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Does a diet high in corn oil lower LDL cholesterol levels in women via an effect on LDL receptor activity?

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To determine if a high intake of corn oil alters LDL receptor activity, a model emphasizing freshly isolated mononuclear cells (MNC) was used with 12 women consuming 40 energy% fat diets based on corn oil or butter in a randomized crossover design. Each phase included 1 week of a prescribed but selfselected high saturated fat (SFA) diet at home followed by 4 weeks of a designated fat-based diet consumed in a metabolic kitchen. There was a 7-week washout period between phases. LDL degradation through and binding to LDL receptors were 63 and 100% higher, respectively, on the corn oil diet than the butter diet ($P < 0.05$). Changes in LDL receptor activity and serum LDL cholesterol concentration were moderately associated ($r = -0.4$ to -0.6). Compared with the butter group, total unsaturated and saturated fatty acid contents of MNC were 166% higher and 150% lower, respectively, in the corn oil group. Changes in saturated and unsaturated fatty acids and receptor-mediated LDL degradation were associated ($r = -0.6$ and 0.6, respectively). These findings suggest that the change in fatty acid composition in MNC may enhance the LDL receptor–mediated pathway. Receptor-mediated LDL degradation may account for only part of the effect of corn oil on serum LDL cholesterol concentrations in healthy women. (J. Nutr. Biochem. 6:88–96, 1995.)

Keywords: LDL receptor; butter; corn oil; mononuclear cells; low density lipoprotein

Introduction

Epidemiologic and experimental studies have linked diets rich in unsaturated fat to reduction of serum low density lipoprotein (LDL) cholesterol concentrations.[1,2] The molecular mechanisms by which unsaturated fats lower LDL cholesterol concentrations are multifactorial but predominantly involve decreased de novo synthesis and increased LDL degradation.[3,4] LDL, taken up by cells through the LDL receptors on the surface of plasma membranes, is degraded, and LDL receptors are recycled into the membranes.[5] This pathway of LDL uptake, called the receptor-mediated pathway, may account for the catabolism of over 60% of LDL depending on species and concentration in plasma.[4,6,7] The clearance of LDL depends on the receptor activity, which is influenced at least in part by the fatty acid composition of the cell membrane.[8]

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LDL receptors have been demonstrated in liver and extrahepatic tissues including skin fibroblasts and blood mononuclear cells (MNCs).[8–11] Persons heterozygous for familial hypercholesterolemia have greatly elevated serum cholesterol levels and experience premature atherogenesis.[11] Freshly isolated MNCs from familial hypercholesterolemia heterozygotes have over 50% reduced receptor activity and those from homozygotes have no receptor activity. Circulating MNCs have been employed to assess the effect of lipid lowering drugs on LDL receptor activity in humans,[11,12] in which more invasive procedures are usually not possible, but they have apparently not been used to study the potentially more subtle effects of fat modification except in animal models.[13,14] MNC isolated from cebus monkeys fed diets with 31 energy percent (en%) fat degraded ^{125}I-LDL five times more rapidly when the habitual diet provided corn oil rather than coconut oil.[8] In similar studies in which radioiodinated LDL was infused, a coconut oil as opposed to a corn oil diet reduced receptor-mediated LDL catabolism by the whole body by 50% with the liver probably accounting for 90% of the receptor activity.[13] Thus, receptor activity of MNCs gives an indication of re-

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ceptor activity in the liver. The effects of dietary polyunsaturated fat (PUFA) compared with SFA on LDL receptor activity have not been studied in healthy women using freshly isolated MNCs. This was the unique aspect of the present study.

The purpose of this research was (1) to determine if a high intake of corn oil compared with the consumption of a diet high in butter increases LDL degradation through LDL receptors and binding to the receptors in a model employing freshly isolated MNC from women, (2) to determine if the intake changes the fatty acid composition of circulating MNC isolated from the female subjects and if fatty acid composition and receptor activity are correlated. In addition the influence of diets high in PUFA and SFA on nonreceptor mediated catabolism of LDL was also determined.

Methods and materials

Research design

This study had a two-phase randomized crossover design with an 8-week wash-out period. Prior to each period subjects self-selected a prescribed diet (a high SFA diet, P:S = 0.4) for a week based on food exchanges in an attempt to establish a common baseline similar to the saturated (butter) experimental diet. Recorded consumption during the week of the prescribed diet, as analyzed by Food Processor II (ESHA Research, Salem, OR USA), is shown in *Table 1*. Twelve healthy females were randomly assigned for 4 weeks to diets providing 40 energy (en)% as fat with corn oil or butter furnishing 54% of the fat. The diets were all prepared in a metabolic kitchen. After the first study period, subjects consumed a free-choice diet for a 7-week wash-out period. Then each group was provided the alternate diet for 4 weeks. Blood (25 ml) was drawn into vacutainer tubes from all subjects after an overnight fast (12 hr) at 0 and 4 weeks during each experimental diet period. Blood was sampled from individual subjects at the same stage of the menstrual cycle.

Subjects

After approval from the Human Subjects Biomedical Review Committee of The Ohio State University, subjects were recruited from among OSU female students and faculty. Volunteers were screened on the basis of information from a health questionnaire and a physical examination. Subjects were 11 healthy premenopausal female students and one postmenopausal faculty, with a mean blood cholesterol concentration of 4.95 ± 0.15 mmol/L and ages ranging from 20 to 55 years. The subject sample consisted of 5 Caucasians and 7 Asians. Mean weight of the subjects was 59.5 ± 5.2 kg.

Initially subjects were randomized into groups on the basis of serum total cholesterol concentration established at screening so that the mean values of each group would be similar. However, two subjects from the same group dropped out of the study during the standardization period and were replaced by two subjects with lower cholesterol levels. Subjects had no known history of metabolic disease and had not taken any medication including oral contraceptives. All 12 subjects entering the first experimental period completed the study.

Diets

Subjects consumed 8.1 MJ/day, on average, in a menu plan that rotated every 4 days. All diets were adjusted individually to provide an appropriate energy content for maintaining a constant body weight within ±1.8% for each subject during the study. They were weighed daily. Subjects requiring more food energy to maintain a stable weight were given extra sandwiches, cookies, and cakes because these foods had a protein-carbohydrate-fat ratio similar to the overall diet. Serving sizes were reduced for subjects needing less food energy. Both diets provided 45.0 ± 0.2 en% of carbohydrate, 15.0 ± 0.2 en% of protein, 40.0 ± 0.1 en% of fat, and 200 mg of cholesterol (*Table 1*). The fatty acid composition of the diets was verified by analysis with gas chromatography (*Table 2*). The lipid composition of the diets was altered by changing the type of lipid used in food preparation. The corn oil and butter were used primarily in cooking or were incorporated into food products. Salad dressing, spread, cookies, and cakes served as vehicles to incorporate lipid into the diet.

Alcohol consumption was limited to two servings per week and restricted for 5 days before blood drawing. The intakes were below the threshold (2 to 4 servings per day) for influencing serum HDL-cholesterol concentrations.[15] Smoking was prohibited.

Food was provided for the subjects as three meals per day and was prepared in our metabolic kitchen. On weekdays subjects consumed breakfast and dinner at the kitchen and were given a sack lunch. On weekends they consumed dinner on Saturday at the kitchen and were given a carry out breakfast and lunch for Saturday and all meals for Sunday.

Table 1 Composition of diets

	Prescribed diet	Butter diet	Corn oil diet
Energy (MJ)	6.8 ± 0.4*	8.1 ± 0.2	8.1 ± 0.2
Carbohydrate (en%)†	51.2 ± 2.0	45.0 ± 0.2	45.0 ± 0.1
Protein (en%)	15.0 ± 1.3	15.0 ± 0.2	15.0 ± 0.2
Fat (en%)	33.4 ± 1.4	40.0 ± 0.1	40.0 ± 0.1
Saturated fatty acids (en%)	13.7 ± 1.6	23.2 ± 0.1	8.0 ± 0.0
Monounsaturated fatty acid (en%)	8.5 ± 0.5	13 ± 0.1	12.2 ± 0.1
Polyunsaturated fatty acid (en%)	4.1 ± 0.5	3.8 ± 0.0	20.0 ± 0.0
Cholesterol (mg)	210 ± 50	258 ± 8	258 ± 10
P:S ratio	0.3 ± 0.1	0.2 ± 0.0	2.5 ± 0.0

*Mean ± SEM; n = 12.
†En% refers to the percentage of diet energy.

Table 2 Mean fatty acid content (weight % of total methyl esters) of five rotating daily menus for the corn oil and butter groups as analyzed by GLC

Fatty Acids*	Corn oil group	Butter group
12:0	trace	2.8 ± 0.1†
14:0	0.6 ± 0.1	9.2 ± 0.2
16:0	13.9 ± 0.4	31.1 ± 0.2
18:0	5.1 ± 0.4	15.2 ± 0.2
18:1	27.6 ± 0.6	28.2 ± 0.2
18:2	48.1 ± 1.5	7.7 ± 0.5
18:3	2.1 ± 0.2	1.2 ± 0.1

*Fatty acid nomenclature: the first number indicates carbon length of fatty acids, and the next number refers to the number of double bonds in the fatty acids.
†Mean ± SEM.

Sample size

The power test was done to determine the number of subjects needed to detect a 10% mean difference in serum total cholesterol concentrations between women consuming a high corn oil diet and a high butter diet. Seven to 8% of the mean was the standard deviation in LDL receptor activities and lipoprotein concentrations obtained in previous studies involving women. It was estimated that 12 subjects for each group were needed for $\alpha = 0.05$ (power = 0.95) using Power Program computer software.

Plasma lipids and apolipoproteins

Serum was separated by centrifugation at 500 *g* for 20 min for total cholesterol, HDL cholesterol, and triglyceride determinations and frozen at −80°C until analysis. The concentration of total cholesterol and triglyceride was measured by an enzymatic method using kits (Sigma Chemical Company, St. Louis, MO USA; procedure #352-50 and GPO, respectively). The concentration of HDL cholesterol was assayed by the same enzymatic procedure as total cholesterol, after removal of VLDL and LDL cholesterol by a precipitation reagent composed of 10 g/L of dextran sulfate and 0.5 mol/L of Mg ions with buffer (Sigma Chemical Company, St. Louis, MO USA; procedure #352-2).[16] The concentration of LDL in mg/dL was calculated by the Friedewald equation[17] and then converted to mmol/L.

Serum apolipoproteins A-1 and B-100 were measured by a radial immunodiffusion (RID) method using RID plates from TAGO (Burlingame, CA USA). Diameters, which represented the concentrations of apoprotein B-100 and apoprotein A-1, were measured, and apoprotein concentrations were calculated using standard curves based on the square of the diameters.

LDL isolation

Pooled plasma (cholesterol levels = 4.7–5.5 mmol/L) was isolated into 0.1% disodium ethylenediaminetetraacetate (EDTA) from volunteers who were not subjects. Whenever needed the same volunteers were used throughout the study. For separation of lipoproteins, the pooled plasma density was adjusted to 1.210 g/mL with solid potassium bromide (KBr) and centrifuged for 24 hr at 65,000 rpm at 10°C in a L7-65 Beckman ultracentrifuge (75 Ti rotor) (Beckman Instruments, Inc., Fullerton, CA USA). The isolated lipoprotein solution was adjusted to a density of 1.25 g/ml with solid potassium bromide, which was overlayered with an equal volume of density 1.10 g/mL of KBr solution and water. The layered solution was centrifuged at 10°C for 24 hr at 35,000 rpm in a Beckman SW 41 rotor. LDL was pooled from the fraction with a density of 1.019–1.062 g/ml.[18] LDL was dialyzed overnight with phosphate buffered saline, pH 7.4, and confirmed to be free of other lipoproteins by SDS–PAGE.[19] The total protein concentration of LDL was determined by the method of Lowry.[20] LDL was radioiodinated by the monochloride method of McFarland,[21] and the percentage of radioactive labeling of the LDL (40 to 50%) produced a LDL with a specific activity of 100–600 cpm/ng of protein.

Mononuclear cell preparation

Twenty-five milliliters of citrated whole blood was diluted by addition of an equal volume of sterilized 0.9% sodium chloride (NaCl). MNC were isolated from the diluted blood overlayered with Nycoprep™ (Life Technologies, Inc., Gaithersburg, MD USA) by centrifugation at 800 *g* for 20 min at room temperature in a JS 7.5 swing-out rotor (Beckman Instruments, Inc., Fullteron, CA USA).[22] The cells harvested from Nycoprep-MNC meniscus were washed twice with Hank's buffer solution, pH 7.4, by centrifugation at 400 *g* for 10 min. Viable cells were counted with Trypan blue exclusion under a microscope, and cell viability was usually greater than 95%.[7,8] Mononuclear cell samples prepared in this fashion were comprised of 72% lymphocytes, 22% monocytes, and 4% neutrophills.[7,8,22]

LDL degradation and binding

The amount of ^{125}I-LDL degraded by MNC was determined according to the following method. An aliquot of freshly isolated MNC in Hank's buffer was incubated with ^{125}I-LDL (20 μg/ml) in the presence or absence of a 20 fold excess of unlabeled LDL at 37°C for 5 hr. Several studies showed that LDL receptors on MNC were saturated at LDL concentrations of about 20 μg of protein/ml in in vitro systems similar to the one used in this study.[13,14] The saturation concentration can be slightly changed by dietary fat modifications. In our pilot study of the validity of the methodology for LDL receptor activity using males and pre- and postmenopausal women, binding was curvilinear with respect to ^{125}I-LDL concentration, but 10, 20, and 40 μg of ^{125}I-LDL/mL did not result in saturation. However, since the slope of binding was decreased after 20 μg of ^{125}I-LDL/mL and differences in binding among subjects believed to have different binding kinetics were demonstrated at 10 μg/mL of ^{125}I-LDL, 20 μg of ^{125}I-LDL appeared to be a reasonable concentration to use. When 20 μg/mL of ^{125}I-LDL were incubated for different periods of time with MNC, degradation was curvilinear as incubation time was increased. Expected differences among subjects were noted at 3, 4, and 5 hr of incubation, so any of these incubation times was apparently adequate to determine differences among treatments. When LDL was incubated with different concentrations of MNC, specific degradation of LDL linearly increased in a range of 2 to 11 × 10^5 cells. This range was used in each assay in this study.

After incubation, the samples were treated with 20% trichloroacetic acid to precipitate the undegraded, protein-bound iodo-

Table 3 Percent change in serum lipid and apoprotein concentrations between prestudy and end of period

	1st period		2nd period	
	Corn oil (n = 6)	Butter (n = 6)	Corn oil (n = 6)	Butter (n = 6)
Total cholesterol	−10.7 ± 4.2*	8.9 ± 4.4†	−5.6 ± 3.2	9.7 ± 2.9†
HDL cholesterol	−8.1 ± 5.9	−3.8 ± 4.4	−5.2 ± 3.9	4.3 ± 5.8
LDL cholesterol	−13.0 ± 4.8	19.0 ± 4.5†	−6.3 ± 5.6	17.9 ± 3.4†
Apoprotein A-1	10.4 ± 7.5	9.0 ± 4.7	2.2 ± 0.5	8.3 ± 1.3
Apoprotein B-100	−7.0 ± 1.8	25.4 ± 6.8†	−8.2 ± 3.3	11.3 ± 9.4†

*Mean ± SEM percent changes in lipoprotein concentration.
†Significantly different from corn oil diet ($P < 0.05$), according to paired t-tests.

tyrosine. The precipitate was sedimented by centrifugation at 14,000 *g* for 3 min. The supernatant was counted to determine the amount of LDL degradation in a gamma counter. Degradation values were expressed as specific degradation, the difference in degradation in the presence and absence of a 20 fold excess of unlabeled LDL. Specific degradation was expressed as nanograms of ^{125}I-LDL protein degraded/10^6 cells/1 hr. The specific binding activity of LDL receptors was determined by incubation at 4°C with ^{125}I-LDL (20 μg/ml) in the presence or absence of excess unlabeled LDL for 30 min. All reagents were cooled to 4°C before use. After incubation cells were washed with RPMI and collected by centrifugation at 450 *g* for 15 min at 4°C. The supernatant was removed by pipette, and the cell pellets, isolated by amputating the tip of the microfuge tube, were dissolved in 1 ml of 0.1 mol/L of sodium hydroxide (NaOH). The radioactivity of the pellet was counted by gamma counter. Bound counts were expressed as nanograms of LDL protein bound per 10^6 cells.

Fatty acid analysis of MNC

After being washed twice in saline, MNCs separated from blood were extracted with hexane-isopropanol (3:2, vol/vol).[23] The fatty acyl groups from the top layer were transesterified with 10% methanolic-HCl at 90°C for 3 hr. The methylated fatty acids were extracted into hexane and neutralized with 6% potassium carbonate (K_2CO_3). The layer containing methyl esters was separated by centrifugation at 1,500 rpm for 10 min and dried with sodium sulfate. Separation and identification of the fatty acid methyl esters was performed on a Hewlett-Packard 5890 gas-chromatograph fitted with automatic sampler 7673 A, integrator 3392 A, and FID detector. A SP-2340 fused silica capillary column (0.32 mm × 3 mm) purchased from Supelco, Inc. (Bellefonte, PA USA) was used. Temperature was programmed from 90 to 180°C at 4°C/min. Each fatty acid was identified using standards from Nu Chek Prep, Inc. (Elysian, MN USA).

Statistical analysis

Prior to statistical analysis the homogeneity of response of the subjects was checked since the subjects were not homogeneous with regard to age and race (SAS, SAS Institute, Cary, NC USA). Age and race did not influence the dietary fat effect. Mean differences in the various lipid parameters between the beginning and end of each period were compared for each diet using two sample *t*-tests. Differences between the corn oil and butter diets were analyzed with one-way analysis of variance (ANOVA) for crossover designs.[24] In this design possible fixed sources of variation include the overall mean, differential carryover, period, and diet effects. If crossover effects had been present, data from period 2 could not be used for statistical analysis. However there were no differential carryover effects and period effects, so data from both periods were combined for further statistical analysis.

Pearson correlation coefficients between specific binding and specific degradation and serum lipid levels were calculated using the combined data set to determine what associations existed among LDL receptor activity and serum lipid levels. Relationships between specific binding and specific degradation and the percentages of fatty acids in MNC were determined by the same procedures. The level of significant probability was set at $\alpha = 0.05$.

Results

Dietary effects on serum lipids and apoproteins

Compared with prestudy values, the concentrations of serum total cholesterol, LDL cholesterol, and apoprotein B-100 fell when subjects consumed corn oil in period 1 and 2 (*Table 3* and *Figures 1* and *2*) although the changes were not statistically significant. However, there was a significant dietary fat effect in each period: compared with the butter diet the change produced by the corn oil diet (a lowering effect) was significantly different for serum total cholesterol, LDL cholesterol, and apolipoprotein B-100 in both periods. The 95% confidence interval for the difference in total cholesterol, LDL cholesterol, and apolipoprotein B-100 between the effect of the corn oil versus the butter diet was (−1.08, −0.48) mmol/L ($P = 0.0002$), (−0.98, −0.42) mmol/L ($P = 0.0003$) and (−0.22, −0.08) g/L ($P = 0.0005$), respectively. Mean serum HDL cholesterol concentrations and apolipoprotein A1 concentrations did not change significantly on either diet (*Table 3* and *Figures 1* and *2*).

Degradation of LDL cholesterol

The effect of the corn oil and butter diets on LDL degradation through the LDL receptors of MNC is shown in *Table 4*. Compared with the butter diet the corn oil diet resulted in 36 to 98% higher specific LDL degradation in periods 1 and 2 ($P > 0.05$). Specific degradation was significantly increased on the corn oil diet compared with the butter diet ($P = 0.019$) when data from both periods were combined. The 95% confidence interval for the difference between the effect of the corn oil versus the butter diet was 17.9 ± 14.6 ng/10^6 cells/hr of incubation. The dietary treatments had no significant effect on nonspecific degradation (*Figure 3*).

Binding of LDL cholesterol

Compared with butter the corn oil diet resulted in 100 to 104% higher specific LDL binding by MNC in period 1 and 2 ($P < 0.05$) (*Table 5*). Mean specific LDL binding was 77% lower than the mean prestudy value in the butter diet group at the end of period 1 ($P = 0.09$). When data from both periods were combined, the mean specific LDL binding was significantly higher on corn oil ($P = 0.01$). The

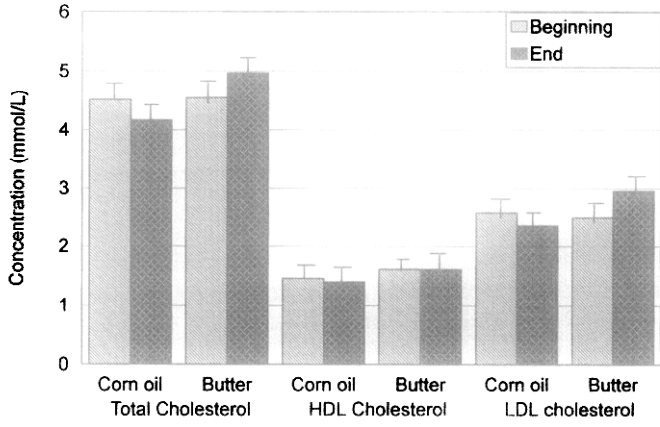

Figure 1 Serum lipoprotein concentrations at the beginning and end of the experimental period. Data are combined for the two periods. Error bars are for SEM.

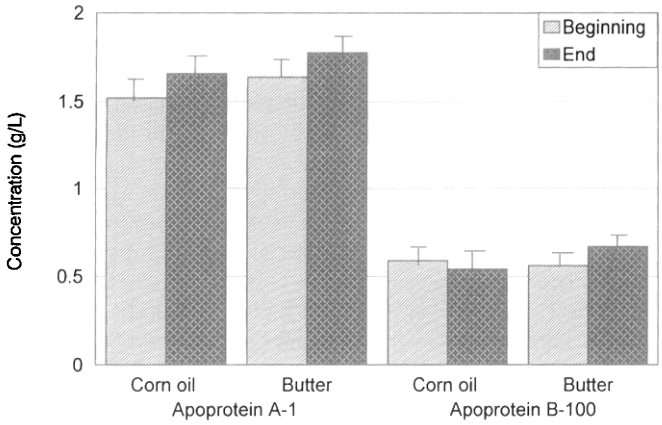

Figure 2 Serum apolipoprotein concentrations at the beginning and end of the experimental period. Data are combined for the two periods. Error bars are for SEM.

95% confidence interval for the difference between the effect of the corn oil versus the butter diet was 5.7 ± 4.0 ng/10^6 cells. There was no significant difference between the corn oil group and the butter group in receptor independent binding by MNC (*Figure 4*).

Fatty acid content of MNC

Individual fatty acid content in MNC was not significantly different between the butter group and the corn oil group. However, there was a trend for every saturated fatty acid in MNC to increase on the butter diet and decrease on the corn oil diet. Percent changes in some of the fatty acids approached significance ($P < 0.2$) in combined data for period 1 and period 2. The 95% confidence intervals for the mean differences between corn oil and butter for the percent changes in palmitic acid, linoleic acid, linolenic acid, arachidic acid, and docosahexaenoic acid were −6.5 ± 8.4, 11.9 ± 13.8, 5.4 ± 5.3, −3.2 ± 4.3, and 0.7 ± 0.9%, respectively.

Percent changes in the sum of fatty acid content between the prestudy and the end of period 1 and period 2 are shown in *Table 6*. In the first period, total saturated fatty acids decreased ($P = 0.02$), and total unsaturated fatty acids increased ($P = 0.02$) on the corn oil diet. When subjects consumed the butter diet, changes tended to proceed in the opposite direction. The mean changes in proportion of total saturated fatty acids and total unsaturated fatty acids on the corn oil diet were different from those on the butter diet ($P = 0.05$) in period 1. Although the pattern of change was similar in period 2, there were no differences between groups. When data were combined, the 95% confidence interval for the mean change in percent total saturated fatty acid content in MNC on corn oil diets compared with butter diets was (−32.4, −7.0) % ($P = 0.01$); the mean change in percent total saturated fatty acid was estimated to be anywhere from 7 to 32.4% greater on the butter diet compared with the corn oil diet. The 95% confidence interval for the mean change in percent total polyunsaturated fatty acid content in MNC for the corn oil diet compared with the butter diet was (4.1, 39.7) % ($P = 0.02$). Proportions of total monounsaturated fatty acids did not vary on either diet.

Correlation between serum lipids and LDL receptor activity

Changes in specific degradation of LDL were negatively correlated with changes in serum LDL cholesterol concentration ($r = -0.40$) and serum apoprotein B-100 concentration ($r = -0.46$). There was also a negative association between changes in specific binding of LDL and the changes in LDL cholesterol ($r = -0.60$) and apoprotein B-100 concentrations ($r = -0.50$), although the correlation between LDL degradation and serum lipids was slightly lower than that between LDL-specific binding and lipids. Other serum lipids such as HDL cholesterol and apoprotein A-1 levels were not associated with LDL-specific binding and degradation.

Correlation between fatty acids and LDL receptor activity

There was a significant correlation between changes in some fatty acids and LDL-specific degradation in combined data from period 1 and period 2. Changes in palmitic acid ($r = -0.51$), arachidic acid ($r = -0.54$), and total saturated fatty acids ($r = -0.61$) were negatively correlated with changes in LDL-specific degradation while changes in linolenic acid ($r = 0.52$), docosahexaenoic acid ($r = 0.41$), total polyunsaturated fatty acids ($r = 0.49$), and total unsaturated fatty acids ($r = 0.61$) were positively correlated. These results indicated that LDL degradation through LDL receptors in MNC was increased when unsaturated fatty acids, including linolenic acid and docosahexaenoic acid, increased in MNC and decreased when saturated fatty acids including palmitic acid and arachidic acid increased. Changes in LDL binding to LDL receptors were not significantly correlated with changes in saturated fatty acids in MNC.

Discussion

Few studies of LDL receptor activity in dietary fat modification have been performed on healthy humans, in part because the methodology frequently employed in animal models requires use of invasive procedures or radioisotopes. In this study we used an easily obtained, LDL receptor–bearing tissue, MNC, to compare the effect of a polyunsaturated (corn oil) and a saturated (butter) diet on LDL receptor activity of healthy women.

Use of MNC to assess LDL receptor activity

Several studies have justified the use of MNC to assess LDL receptor activity in vivo.[11,12,25,26] These studies compared LDL receptor activity from freshly isolated MNC between familial hypercholesterolemia (FH) heterozygotes and normal individuals, some of whom were treated with cholesterol lowering drugs.[11,12,25,26] A study of a large family with a pattern of inheritance of the FH gene through five generations showed that the mean plasma LDL concentra-

Table 4 Specific degradation of LDL cholesterol by freshly isolated MNC (ng/10^6cell/hr)

Values for each period	1st period		2nd period	
Diet	Prestudy	End	Prestudy	End
Corn oil (n = 6)	26.6 ± 8.7*	43.7 ± 8.1	27.2 ± 6.2	48.9 ± 10.0
Butter (n = 6)	30.9 ± 6.7	32.0 ± 4.9	28.7 ± 4.9	24.6 ± 7.5

Combined data from period 1 and period 2 Diet	Prestudy	End	Difference†
Corn oil (n = 12)	26.9 ± 6.7	46.3 ± 9.6	19.4 ± 7.0
Butter (n = 12)	29.8 ± 5.8	28.3 ± 11.7‡	−1.5 ± 5.7§

*Mean ± SEM.
†End of study minus prestudy value.
‡Significantly different from corn oil diet ($P < 0.05$) according to analysis of variance for crossover design.
§Significantly different from corn oil diet ($P < 0.02$) according to analysis of variance for crossover design.

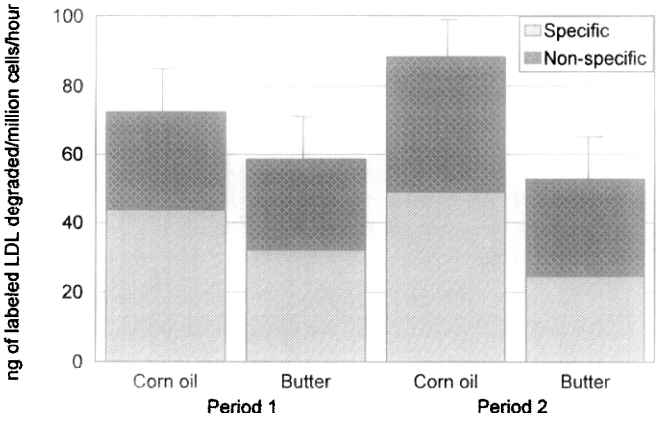

Figure 3 Total degradation (specific plus nonspecific) of LDL cholesterol by freshly isolated MNC at the end of each period. Error bars are SEM for total degradation.

tion in the FH heterozygotes was elevated about 2 fold. There was a significant difference between the mean values for LDL degradation in freshly isolated MNC from the normal and FH heterozygotes in this family.[25]

In another study with hypercholesterolemic patients, LDL receptor defects in lymphocytes were identified.[26] When lymphocytes were cultured in lipoprotein-depleted medium and endogeneous sterol biosynthesis was suppressed with mevinolin, a small concentration of exogeneous LDL cholesterol gave a maximum response in normal lymphocytes but even higher concentrations (5 to 12 times) of exogeneous LDL were unable to cause an LDL receptor response in lymphocytes from patients with homozygous FH. The response of lymphocytes from patients with heterozygous FH was intermediate between that of homozygotes and normal or hyperlipidemic controls.[12] These studies showed that LDL receptors in MNC reflect the in vivo receptor activity.

In this study, dietary fat modification did not affect the LDL receptor–independent pathway of LDL degradation, which accounted for about 37 to 58% of total mean in vitro LDL degradation. The finding of increased receptor-mediated LDL degradation with increased consumption of PUFA agreed with results of animal studies.[3,13,14,27] In cebus monkeys, specific LDL degradation in vitro in MNC was enhanced 5 fold in animals with long-term consumption of corn oil in comparison to primates fed coconut oil.[8,13] In our short-term study in humans, specific degradation of LDL by MNC was less than 2 fold higher when corn oil was fed instead of butter.

In the whole body kinetic study performed by Nicolosi et al.[10] using cebus monkeys the correlation between plasma cholesterol concentrations and LDL catabolized by receptor-mediated pathways was stronger ($r = -0.732$) than our calculated relationship ($r = -0.39$) for the association between changes in specific degradation and serum cholesterol. This result is logical since Nicolosi et al.[13] used another species and studied whole body kinetics in vivo while we studied in vitro kinetics in MNC, a tissue not importantly associated with whole body cholesterol catabolism. Nevertheless, our results support the usefulness of the MNC model to predict, at least qualitatively, the effect of diet on rates of whole body cholesterol catabolism. Specific binding was more affected than specific degradation by the dietary fat manipulations since differences between dietary fats were seen in both periods for specific binding whereas a significant dietary fat effect was seen for specific degradation only when data for the two periods were combined. The rate of LDL-specific degradation is a function of recycling rates of LDL receptors as well as the number of receptors. These processes are related to such cellular conditions as cholesterol levels, conformation of membrane, and membrane fluidity. Accumulation of cholesterol in cells decreases the recycling rate of LDL receptors (down-regulation).[3,28]

The most important findings of this study were that corn oil feeding enhanced LDL binding to and degradation through the LDL receptor of MNC and that enhanced activity was associated with an increase in unsaturated fatty acids in the MNC. However, according to the strength of the calculated correlation coefficients between the lipid parameters, the change in LDL receptor activity accounted for

Table 5 Specific binding of LDL cholesterol by freshly isolated MNC (ng/10^6 cell/hr)

Values for each period	1st period		2nd period	
Diet	Prestudy	End	Prestudy	End
Corn oil (n = 6)	5.8 ± 1.8*	10.8 ± 2.2	7.0 ± 1.4	14.4 ± 1.6
Butter (n = 6)	7.5 ± 3.6	5.3 ± 0.6†	6.1 ± 1.8	7.2 ± 2.2†

Combined data from period 1 and period 2 Diet	Prestudy	End	Difference‡
Corn oil (n = 12)	6.4 ± 1.0	12.6 ± 1.2	6.2 ± 1.1
Butter (n = 12)	6.8 ± 1.3	6.3 ± 1.0§	−0.5 ± 0.8‖

*Mean ± SEM.
†Significantly different from corn oil ($P < 0.05$) according to two sample t-test.
‡End of study minus prestudy value.
§Significantly different from corn oil diet ($P < 0.03$) according to analysis of variance for crossover design.
‖Significantly different from corn oil diet ($P < 0.01$) according to analysis of variance for crossover design.

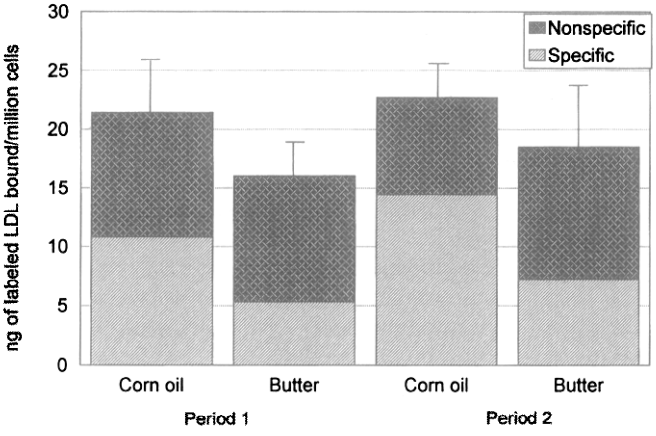

Figure 4 Total binding (specific plus nonspecific) of LDL cholesterol by freshly isolated MNC at the end of each period. Error bars are SEM for total binding.

only part of the change in serum LDL cholesterol concentrations while the change in the fatty acid composition of the MNC was associated with only part of change in receptor activity.

Fatty acids and serum lipid levels

Butter fat is high in myristic acid (10 to 12% of total fatty acids) and palmitic acid (20 to 30% of total fatty acids).[29] The butter diet in this study provided about 3.6 en% as myristic acid compared with approximately 1.3 en% in a normal diet and about 3.6 en% as linoleic acid.[30] Corn oil contains no myristic acid; palmitic and linoleic acids contribute 11 and 50%, respectively, of the total fatty acids.[29] The corn oil diet provided about 19.2 en% as linoleic acid. PUFA in the corn oil diet and SFA in the butter diet in our study were given at twice the amount recommended by various national health organizations in order to maximize the effect of dietary fat type. The ratio of linoleic to myristic acid was 83 in the corn oil diet and 0.83 in the butter diet. These ratios are below and above, respectively, the threshold associated with fat-induced changes in plasma cholesterol.[31] Vega et al. reported that PUFA decreased mean plasma total cholesterol and apoprotein B by 25% and LDL cholesterol decreased by 26% when 8 men and 2 women were given a diet high in SFA for 4 weeks and then fed a diet high in PUFA for 4 weeks.[32] Our study showed a similar result to Vega's research; PUFA diets decreased total cholesterol, LDL cholesterol, and apoprotein B-100 by 19.3, 26.6, and 23%, respectively. Hayes and colleagues[14,31,33] suggested that a linoleic acid intake below 4 en%, a cholesterol sensitivity, and a higher level of dietary cholesterol (>400 mg/day) are needed to see a hypercholesterolmic effect of palmitic acid. In this study, dietary cholesterol was <300 mg/day. These results were also consistent with previous studies conducted with men in our laboratory by Wardlaw et al.[34,35] and with many previous studies in men and women performed by Grundy et al.[32,36,37] HDL cholesterol concentration decreased a little more on the corn oil diet than on the butter diet in this study, but differences were not significant. Apoprotein A-1, the apolipoprotein associated with HDL, was not affected by diet during either period. The effect of PUFA diets on serum HDL cholesterol and apolipoprotein concentrations is not consistent. Grundy et al.[32,36,37] have suggested that blood HDL cholesterol concentrations were not always changed by dietary fat modification, but Fernandez and McNamara[27] noted that high PUFA diets decreased blood HDL cholesterol in guinea pigs.

Diet and LDL-receptor activity

On high PUFA diets, cell membranes are highly disordered and more fluid.[8,28] Changes in the LDL receptor activity are associated with modifying the lipid composition of cell membranes.[8,13] The incorporation of fatty acids into cell membranes occurs rapidly when monocytes are incubated with unsaturated fatty acids in vitro. The amount of cholesterol in cell membranes can influence membrane fluidity and cholesterol increases the rigidity of the cell membrane.

In this research, total polyunsaturated fatty acids in the MNC were significantly higher and total saturated fatty ac-

Table 6 Mean fatty acid content of MNC (weight % of total methyl esters).

Fatty acid	Prestudy		End	
	Corn oil	Butter	Corn oil	Butter
1st period				
Sum of saturated fatty acids	45.2 ± 3.6*	41.8 ± 5.4	34.0 ± 1.2	50.1 ± 3.0†
Sum of monounsaturated fatty acids	32.5 ± 2.9	26.1 ± 2.5	30.3 ± 2.8	26.6 ± 2.1
Sum of polyunsaturated fatty acids	22.3 ± 3.6	32.0 ± 6.7	35.6 ± 3.4	23.3 ± 3.5†
Sum of unsaturated fatty acids	54.8 ± 3.6	58.1 ± 5.4	65.9 ± 1.2	49.9 ± 3.0†
P:S ratio	0.5 ± 0.1	0.6 ± 0.3	1.1 ± 0.1	0.5 ± 0.1
U:S ratio	1.3 ± 0.2	1.6 ± 0.4	2.0 ± 0.1	1.0 ± 0.1
2nd period				
Sum of saturated fatty acids	39.8 ± 6.3	27.6 ± 4.7	35.4 ± 4.9	43.1 ± 9.5
Sum of monounsaturated fatty acids	23.5 ± 2.6	29.4 ± 5.4	22.1 ± 2.4	29.9 ± 3.3
Sum of polyunsaturated fatty acids	36.8 ± 4.6	42.9 ± 9.1	42.5 ± 6.7	27.0 ± 7.2
Sum of unsaturated fatty acids	60.2 ± 6.3	72.4 ± 4.7	64.6 ± 4.9	56.9 ± 6.4
P:S ratio	1.1 ± 0.2	2.0 ± 0.6	1.4 ± 0.3	0.8 ± 0.3
U:S ratio	1.7 ± 0.3	3.1 ± 0.6	2.1 ± 0.4	1.6 ± 0.4

*Mean ± SEM.
†Significantly different from corn oil diet ($P < 0.03$) according to analysis of variance for crossover design.

ids lower on the high corn oil diet than the butter diet. Thus, high PUFA diets could make the cell membranes more disorderly and more fluid than high SFA diets. Since linoleic acid, as an essential fatty acid, is of food origin, changes in linoleic acid composition represent the dietary fat effect. The amount of linoleic acid in the cells was about 24% higher on the corn oil diet than the butter diet. However, the individual fatty acids including linoleic acids in MNC did not differ significantly between high PUFA and high SFA diets. Even in cebus monkeys fed high corn oil or coconut oil for long periods, individual fatty acid composition of cell membranes was not significantly different.[8] Thus, the length of the feeding period may not be a significant factor associated with change in individual fatty acid composition of cells.

As mentioned previously, there was a significant positive correlation between unsaturated fatty acid content in MNC and LDL degradation through LDL receptors, and also between unsaturated fatty acid content and LDL binding to the receptors. Increased polyunsaturated fatty acids in cell membranes may increase membrane fluidity which may increase binding affinity and/or recycling rates. However, other unidentified factors, possibly including changes in levels of LDL receptor mRNA, may account for part of the response of LDL receptor activity to dietary fat modifications. In hamsters, changes in receptor-mediated LDL uptake by liver occur in parallel with alteration in LDL receptor protein and mRNA levels.[38,39]

Effect of race and menopause status

The sample population, consisting of 5 Caucasians and 7 Asians, was not homogeneous. In the crossover design, one group had 2 Caucasians and 4 Asians, and the other had 3 Caucasians and 3 Asians. No statistically significant effect of race was found in a complete block design on any variable measured in this study including those related to LDL receptor activity. Even though no block effect was present in this study, Caucasians seemed to have larger changes than Asians in some lipid parameters on the high corn oil diet. In a previous study[40] lipid metabolism of Caucasian women was changed more by a PUFA diet than was that of Chinese. More research is needed to compare the effect of dietary fat on lipoprotein metabolism as well as on alteration of LDL degradation in different races.

Since menopause status could influence the LDL receptor activity, the data were statistically analyzed with and without the data of a postmenopausal subject. There was no significant difference between the two analyses, even though her LDL cholesterol level was on the high side among subjects and her LDL receptor activity was on the low side.

In conclusion, the precise mechanism for the regulation of LDL cholesterol concentration in the blood remains unclear. Our data indicate that high PUFA diets can decrease blood LDL cholesterol in part through enhanced LDL receptor activity, and the LDL receptor-mediated pathway could be increased in part by changes in fatty acid composition. However, changes in LDL receptor activity only explained part of the variance in diet-induced changes in serum concentrations of LDL. In this study we also demonstrated that circulating MNC could be used to detect the effect of dietary fat on LDL receptor activity in humans.

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