diets, which provided 13 240 kJ as 16% protein, 51% carbohydrates, 33–34% lipids, and 21 g fiber/d. The ratio of polyunsaturated to saturated fat was 1.3:1 for the margarine diet and 0.3:1 for the milk-fat diets. The cholesterol content of the modified milk-fat and margarine diets was similar (248 and 254 mg/d, respectively), but was significantly higher (428 mg/d) for the regular milk-fat diet.

Results: Modified and regular milk fats did not change plasma total and LDL cholesterol significantly, but margarine did ($P < 0.01$). Furthermore, modified milk fat maintained initial HDL_2-cholesterol concentrations, but margarine reduced this variable significantly ($P < 0.05$). These results can be explained by the lower ratio of polyunsaturated to saturated fat in the modified and regular milk-fat diets than in the margarine diet. Men who ingested modified milk fat had significantly ($P < 0.05$) lower total and VLDL-triacylglycerol and VLDL-cholesterol concentrations than did those who ingested either regular milk fat or margarine. This may have been, in part, because of the lower intestinal fat absorption with modified milk fat than with regular milk fat and margarine arising from changes in the melting properties of milk fat with fractionation.

Conclusion: A reduction in plasma triacylglycerol concentrations after the consumption of modified milk fat may prevent the onset of hypertriacylglycerolemia. *Am J Clin Nutr* 1999;70:983–91.

KEY WORDS Modified milk fat, milk fat, triacylglycerols, margarine, plasma lipoproteins, men

INTRODUCTION

The consumption of butter and full-fat dairy products has gradually declined in the past 2 decades because of public awareness that dairy fat is rich in saturated fat, which has been shown to significantly elevate total, LDL-, and HDL-cholesterol and apolipoprotein B and A concentrations (1–3) compared with either polyunsaturated vegetable oils or soft margarines. Dietary cholesterol and 2 of the principal saturated fatty acids in butterfat, myristic and palmitic acids, have been identified as major dietary factors that raise total- and LDL-cholesterol and apolipoprotein B concentrations (3, 4). Elevated concentrations of plasma LDL cholesterol and apolipoprotein B have long been associated with increased risk of cardiovascular disease.

Because consumers have become more health conscious, they tend to choose products containing less saturated fats and cholesterol. To meet consumer demands, low-fat dairy products have been produced and are now available in the marketplace. Another strategy that has been proposed to reduce the plasma cholesterol-raising potential of milk fat involves the modification of milk-fat composition either via the feeding regimen of the cows (5, 6) or via fractionation technologies (7, 8). The fractionation process of milk fat is used widely by the international dairy industry to improve physical and functional properties, such as the melting point and crystallization behavior (7, 9). The removal of cholesterol naturally occurring in milk fat, which is one of the factors contributing to the elevation of LDL cholesterol, may also be achieved by physical fractionation processes (7) and could be nutritionally desirable (10). Modification of milk-fat composition by fractionation could result in milk-fat fractions with favorable technical and nutritional qualities, and this option appears to hold promise. It is essential that the possible beneficial effects of such modified milk fats on lipid metabolism be nutritionally evaluated.

The nutritional effect of milk fat, modified by fractionation processes, on the human plasma lipid and lipoprotein responses has not yet been reported. The objective of this study was to test the effect of this modified milk fat on human plasma lipid and

[1] From the Département des Sciences des Aliments et de Nutrition, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Canada, and the Centre de Recherche sur les Maladies Lipidiques, Centre Hospitalier de l'Université Laval, Canada.

[2] Supported by a grant from the Dairy Farmers of Canada.

[3] Address reprint requests to H Jacques, Département des Sciences des Aliments et de Nutrition, Faculté des Sciences de l'Agriculture et de l'Alimentation, Pavillon Paul-Comtois, Université Laval, Québec, Canada, G1K 7P4. E-mail: helene.jacques@aln.ulaval.ca.

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TABLE 1
Physical characteristics and lipid profile of participants[1]

	Value
Age (y)	22.4 ± 0.9
Body weight (kg)	74.6 ± 1.6
Height (cm)	176.3 ± 1.4
BMI (kg/m^2)	24.0 ± 0.5
Plasma lipids	
Total cholesterol (mmol/L)	4.11 ± 0.12
LDL cholesterol (mmol/L)	2.45 ± 0.10
HDL cholesterol (mmol/L)	1.27 ± 0.04
Total:HDL cholesterol	3.31 ± 0.14
Total triacylglycerols (mmol/L)	0.89 ± 0.07

[1] $\bar{x}$ ± SEM; n = 21.

lipoprotein concentrations. The effects of this modified milk fat were compared with those of regular milk fat and nonhydrogenated margarine on plasma cholesterol, triacylglycerols, and lipoproteins in normolipidemic men; regular milk fat and nonhydrogenated soft margarine served as reference fats. Normolipidemic men representing a large proportion of the general population were selected as subjects for this study.

SUBJECTS AND METHODS

Subjects

Twenty-one free-living, normolipidemic men, students at Laval University, were enrolled in the study. They were initially screened on the basis of a complete physical examination and medical history. Exclusion criteria included dyslipoproteinemia, use of medication that could affect lipid metabolism, a weight change >10% of usual body weight within the 6 mo preceding the experiment, and chronic, metabolic, or acute disease or major surgery within the 3 mo preceding the study. All participants were in good general health, exercised regularly (muscular training, jogging, cycling, or swimming: 17 participants, 1–5 h/wk; 4 participants, 10 h/wk), took no medication regularly, and were nonsmokers. The consumption of alcoholic beverages before the study was as follows: 7 participants, 0–4 drinks/mo; 10 participants, 1–3 drinks/wk; and 4 participants, 5 drinks/wk. Inclusion criteria included availability, reliability, and regular meal patterns. Physical characteristics and lipid profiles of the participants at screening are presented in **Table 1**. Body mass indexes (BMIs; in kg/m^2) ranged from 19 to 25 in 17 subjects and from 26 to 31 in 4 subjects. For 2 of these latter subjects, the large BMI was the result of larger muscle mass and for the 2 others it was the result of larger fat mass. According to data from the third National Health and Nutrition Examination Survey, individual plasma total and LDL-cholesterol values were distributed between the 5th and the 75th percentiles of the population for the corresponding age group, whereas HDL-cholesterol values were between the 15th and 100th percentiles (11). According to the Lipid Research Clinics Program Prevalence Study, individual plasma triacylglycerol values were distributed between the 10th and 90th percentiles (12). The experimental protocol was fully explained to the participants, who gave their informed consent. The protocol was approved by the Clinical Research Ethical Committee of Laval University.

Experimental fats: cholesterol and solid-fat contents and fatty acid composition

Modified milk fat was produced by fractionation technology (13, 14). The regular milk fat used in this study was from the same lot used to produce the modified milk fat. The nonhydrogenated soft margarine (Becel; Thomas J Lipton, Toronto) was a blend of 64% canola oil, 28% linola oil, and 8% modified palm and palm kernel oils and contained no *trans* fatty acids. Linola oil is linseed oil that has been genetically improved to contain a higher essential fatty acid content; its main constituent fatty acid is linoleic acid. The mean cholesterol and fatty acid contents of the experimental fats are presented in **Table 2.** The free cholesterol content of experimental fats was measured by gas chromatography (14). The fatty acid composition of experimental fats was determined by capillary gas chromatography as described previously (15). The solid-fat content profile at various temperatures (**Figure 1**) was determined from the melting profile of the fats by differential scanning calorimetry with a model 990 Thermal Analyzer (Dupont Co, Wilmington, DE) by the method of Timms (16).

Study design

A balanced crossover design for 3 experimental periods (17) was used to compare the effects of modified milk fat on the plasma lipoprotein profile with those of regular milk fat and nonhydrogenated margarine. Before each experimental period, the participants were asked to follow a diet similar to their usual diet

TABLE 2
Mean cholesterol and fatty acid contents of the experimental milk fats

	Regular milk fat	Modified milk fat	Nonhydrogenated margarine
Cholesterol			
(% by wt/milk fat)	0.41	0.03	0.00
(mg/1g milk fat)	4.15	0.31	0.00
Fatty acids (% by wt of total fatty acids)			
Saturated short-chain			
Butyric (4:0)	4.2	3.4	0.0
Caproic (6:0)	2.4	2.0	0.0
Caprylic (8:0)	1.4	1.2	0.1
Total	8.0	6.6	0.1
Saturated medium-chain			
Capric (10:0)	3.1	2.9	0.1
Lauric (12:0)	3.5	3.4	1.6
Tridecanoic (13:0)	1.3	1.2	0.0
Total	7.9	7.5	1.7
Saturated long-chain			
Myristic (14:0)	11.1	10.9	0.9
Pentadecanoic (15:0)	1.2	1.2	0.0
Palmitic (16:0)	30.4	30.4	9.0
Margaric (17:0)	0.6	0.6	0.1
Stearic (18:0)	12.5	12.7	2.7
Arachidic (20:0)	0.2	0.2	0.4
Total	56.0	56.0	13.1
Unsaturated long-chain			
Myristoleic (14:1)	1.1	1.1	0.0
Palmitoleic (16:1)	1.5	1.6	0.2
Oleic (18:1)	22.6	24.1	39.1
Linoleic (18:2)	2.4	2.5	36.5
Linolenic (18:3)	0.7	0.7	9.4
Total	28.3	30.0	85.2

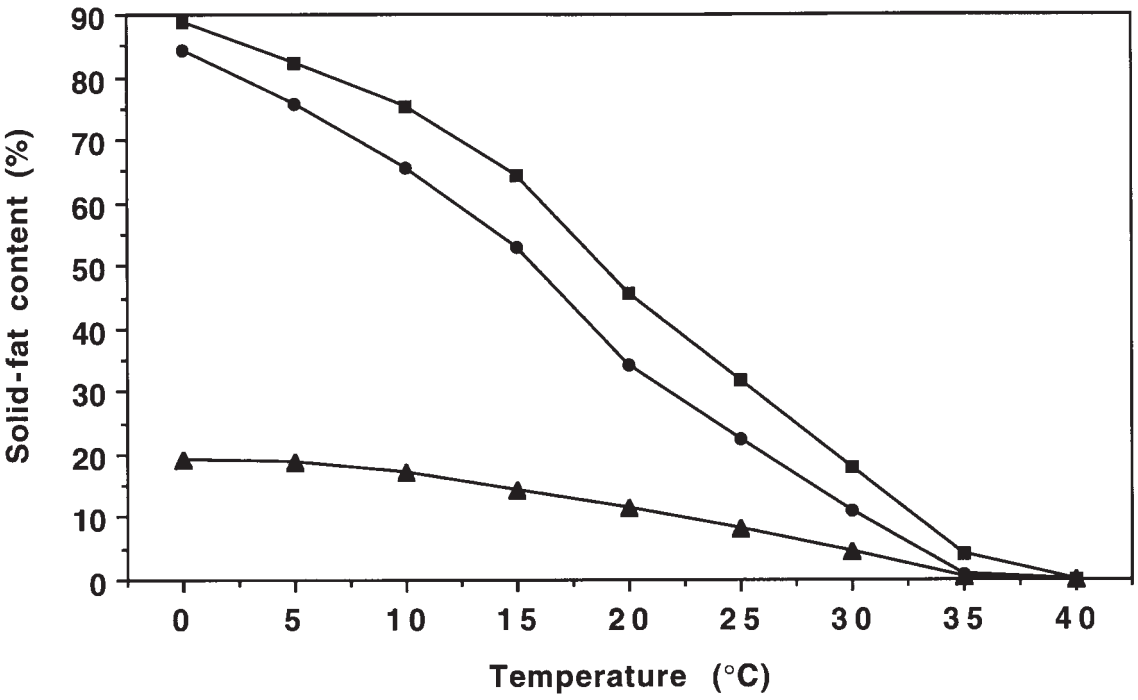

FIGURE 1. Solid-fat content of the test fats according to the temperature: regular milk fat (●), modified milk fat (■), and nonhydrogenated margarine (▲).

for 2 wk. Then, each subject randomly rotated through three 4-wk experimental periods, alternately consuming the 3 experimental diets. Each subject was thus his own control. At the end of each experimental period, the subjects resumed their usual diets for 6-wk washout periods to remove the residual effects of the preceding experimental diet on the tested variables. Because the second washout period included Christmas holidays, the chosen length for the washout periods was 6 wk instead of 4 wk to remove the residual effects of holiday meals in addition to those of experimental diets on plasma lipids. Subjects were blinded to dietary assignments and were not informed of their lipid responses until the study was completed. Principal investigators and staff supervising the laboratory analyses were also blinded to dietary assignments. Throughout the study, participants were asked to maintain their usual lifestyle, including mealtime patterns, work schedules, and usual patterns of physical activity. Alcohol consumption was forbidden 2 wk before and during each experimental period.

Diets

Participants who were selected on the basis of the inclusion and exclusion criteria were asked to record their food intake for 3 consecutive days (including 2 weekdays and 1 weekend day), with the advice of a qualified dietitian, to evaluate individual energy and nutrient intakes. Seven-day rotating menus were developed for each experimental diet according to preference and usual meal patterns to ensure compliance. The Canadian Nutrient File database was used to calculate the nutritional composition of experimental diets and food records (18).

TABLE 3
Nutrient composition of the preexperimental and experimental diets[1]

	Preexperimental diet	Experimental diets		
		Regular milk fat	Modified milk fat	Nonhydrogenated margarine
Energy (kJ)	13 343 ± 732[2]	13 292 ± 716	13 316 ± 720	13 328 ± 702
Protein (% of energy)	17	16	16	16
Carbohydrate (% of energy)	52	51	51	51
Lipids (% of energy)	32	34	34	33
Polyunsaturated fatty acids (g)	18 ± 1	16 ± 1	16 ± 1	36 ± 2[3,4]
Monounsaturated fatty acids (g)	43 ± 2	47 ± 3	48 ± 3	48 ± 3
Saturated fatty acids (g)	39 ± 3	52 ± 3	51 ± 3	27 ± 1[3,4]
P:M:S[5]	0.5:1.1:1	0.3:0.9:1	0.3:0.9:1	1.3:1.8:1[3,4]
Cholesterol (mg)	388 ± 30	428 ± 21	254 ± 11[3]	248 ± 11[3]
Total fiber (g)	19 ± 2	21 ± 1	21 ± 1	21 ± 1

[1] $n = 21$.
[2] $\bar{x}$ ± SEM.
[3] Significantly different from the regular milk-fat diet, $P < 0.01$.
[4] Significantly different from the modified milk-fat diet, $P < 0.01$.
[5] Ratio of polyunsaturated to monounsaturated to saturated fatty acids.

The experimental diets were designed to have an identical food composition, except for the test fat, which was either regular milk fat, modified milk fat, or nonhydrogenated margarine. The nutrient compositions of the preexperimental and experimental diets are shown in **Table 3**. All experimental fats represented 13% of total energy, or 37% of total fat. The fats were used in a variety of recipes but were incorporated in larger quantities in desserts such as cakes, cookies, and breads. Regular and modified milk fats were melted slowly and used in the form of oil in the recipes.

Protein sources included lean beef (4 meals/wk), poultry without skin (4 meals/wk), trimmed pork (2 meals/wk), lean ham (2 meals/wk), eggs (1 meal/wk), and salmon (1 meal/wk). Among milk products, only skim milk and nonfat yogurt were used, to avoid introducing other important milk fat sources in experimental diets. Other milk products were not permitted.

Diets were designed to provide the Canadian daily recommended allowances of all essential nutrients (19). A sample 1-d menu of the nonhydrogenated-margarine diet, with 3 different energy levels, is presented in **Table 4**. Ten different energy levels (8815–22 065 kJ) were formulated for each experimental diet. Participants started the study at the energy level closest to their usual intake and were moved from one level to another if their weight fluctuated by >1 kg. Body weight was monitored every 2 d before lunch, and variables such as clothing, exercise levels, and the consumption of breakfast, snacks, and beverages were taken into account. Variations in body weight did not exceed 2.5 kg within each dietary period. The largest fluctuations in body weight were mostly related to corporal water losses after intense exercise, such as football or basketball games, but the weight was restored on the following day.

Participants followed a preapproved list to prepare their breakfast and snacks at home. Lunch and dinner were prepared and served by 2 professional dietitians and 2 dietary technicians in our Human Nutrition Research Laboratory. All food items were weighed or measured before being used in recipes. As described in Table 4, different quantities of food were served to participants according to their energy requirements. Weekend meals were prepared on Thursdays and distributed on Friday afternoons. No other food items were permitted during the experimental periods. Dietary compliance was evaluated every 2 d by means of a verbal questionnaire, and participants were frequently encouraged to strictly follow the experimental diets. Subjects were asked to report any illness, use of medications, and deviations from the diets. No side effects of the experimental diets were reported and few deviations from the experimental diets were noted because dietary intake was strictly monitored. Five participants reported a cold or headache and took acetaminophen-containing pills, which are known to not affect lipid metabolism.

TABLE 4
Sample 1-d menu for 3 energy levels of the nonhydrogenated margarine diet[1]

	Energy		
Food item	12 200 kJ	14 800 kJ	17 575 kJ
		g	
Breakfast			
Whole-wheat bread	56	56	84
Peanut butter	11	11	22
Strawberry jam	13	13	26
Skim milk	259	259	259
Lunch			
Carrot soup	264 [5.2][2]	264 [5.2]	396 [8.4]
Turkey sandwich			
White bread	50	50	100
Sliced turkey	60	80	100
Nonhydrogenated margarine	11	14	16
Cucumber slices	34	34	34
Celery sticks	33	33	33
Chocolate-chip cookies	48 [20]	48 [20]	48 [20]
Stirred-fruit nonfat yogurt	0	175	175
Dinner			
Spaghetti noodles	178	178	178
Meat and vegetable sauce	447 [10.6]	668 [15.8]	668 [15.8]
Lean beef	125	190	190
Romaine lettuce	59	59	59
Caesar dressing	16	16	16
White bread bun	0	28	28
Nonhydrogenated margarine	0	9	9
Date squares	115 [15.8]	115 [15.8]	173 [24.3]
Snacks			
Skim milk	195	195	259
Orange	0	131	131

[1] Quantities of milk fat were adjusted on the basis of the water content (%) of the nonhydrogenated margarine to reach the same proportion of experimental fat in each of the 3 diets. Items represent those on the second day of the 7-d rotating menu.
[2] The amount of experimental fat in the recipes is indicated in brackets.

Blood analysis

Blood was obtained by venipuncture at the beginning and end of each experimental period after a 12-h overnight fast. It was collected into tubes containing EDTA and was centrifuged immediately at 4°C for 10 min at 1500 × *g* to obtain plasma samples, which were then stored at 4°C and analyzed within 5 d. Lipoprotein fractions (VLDL, LDL, and HDL) were separated by a combination of ultracentrifugation (256 000 × *g* for 10 h at 11°C) and heparin-manganese precipitation (20, 21). HDL_2 and HDL_3 were separated by dextran-sulfate precipitation (22). Lipoproteins were assayed for their cholesterol and triacylglycerol contents with enzymatic methods (model RA-500; Technicon Autoanalyzer (Tarrytown, NY). LDL apolipoprotein B and HDL apolipoprotein A-I were determined by rocket immunoelectrophoresis (23). Quality control of the ultracentrifugation procedures was ensured by certification for traceability of the National Reference System for Cholesterol and by continuous participation in clinical laboratory certification programs for ultracentrifugation and apolipoprotein and lipid measurements with the Canadian Reference Laboratory Ltd. Precision of all components of ultracentrifugation was <2.0%, whereas accuracy, estimated by total error, was <5.0%. The CVs of the instruments used were <1%.

Statistical analysis

The data were analyzed with SAS software (Statistical Analysis System Institute, Cary, NC) and the results are expressed as means ± SEMs. The nutrient composition of the experimental diets was compared by using Student's *t* test. Analysis of variance with adjustment for crossover designs with >2 periods (17), using the general linear model followed by Duncan's new multiple-range test (24), was performed to identify differences between experimental treatments. Analysis of variance was also performed separately in subjects with higher (10 h/wk) and lower (1–5 h/wk) physical activity levels. Because plasma lipids, lipoproteins, and apolipoproteins responded similarly in both groups, the data of all participants were pooled and analyzed as a whole. Moreover, no

significant effects of experimental period or sequence were observed and no residual effects of the first experimental period over the second experimental period or of the second experimental period over the third experimental period were seen for any lipid variable. Therefore, the data for experimental periods, sequence, and dietary treatments were pooled. The data of only 20 participants consuming the modified milk-fat and nonhydrogenated-margarine diets were used because a snow storm prevented one participant following each of these diets to be present for blood withdrawal. Pearson's correlation coefficients were performed between BMI and lipid variables.

RESULTS

Free cholesterol content, fatty acid composition, and solid-fat content of the experimental fats

The modified milk fat contained 93% less cholesterol than the regular milk fat (Table 2). The removal of cholesterol was the major modification in lipid content of milk fat, with lesser changes made in fatty acids to obtain modified milk fat. Contents of saturated short- and medium-chain fatty acids were 18% and 5% lower in modified milk fat than in regular milk fat, respectively. The proportions of unsaturated long-chain fatty acids, mainly of oleic and linoleic acids, were 7% and 5% higher in modified milk fat than in regular milk fat, respectively. The proportion of saturated long-chain fatty acids remained unchanged. Contents of saturated short-, medium-, and long-chain fatty acids were lower and the content of unsaturated long-chain fatty acids was higher in nonhydrogenated margarine than in both the regular and modified milk fats. The nonhydrogenated margarine contained no cholesterol.

The solid-fat content profile of modified milk fat was higher than that of regular milk fat and nonhydrogenated margarine in the temperature range of 0 to 40°C (Figure 1). For example, at room temperature (20°C), modified milk fat contained 46% solids, regular milk fat contained 34% solids, and nonhydrogenated margarine contained 11% solids. At 35°C, regular milk fat and nonhydrogenated margarine were completely liquid, whereas modified milk fat was partially liquid with 4% of fat still solid.

Body weight and BMI

Mean body weight and BMI did not change significantly during the experimental periods, indicating that body weight had no significant effect on the lipid profile. Mean initial body weights were 75.5 ± 1.7, 75.2 ± 1.5, and 75.9 ± 1.5 kg with the regular milk-fat, modified milk-fat, and nonhydrogenated-margarine diets, respectively. These values changed slightly, but not significantly, to 75.4 ± 1.6, 75.3 ± 1.5, and 75.6 ± 1.5 kg, respectively, after 4 wk of the experimental diets. BMIs were 24.3 ± 0.6, 24.2 ± 0.5, and 24.4 ± 0.5 with the regular milk-fat, modified milk-fat, and nonhydrogenated-margarine diets, respectively. These values were the same or nearly the same after 4 wk of the experimental diets: 24.3 ± 0.6, 24.2 ± 0.5, and 24.3 ± 0.5, respectively. Furthermore, BMIs were not correlated with total triacylglycerols (n = 21; r = 0.08, P = 0.72) or any of the other lipid variables. Therefore, BMI did not influence blood lipid, lipoprotein, or apolipoprotein concentrations significantly.

Plasma total and lipoprotein-cholesterol concentrations

The effects of the experimental diets on the cholesterol profile are shown in **Table 5**. When compared with initial plasma concentrations, modified milk fat did not change total nor LDL plasma

TABLE 5
Plasma cholesterol and lipoprotein-cholesterol concentrations before and after the experimental diets[1]

	Regular milk fat (n = 21)	Modified milk fat (n = 20)	Nonhydrogenated margarine (n = 20)
Total cholesterol (mmol/L)			
Before	3.95 ± 0.12	4.08 ± 0.15	4.04 ± 0.10
After	4.01 ± 0.13	3.93 ± 0.12	3.56 ± 0.12[2–4]
VLDL cholesterol (mmol/L)			
Before	0.24 ± 0.03	0.28 ± 0.04	0.23 ± 0.03
After	0.22 ± 0.03	0.17 ± 0.02[5,6]	0.19 ± 0.03[7]
LDL cholesterol (mmol/L)			
Before	2.56 ± 0.11	2.65 ± 0.12	2.64 ± 0.10
After	2.74 ± 0.12	2.67 ± 0.11	2.33 ± 0.11[2,3,8]
HDL cholesterol (mmol/L)			
Before	1.15 ± 0.04	1.15 ± 0.05	1.17 ± 0.06
After	1.06 ± 0.05[6]	1.09 ± 0.05[9]	1.03 ± 0.04[8]
HDL_2 cholesterol			
Before	0.36 ± 0.03	0.35 ± 0.03	0.35 ± 0.03
After	0.31 ± 0.04	0.34 ± 0.04	0.29 ± 0.03[7,9]
HDL_3 cholesterol (mmol/L)			
Before	0.79 ± 0.03	0.80 ± 0.02	0.82 ± 0.03
After	0.74 ± 0.02[6]	0.75 ± 0.02[8]	0.75 ± 0.02[9]
Total:HDL cholesterol			
Before	3.52 ± 0.16	3.65 ± 0.19	3.61 ± 0.20
After	3.94 ± 0.21[9]	3.76 ± 0.21	3.52 ± 0.15[2]

[1] $\bar{x}$ ± SEM.
[2,5] Significantly different from the regular milk-fat diet: [2] $P < 0.01$, [5] $P < 0.05$.
[3,7] Significantly different from the modified milk-fat diet: [3] $P < 0.01$, [7] $P < 0.05$.
[4,6,8,9] Significantly different from initial (before) concentrations: [4] $P < 0.0001$, [6] $P < 0.01$, [8] $P < 0.001$, [9] $P < 0.05$.

TABLE 6
Mean plasma total and lipoprotein-triacylglycerol concentrations before and after the experimental diets[1]

	Regular milk fat (n = 21)	Modified milk fat (n = 20)	Nonhydrogenated margarine (n = 20)
		mmol/L	
Total triacylglycerols			
Before	0.92 ± 0.07	0.97 ± 0.10	0.86 ± 0.07
After	0.89 ± 0.07[2]	0.78 ± 0.06[3]	0.86 ± 0.07[2]
VLDL triacylglycerols			
Before	0.52 ± 0.07	0.55 ± 0.08	0.45 ± 0.06
After	0.51 ± 0.06[2]	0.40 ± 0.06[3]	0.49 ± 0.06[2]
LDL triacylglycerols			
Before	0.19 ± 0.01	0.21 ± 0.02	0.20 ± 0.01
After	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
HDL triacylglycerols			
Before	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01
After	0.17 ± 0.01[3]	0.17 ± 0.01[3]	0.17 ± 0.01[3]

[1] $\bar{x}$ ± SEM.
[2] Significantly different from the modified milk-fat diet, $P < 0.05$.
[3] Significantly different from initial (before) concentrations, $P < 0.01$.

cholesterol significantly, whereas nonhydrogenated margarine did reduce both these concentrations significantly (by 12%). As a result of these variations, total and LDL plasma cholesterol were significantly higher after the modified milk-fat and regular milk-fat diets than after the nonhydrogenated-margarine diet. However, modified milk fat significantly lowered VLDL-cholesterol concentrations whereas regular milk fat and margarine did not. All 3 experimental fats reduced HDL and HDL_3 cholesterol significantly from initial values. However, plasma HDL_2 cholesterol was significantly higher after the modified milk-fat diet than after the nonhydrogenated-margarine diet, mainly because of a 17% reduction in HDL_2 cholesterol with the nonhydrogenated-margarine diet compared with a 3% reduction with the modified milk-fat diet. The ratio of total to HDL cholesterol was significantly higher with regular milk fat, which increased it by 12% from initial concentrations compared with nonhydrogenated margarine.

Plasma total and lipoprotein-triacylglycerol and apolipoprotein concentrations

Men ingesting modified milk fat had significantly lower concentrations of both total- and VLDL triacylglycerols than did those ingesting regular milk fat and nonhydrogenated margarine, after reductions of 20% and 27% from initial concentrations, respectively (**Table 6**). The reducing effect of modified milk fat on triacylglycerol concentrations was homogeneous when individual responses were compared (**Figure 2**). Indeed, of the 21 subjects who consumed the modified milk-fat diet, triacylglycerols decreased in 17, rose in 3, and stayed the same in 1. Participants with the highest total triacylglycerol concentrations (> 1.2 mmol/L) at the beginning of the study responded with the larger reductions (25–40%). Initial HDL-triacylglycerol concentrations were significantly lowered by all experimental diets, but these variations were not significantly different when experimental diets were compared.

The effects of the experimental diets on apolipoprotein concentrations are shown in **Table 7**. Although nonhydrogenated margarine significantly reduced initial total- and LDL-apolipoprotein B concentrations and modified milk fat significantly reduced initial VLDL-apolipoprotein B and increased the ratio of apolipoprotein B to apolipoprotein A-I, there were no significant differences in these variables between the 3 experimental diets. Initial HDL-apolipoprotein A-I concentrations were also significantly reduced by the 3 experimental diets but no significant differences were observed when the experimental diets were compared. Finally, there was no significant effect of diet on the ratios of triacylglycerols and cholesterol to apolipoproteins or on the ratios of triacylglycerols to cholesterol, indicators of lipoprotein composition in VLDL, LDL, and HDL particles (data not shown).

DISCUSSION

This study was designed to examine the effects of a milk fat, the cholesterol content of which was reduced by fractionation technology, compared with those of regular milk fat and nonhydrogenated margarine on the lipid profile in humans. To ensure that the observed effects were truly the result of the experimental fats, variables that can also affect lipid metabolism—such as physical activity, smoking, and dietary intake—were strictly controlled for in the experiment. Body weight did not change significantly and was not significantly different among dietary groups, and physical activity remained stable during the experimental periods. Moreover, participants were asked to consume a preexperimental diet closely similar to their usual diet to avoid unusual excess fat or carbohydrate consumption and to abstain from alcohol consumption 2 wk before each experimental period. The most important feature of this study was that the content of all dietary constituents, including that of simple and complex carbohydrates (and, therefore, the glycemic index), were identical in all 3 experimental diets, except for the main source of fat, which was either regular milk fat, modified milk fat, or nonhydrogenated margarine. Therefore, the effects of experimental diets on lipid variables can be largely attributed to experimental fats.

Modified milk fat was obtained by a fractionation process that involves short-path distillation under high vacuum to reduce essentially the free cholesterol content, followed by melt crystallization (7). The fractionation process separated cholesterol and triacylglycerol molecules on the basis of molecular weight

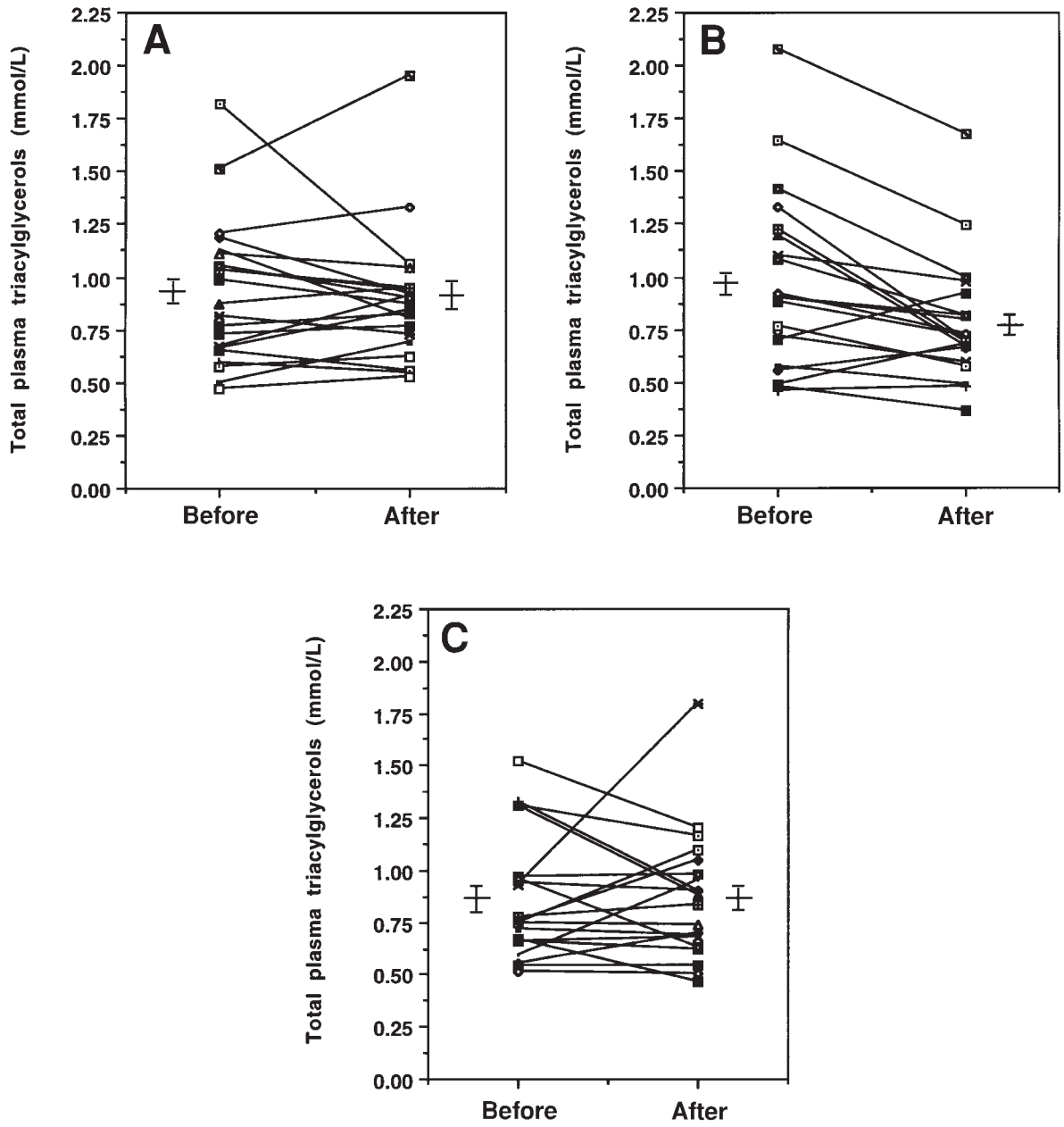

FIGURE 2. Individual total plasma triacylglycerol concentrations before and after 4 wk of the regular milk-fat (A), modified milk-fat (B), and nonhydrogenated-margarine (C) diets. After the modified milk-fat diet, total triacylglycerol concentrations were significantly lower than both initial concentrations ($P < 0.01$) and concentrations after the regular milk-fat and nonhydrogenated-margarine ($P < 0.05$) diets. Mean (±SEM) values are presented before and after each experimental diet. $n = 20–21$ subjects.

only and involved no change in pH conditions and, thus, no change in the position of milk-fat fatty acids on the glycerol molecule. Cholesterol removal (93%) also entails removal of a small proportion of short-chain (18%) and medium-chain (5%) fatty acids, which results in an alteration of the physical properties of milk fat, especially its melting behavior. As a consequence, modified milk fat was more solid than was regular milk fat and nonhydrogenated margarine at various temperatures <40 °C.

The consumption of modified milk fat altered the plasma lipid response of normolipidemic men. Subjects consuming modified milk fat had significantly lower total triacylglycerol, VLDL triacylglycerol, and VLDL cholesterol concentrations than those consuming either regular milk fat or nonhydrogenated margarine. These results agree well with those of Labat et al (25), who observed significantly lower concentrations of plasma triacylglycerols in postmenopausal women consuming an animal fat in which cholesterol content was reduced by distillation technology than in women consuming either a linoleic acid–rich safflower oil, cholesterol-reduced animal fat, or an unmodified animal fat. It might be suggested that the low cholesterol content of the modified milk fat may have been responsible for the significantly lower concentrations of plasma total- and VLDL-triacyl-

TABLE 7
Apolipoprotein concentrations before and after the experimental diets[1]

	Regular milk fat (n = 21)	Modified milk fat (n = 20)	Nonhydrogenated margarine (n = 20)
Apolipoprotein B (g/L)			
Total			
Before	0.75 ± 0.03	0.75 ± 0.03	0.74 ± 0.03
After	0.76 ± 0.03	0.75 ± 0.03	0.69 ± 0.02[2]
VLDL			
Before	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
After	0.07 ± 0.00	0.07 ± 0.01[3]	0.07 ± 0.00
LDL			
Before	0.68 ± 0.03	0.66 ± 0.03	0.66 ± 0.02
After	0.69 ± 0.03	0.67 ± 0.03	0.62 ± 0.02[3]
Apolipoprotein A-I (g/L)			
HDL			
Before	1.17 ± 0.03	1.17 ± 0.03	1.15 ± 0.03
After	1.11 ± 0.02[3]	1.09 ± 0.03[2]	1.08 ± 0.02[2]
Apolipoprotein B:A-I			
Before	0.65 ± 0.03	0.64 ± 0.02	0.65 ± 0.03
After	0.69 ± 0.03	0.69 ± 0.03[2]	0.64 ± 0.02

[1] $\bar{x}$ ± SEM.
[2,3] Significantly different from initial (before) concentrations: [2] $P < 0.01$, [3] $P < 0.05$.

glycerol concentrations, as compared with regular milk fat, by improving the efficiency of hepatic B/E receptors and hence increasing the cellular uptake of triacylglycerol-rich lipoproteins. Dubois et al (26) showed a higher plasma clearance of triacylglycerol-rich lipoproteins (chylomicrons) after a meal low in cholesterol than after a meal rich in cholesterol. However, it is difficult to attribute the lower plasma triacylglycerol concentrations obtained with modified milk fat to a reduction in its cholesterol content because these effects were not observed with the nonhydrogenated-margarine diet, which had a similar cholesterol content.

It is likely that the reduction in plasma total and VLDL triacylglycerols with modified milk fat may have been the result of the greater diminution of intestinal fat absorption with this fat than with either regular milk fat or nonhydrogenated margarine. Preliminary measurements made on 13 of our subjects in the present study showed a 25% smaller increase in plasma triacylglycerol concentrations 3 h postprandially with modified milk fat than with regular milk fat and nonhydrogenated margarine (K Thibault, N Bergeron, H Jacques et al, unpublished observations, 1997), suggesting a reduction in intestinal fat absorption (27). Stearic acid, which has been reported to be absorbed less than other saturated long-chain fatty acids (28, 29), may have played a role in lowering plasma triacylglycerols in subjects fed modified milk fat. However, in the present study, the stearic acid content in regular and modified milk fats was similar and therefore could not explain the differences in plasma triacylglycerol concentrations between these 2 milk fats. On the other hand, the higher solid-fat content (Figure 1) and the undercooled state (liquid crystalline phase) of the melting triacylglycerols of modified milk fat at body temperature could have induced a higher viscosity of the fat emulsion, resulting in lower intestinal fat absorption. According to Small (30), fats containing triacylglycerols in a solid state are less digested at body temperature than are those containing triacylglycerols in liquid form only. It is noteworthy that the estimated amount of modified milk fat not being absorbed (4%) would be insufficient to induce large increases in the proportions of absorbed carbohydrates and proteins, leading to increases in plasma triacylglycerol concentrations. Nevertheless, a complete study of the postprandial lipid responses to this modified milk fat will verify this assumption. Notably, reductions in plasma triacylglycerol were observed in a group of normolipidemic young men in the current study. Because participants having the highest plasma triacylglycerol concentrations appear to be more responsive to modified milk fat, there is thus a possibility that greater reductions would be observed in subjects with hypertriacylglycerolemia.

Neither modified nor regular milk fat adversely affected total or LDL cholesterol in normolipidemic men. Indeed, when modified and regular milk fats were consumed, total and LDL-cholesterol concentrations remained unchanged. In contrast and as expected, nonhydrogenated margarine did reduce the concentrations of these lipids. The fact that regular and modified milk fats did not significantly reduce total and LDL-cholesterol concentrations, whereas margarine did, can be explained by their lower ratio of polyunsaturated to saturated fats (0.3:1) compared with that of the margarine diet (1.3:1) (2, 31). The modified milk fat maintained HDL_2-cholesterol concentrations whereas nonhydrogenated margarine diminished them. This can be explained by high quantities of n−6 polyunsaturated fatty acids in margarine, which have been shown to reduce HDL cholesterol in addition to LDL cholesterol (31). Moreover, there was no inverse relation between VLDL triacylglycerols and HDL_2 cholesterol (n = 20; r = 0.35, P = 0.12) in subjects fed the various diets. Thus, the lower VLDL-triacylglycerol concentrations with modified milk fat are more likely attributable to lower fat absorption than to higher VLDL-triacylglycerol hydrolysis by lipoprotein lipase, which is associated with higher plasma HDL_2-cholesterol concentrations (32). This hypothesis should nevertheless be examined in future studies.

In conclusion, an average 20% reduction in triacylglycerol concentrations with modified milk fat, in addition to other improvements in lipids such as the maintenance of HDL_2-cholesterol concentrations, may be viewed as favorable in preventing the onset of hypertriacylglycerolemia. Moreover, because

these results were obtained from a group of normolipidemic young men whose plasma lipids are usually less responsive to dietary alterations than are those of subjects with elevated plasma triacylglycerols, this study warrants further research to determine whether larger reductions in triacylglycerols would be observed in individuals with hypertriacylglycerolemia. Finally, to further understand the underlying physiologic action of modified milk fat, our future animal and human studies will focus on the effects of this milk fat on intestinal fat absorption, triacylglycerol production, and plasma postheparin lipoprotein lipase activity.

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