

# Relative Effects of High Saturated Fatty Acid Levels in Meat, Dairy Products, and Tropical Oils on Serum Lipoproteins and Low-Density Lipoprotein Degradation by Mononuclear Cells in Healthy Males

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To determine the effects of three saturated fatty acid combinations on lipoprotein metabolism, we fed 18 21- to 32-year-old men three diets in a crossover design for 28-day periods separated by washout periods of 4 to 6 weeks. The men self-selected a prescribed diet at home emphasizing saturated fat as the visible fat for 1 week. Then, they ate experimental diets providing 40%, 15%, 17%, and 7% of food energy, respectively, as total, saturated, monounsaturated, and polyunsaturated fatty acids, levels representing amounts available in the US diet. Different test fatty acid combinations, given at 4 to 6 energy% (en%) each, were incorporated into food products: 12:0 + 14:0, 14:0 + 16:0, and 16:0 + 18:0. Test fatty acids were equalized by giving free myristic acid (14:0) with palm kernel oil or butter and sheanut butter (high in 18:0) with lard. The diet highest in 12:0 + 14:0 also provided 4.2 en% 16:0, the most common saturated fatty acid in the US diet. Mean apparent absorption of all fatty acids was at least 90%. The three diets produced similar concentrations of serum total and low-density lipoprotein (LDL) cholesterol and apolipoprotein (apo) B-100 regardless of the apo E phenotype of the subjects. Compared with baseline, the experimental diets affected serum high-density lipoprotein (HDL) concentrations ( $P < .06$ ), with the highest values occurring on diet 12:0 + 14:0. When the change from baseline in receptor-mediated degradation of  $^{125}\text{I}$ -LDL in freshly isolated mononuclear cells (MNC) was stratified by apo E phenotype, diet 16:0 + 18:0 produced a 30% increase, compared with a 9% decrease on diet 12:0 + 14:0 and a 6% increase on diet 14:0 + 16:0 in subjects with the apo E3/3 phenotype. These results suggested that different saturated fatty acid combinations, consumed at levels typical of availability in the United States and with diets providing ample unsaturated fat, had similar cholesterolemic properties in healthy males despite some subtly different effects on lipoprotein metabolism.

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**C**URRENT NUTRITION guidelines advocate reduction of saturated fatty acids in the diet. Yet individual saturated fatty acids may not have identical effects. Hegsted et al.<sup>1</sup> using multiple regression statistical techniques with data compiled in studies involving 36 experimental diets, predicted the most hypercholesterolemic saturated fatty acid to be 14:0 (myristic acid), followed by 16:0 (palmitic acid) and then 12:0 (lauric acid). Stearic acid (18:0) had a neutral effect on blood cholesterol concentrations similar to that of oleic acid (18:1).

Other investigators also showed that stearic acid had a neutral effect on blood cholesterol in controlled feeding studies in which 18:0 provided 10% to 11%<sup>2</sup> or 17%<sup>3</sup> of dietary energy (en%). The cholesterolemic properties of palmitic acid are controversial. In some studies, 16:0 increased cholesterol more than 18:1 in humans when it comprised 15 to 18 en% of diets with low or moderate amounts of dietary cholesterol and approximately 4 en% 18:2.<sup>4,5</sup> In other investigations,<sup>6-8</sup> 16:0 fed at varying levels was neutral in humans and primates when contrasted with 18:1 and especially with 12:0 and 14:0. In these studies, the amount of cholesterol fed was low and polyunsaturated fat comprised less than 4 en% of the diet, possible key

considerations when the cholesterol-raising effects of fatty acids are evaluated.<sup>9</sup>

Myristic acid consistently seems to increase blood cholesterol concentrations more than other saturated fatty acids.<sup>9</sup> Yet it is not the major saturated fatty acid in fats consumed in the United States. Tropical oils, such as palm kernel oil and coconut oil, contain more 12:0 than 14:0. Butterfat provides more 16:0 than 14:0.<sup>10</sup> Although beef tallow and lard are good sources of 18:0, they are better sources of 16:0.

A study had not been performed that involved equalizing amounts of the two major saturated fatty acids in diets providing added fat as tropical oil, dairy fat, and meat fat to compare their effects in equal combination on blood lipids. We did this by feeding free myristic acid with palm kernel oil and butter and by giving a novel source of stearic acid (sheanut butter) with lard. The research questions asked were, "Is the combination of 16:0 and 18:0 fed with only low amounts of 12:0 and 14:0 less cholesterolemic than the other combinations, and does a diet with equal amounts of 12:0 and 14:0 increase blood cholesterol more than a diet with 14:0 and 16:0 but low 12:0?" The total amount of saturated, monounsaturated, and polyunsaturated fatty acids fed in this study equaled their availability in the US food supply: 15 en%, 17 en%, and 7 en%, respectively.<sup>11</sup> The two test fatty acids in combination provided approximately 10 en% of each diet, an amount more in line with what might actually be consumed in the United States than the amounts fed in many previous studies. Dietary cholesterol intake (~450 mg) approached dietary cholesterol availability in the United States.<sup>11</sup>

We also examined some of the mechanisms by which fatty acids influence lipid metabolism. We studied low-density lipoprotein (LDL) binding by and degradation via LDL

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receptors in a readily available tissue in humans, mononuclear cells (MNC). The MNC model was effectively applied in a previous study in which we determined that LDL receptor activity was significantly higher when humans were fed diets prepared with corn oil rather than butter.<sup>12</sup> Although used in animal studies, this model has not been used by investigators studying saturated fat in humans.

## SUBJECTS AND METHODS

### Subjects

In this three-period crossover study, the subjects were 18 healthy males, the minimum number needed to detect a mean difference for serum lipoprotein levels between treatments of approximately 10% with a standard deviation of approximately 8% and a power of 0.9. The subjects were 21 to 32 years of age and weighed between 59.1 and 81.8 kg. According to results of a 7-day activity recall,<sup>13</sup> daily energy expenditure was between 10.7 and 13.7 mJ. Serum cholesterol concentration at screening (mean  $\pm$  SD) was  $4.89 \pm 0.03$  mmol  $\cdot$  L<sup>-1</sup>, with a range of 3.49 to 5.22 mmol  $\cdot$  L<sup>-1</sup>. Ethnic background was as follows: American or European white, 14; African-American, one; Asian, two, and Middle-Eastern, one. The subjects had the following apolipoprotein (apo) E phenotypes: E 3/3, 14; E 3/2, three; and E 4/3, one. Before starting the study, subjects signed a consent form approved by The Ohio State University Biomedical Human Studies Committee. One subject left the study at the end of the second period; 17 completed all three periods.

### Diets

All food was prepared in a metabolic kitchen in the Department of Human Nutrition and Food Management. Subjects ate breakfast and dinner on the premises and took out a sack lunch. Weekend meals were also taken out, except for Saturday night dinner. The three experimental diets were formulated to provide 39 en% as fat, 15 en% as saturated fat, 17 en% as monounsaturated fat, 7 en% as polyunsaturated fat, and approximately 450 mg cholesterol. The goal was to give two thirds of the saturated fat (or  $\sim 30$  g  $\cdot$  d<sup>-1</sup>) as one of three equal combinations of fatty acids: (1) 12:0 + 14:0, (2) 14:0 + 16:0, and (3) 16:0 + 18:0. Fats and oils were blended (except for the free myristic acid) after taking into account the fatty acid composition of the meat in the diet (120 g lean beef and 60 g turkey breast or turkey ham). All fat blends included some high-oleic safflower oil, peanut oil, and safflower oil. Additional sources of fatty acids in the 12:0 + 14:0 diet were palm kernel oil and free myristic acid administered in capsules with meals. Butter and encapsulated myristic acid provided additional fatty acids in the 14:0 + 16:0 diet. Lard, sheanut butter, and small amounts of coconut oil (added to furnish some lower-chain saturated fatty acids) were also given with the 16:0 + 18:0 diet. The fat blends were incorporated into cakes and cookies, used as fat spreads on bread, and added to the main dishes at dinner. A basal menu providing 11.3 mJ (2,700 kcal) was given initially. Subjects requiring more calories to maintain body weight (as determined by daily weighing) were given extra cakes and cookies made up of fat blends with the same proportion of fatty acids as the overall diet. Adjustments over 1.2 mJ were met by altering menus to furnish additional protein, fat, and carbohydrate.

Daily records of the subjects' food intake were kept and analyzed using Food Processor II software (ESHA Research, Salem, OR) with added data on fatty acid composition of the foods in the menu (Table 1). Composites of the rotating 4-day menus were made and analyzed for specific fatty acid composition by gas-liquid chromatography (Table 1).

**Table 1. Daily Energy and Nutrient Intake on Saturated-Fat Diets (mean  $\pm$  SD)**

Dietary Variable	Diet		
	12:0 + 14:0 (n = 18)	14:0 + 16:0 (n = 18)	16:0 + 18:0 (n = 17)
Energy (mJ)*	11.7 $\pm$ 0.7	12.4 $\pm$ 0.8	11.7 $\pm$ 0.4
Protein (g)*	99 $\pm$ 3 (14.2)	101 $\pm$ 3 (13.6)	99 $\pm$ 2 (14.2)
Carbohydrate (g)*	334 $\pm$ 23 (47.8)	355 $\pm$ 26 (47.9)	335 $\pm$ 12 (47.9)
Total fat (g)*	125 $\pm$ 7 (40.2)	131 $\pm$ 8 (39.8)	125 $\pm$ 4 (40.2)
Fatty acids (g)†			
8:0 + 10:0	1.9 $\pm$ 0.1 (0.6)	1.4 $\pm$ 0.1 (0.4)	1.4 $\pm$ 0.0 (0.5)
12:0	13.0 $\pm$ 0.7 (4.2)	2.5 $\pm$ 0.1 (0.7)	4.9 $\pm$ 0.2 (1.6)
14:0	14.0 $\pm$ 0.8 (4.5)	14.2 $\pm$ 0.8 (4.3)	2.7 $\pm$ 0.1 (0.9)
16:0	13.1 $\pm$ 0.7 (4.2)	19.9 $\pm$ 1.2 (6.0)	17.1 $\pm$ 0.6 (5.5)
18:0	5.4 $\pm$ 0.3 (1.7)	7.7 $\pm$ 0.4 (2.3)	15.4 $\pm$ 0.5 (5.0)
18:1	52.8 $\pm$ 3.0 (17.0)	59.7 $\pm$ 3.5 (18.1)	52.1 $\pm$ 1.8 (16.8)
18:2	21.5 $\pm$ 1.2 (6.9)	21.6 $\pm$ 1.2 (6.6)	25.4 $\pm$ 0.9 (8.2)
Cholesterol (mg)*	438 $\pm$ 22	454 $\pm$ 27	438 $\pm$ 12

NOTE. Values in parentheses are calculated values for en% provided by the nutrient.

\*Analyzed from daily dietary records using Food Processor II Nutrition Analysis Software (ESHA Research, Salem, OR).

†As determined by gas-liquid chromatography, the basic menu for diet 12:0 + 14:0 provided significantly ( $P < .05$ ) more 8:0 + 10:0 and 12:0 and less 16:0 than the other diets. The basic menu for diet 16:0 + 18:0 provided significantly more 18:0 and less 14:0 than the other diets. Amounts of 18:1 and 18:2 did not differ ( $P > .05$ ) among menu composites, but subjects who ate extra food energy to maintain weight tended to consume more 18:1 on diet 14:0 + 16:0 and more 18:2 on diet 16:0 + 18:0.

### Design

The 18 subjects were randomized into three groups using a three-period and six-sequence design ( $3 \times 3$  crossover design), except that only five rather than six subjects consumed the 16:0 + 18:0 diet during period 3. Each dietary treatment lasted 4 weeks with washout periods of 4 to 6 weeks between experimental periods. Before each period, subjects were instructed to consume a saturated-fat diet similar in general composition to the experimental diets and based on a prescribed food-exchange list. The subjects recorded all food and beverages consumed on the self-selected diet for 7 days preceding each period. The records were analyzed by Food Processor II software. In general, fat and cholesterol intake during the 7-day prestudies was somewhat lower than prescribed, but the polyunsaturated to saturated (P/S) ratio was approximately 0.4. This information was used in the statistical analyses to adjust for the random effects of baseline.

To assess apparent digestibility of the fatty acids, we administered 50 mg Brilliant Blue before breakfast and then again 3 days later during the third week of period 1. Feces collected between the appearance of the two markers were composited, mixed thoroughly with a known amount of water, and stored at  $-20^{\circ}\text{C}$  until analysis.

Blood samples were obtained by venipuncture after an overnight fast twice in the 3-day period preceding each diet phase and twice during the last 3 days of each feeding period. Depending on the requirements of the procedure, analyses either commenced immediately or serum, obtained by centrifugation ( $500 \times g$  for 20 minutes), was frozen at  $-80^{\circ}\text{C}$  for later analysis.

### Biochemical Procedures

**Serum lipoproteins.** Previously frozen serum samples were analyzed for total cholesterol and free cholesterol by an enzymatic assay.<sup>14</sup> Total high-density lipoprotein (HDL), HDL<sub>2</sub>, and HDL<sub>3</sub>

cholesterol was determined by the same enzymatic procedure after precipitation of apo B-containing lipoproteins with dextran sulfate- $Mg^{2+}$  solution and then removal of HDL<sub>2</sub> with a stronger dextran sulfate- $Mg^{2+}$  solution.<sup>15</sup> LDL cholesterol (in milligrams per deciliter) was calculated as total cholesterol minus HDL cholesterol and 0.2 triglycerides. Results for the two preperiod and postperiod analyses were averaged and converted to millimoles per liter by dividing by 38.67 for cholesterol values and 88.54 for triglyceride values. Serum from a previously frozen sample pool was analyzed with each analysis to check consistency of enzymatic assays. The precision (coefficient of variation) of the sequenced runs of this pool was as follows: total cholesterol, 1.02; HDL cholesterol, 2.57; and triglycerides, 3.89. Serum apolipoproteins A-1 and B-100 from one preperiod and postperiod blood sample were assayed by a radioimmunoassay method (TAGO, Burlingame, CA).

**LDL receptor activity.** First, iodinated LDL was prepared as previously described<sup>12</sup> from pooled blood obtained from all subjects (10 mL per subject) 3 days before the beginning of each period when subjects consumed their self-selected diet. Fernandez et al<sup>16</sup> have indicated that the composition of LDL, as well as receptor activity, influences LDL turnover. Because the blood was pooled and the diet of the individual donors did not differ appreciably (according to diet records), we assumed LDL composition remained constant throughout the study, but this was not specifically assessed. Briefly, plasma from pooled blood treated with 0.1% disodium EDTA was subjected to sequential ultracentrifugation with potassium bromide first to isolate lipoproteins and then to obtain LDL from the fraction with a density of 1.019 to 1.062. LDL was dialyzed overnight with phosphate-buffered saline, confirmed to be free of other lipoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis,<sup>17</sup> and radioiodinated by the monochloride method of McFarlane.<sup>18</sup> This procedure produced a LDL with a specific activity of 100 to 600 cpm/ng protein. Free iodine content was less than 3%.

For measurement of LDL degradation and binding, MNC were isolated by centrifugation ( $800 \times g$  for 20 minutes at room temperature) from saline-diluted whole blood anticoagulated with sodium citrate and overlaid with Nycoprep (Life Technologies, Gaithersburg, MD). After centrifugation, the meniscus layer of MNC was collected. After washing with Hanks buffer solution (pH 7.4), viable cells were counted with Trypan blue exclusion under a microscope.

Degradation of <sup>125</sup>I-LDL by MNC was determined as previously described<sup>12,19</sup> by incubation with <sup>125</sup>I-LDL ( $20 \mu g \cdot mL^{-1}$ ) in the presence and absence of a 20-fold excess of unlabeled LDL at 37°C for 5 hours. As reported previously,<sup>12</sup> the optimal time of incubation was assessed in a preliminary study in which MNC from three subjects—a male, a premenopausal female, and a postmenopausal female—were incubated with <sup>125</sup>I-LDL for 3 to 5 hours. The amount of LDL degraded increased as time elapsed. Differences among subjects, where they existed, were apparent at 3, 4, and 5 hours. Undegraded, protein-bound iodotyrosine was removed by precipitation with trichloroacetic acid (final vol, 20%) in the presence of bovine serum albumin followed by centrifugation. Other researchers showed that less than 2% of the radioactivity of LDL was soluble in 10% trichloroacetic acid.<sup>20</sup> The decanted supernatant was counted in a gamma counter. Nonspecific and specific degradation represent, respectively, degradation in the presence of a 20-fold excess of unlabeled LDL and the difference between the latter and degradation in the absence of excess unlabeled LDL.

Specific and nonspecific binding of LDL was measured as previously described,<sup>12,19</sup> except that MNC were incubated for 1 hour rather than 30 minutes at 4°C with 10, 20, and 40  $\mu g \cdot mL^{-1}$

<sup>125</sup>I-LDL in the presence and absence of excess unlabeled LDL. After incubation, cells were washed three times with RPMI, collected, and counted in a gamma counter. Values for the 1-hour incubation with 20  $\mu g \cdot mL^{-1}$  <sup>125</sup>I-LDL were in a comparable range with those obtained in our previous study<sup>12</sup> of women subjects fed butter diets.

**Other analyses.** The rate of cholesterol biosynthesis by MNC was determined by the method of McNamara et al.<sup>21</sup> Briefly, MNC were incubated with 2.5 mmol/L (2-<sup>14</sup>C) acetate (DuPont NEN, Boston, MA) and autologous serum obtained at the time of the test at 37°C for 4 hours in a metabolic shaker. Incubation was terminated by adding 5 mol/L KOH. An internal standard ([1,2-<sup>3</sup>H] cholesterol) and the mixture were saponified by heating. Nonsaponified lipids were extracted with hexane and applied to a column of aluminum oxide. The eluted (acetone:diethyl ether) sterol fraction was reduced in volume and counted in a scintillation counter.

The rate of cholesterol esterification (LCAT activity) was assessed by Channon et al.<sup>22</sup> Briefly, fresh plasma was preincubated at 4°C for 1 hour with <sup>3</sup>H-cholesterol-albumin emulsion; after equilibration of the added cholesterol and endogenous cholesterol, the mixture was incubated at 37°C for 3 hours. Lipids from the incubates were extracted and then separated by thin-layer chromatography, and radioactivity of cholesterol ester and free cholesterol spots was counted by liquid scintillation.

Fatty acid composition of food composites and feces was determined by the method of Sukhija and Palmquist.<sup>23</sup> One milligram each of tritonanoin and tritonadecanoin (Nu Chek Prep, Elysian, MN) were added as internal standards to the samples, and then lipids were extracted with hexane:isopropanol (3:2). The condensed upper layer was methylated with 10% methanolic HCl. Fatty acid methyl esters were separated by chromatography on a Hewlett-Packard (Avondale, PA) 5890 gas-liquid chromatograph using a DB 25 capillary column, and were identified by comparison to authentic standards from NuChek Prep.

Apo E phenotype was determined by isoelectrofocusing of delipidated plasma followed by immunoblotting using polyclonal goat anti-human apo E antiserum as first antibody.<sup>24,25</sup> The apo E isoforms were separated in 7.5% polyacrylamide gels containing 8 mol/L urea and 2.0% ampholytes (pH 4 to 6 and pH 5 to 8) in the proportion of 1:3. Samples of known phenotypes were included in each run. After isoelectrofocusing, proteins were electrophoretically blotted onto a nitrocellulose membrane, soaked in TBS buffer containing 2% to 3% nonfat dry milk, and then incubated with goat anti-human apo E antibody. Apo E bands were visualized<sup>24</sup> and identified.

Whole-blood (citrate) platelet aggregation and release of platelet adenosine triphosphate were measured as previously described<sup>26</sup> using the Whole Blood Lumi Aggregometer (model 500; Chronolog, Havertown, PA) after addition of 0.5, 1, and 2  $\mu g$  collagen/mL of sample. Tests were performed within 2 hours after blood was drawn from fasting subjects, who were not taking antiplatelet drugs.

### Statistical Analyses

The end-of-period values and mean differences between the beginning and end of each period were analyzed statistically. Differences among the three experimental diets, of which the main ones were the three saturated fatty acid combinations, were analyzed using a linear model that contained terms for the subjects, periods, direct treatments, and carryover effects. To allow for correlations among the biochemical values when subjects entered the study or reentered the study after washout periods, a random effect with each subject was used. Differences in dietary fat intake

during the baseline periods were adjusted for, using the recorded dietary fat intake during the 1-week baseline period as covariates. Covariates were incorporated as random effects in the linear model. Linear models with random effects were fitted using PROC MIXED in the Statistical Analysis System (SAS Institute, Cary, NC) program. The significance level was set at  $\alpha = .05$ . Means that were significantly different among diets were separated in the linear model by PROC MIXED. In an additional analysis, subjects were divided into two categories by apo E phenotype, 3/3 and 3/2. The biochemical values were analyzed with and without apo E3/2 using the same linear models described above that were fitted using PROC MIXED of SAS. Correlation coefficients between LDL receptor activity (specific degradation and binding) and lipoprotein concentrations were determined.

## RESULTS

### *Diet Composition and Uptake of Fatty Acids From the Digestive Tract*

The goal for fatty acid composition was achieved for diets 14:0 + 16:0 and 16:0 + 18:0 but not for diet 12:0 + 14:0, according to analysis of food composites (Table 1). Amounts of 16:0 in diet 12:0 + 14:0 were one third less than in diet 14:0 + 16:0 and one fourth less than in diet 16:0 + 18:0 due to the high proportion of this fatty acid in meat. All the diets contained this source of 16:0. The extra 16:0 in diets 14:0 + 16:0 and 16:0 + 18:0 came from butter or lard, an extra 6.8 g and 4.0 g, respectively. Butter and lard tend to have more 16:0 in the sn-2 position of the triglyceride molecule than beef and turkey fat.<sup>27</sup> Although the term, diet 12:0 + 14:0, may be a misnomer, we will use it to designate this diet, since it was the diet that provided the least 16:0.

Despite concerns about bioavailability of the free myristic acid, which might have formed a nonabsorbable salt in the intestine, and of the fats with 18:0, which might have high melting points, the dietary fatty acids were well utilized (Table 2) by the subset of subjects included in the digestibility study. The apparent digestibility of shorter-chain saturated fatty acids and the unsaturated fatty acids fed as triglycerides averaged 99%, whereas an amount representing less than 5% of the dietary 14:0 and 10% of the dietary 18:0 appeared, on average, in the feces. The highest excretion of 14:0 by an individual was about 16%.

### *Serum Lipoprotein Concentrations*

The saturated fatty acid variables had a significant ( $P < .05$ ) effect on postprandial concentrations of HDL

**Table 2. Percent (mean  $\pm$  SD) of Dietary Fatty Acids Excreted in Feces on Three Saturated-Fat Diets**

Fatty Acid	12:0 + 14:0 (n = 6)	14:0 + 16:0 (n = 6)	16:0 + 18:0 (n = 5)
12:0	0.15 $\pm$ 0.12	0.34 $\pm$ 0.21	0.09 $\pm$ 0.03
14:0	4.77 $\pm$ 5.79	5.00 $\pm$ 3.30	2.68 $\pm$ 0.89
16:0	2.69 $\pm$ 2.70	2.33 $\pm$ 1.64	1.76 $\pm$ 0.97
18:0	10.30 $\pm$ 10.78	5.04 $\pm$ 3.07	7.31 $\pm$ 5.44
18:1	0.89 $\pm$ 0.56	1.79 $\pm$ 2.25	0.58 $\pm$ 0.70
18:2	0.51 $\pm$ 0.43	1.02 $\pm$ 0.90	0.35 $\pm$ 0.28

NOTE. Fecal samples were collected for 3 days during the final week of diet period 1.

**Table 3. Serum Lipoproteins on Three Saturated-Fatty Acid Mixtures (mean  $\pm$  SD)**

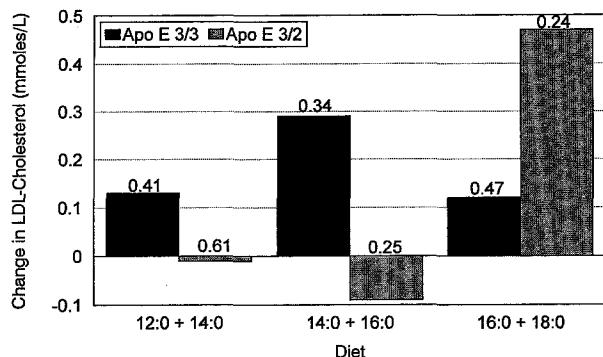
Serum Lipoprotein	12:0 + 14:0 (n = 18)	14:0 + 16:0 (n = 18)	16:0 + 18:0 (n = 17)
Total cholesterol (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	4.39 $\pm$ 0.81	4.32 $\pm$ 0.71	4.40 $\pm$ 0.63
Postperiod	4.63 $\pm$ 0.66	4.58 $\pm$ 0.70	4.52 $\pm$ 0.62
Difference	0.24 $\pm$ 0.43	0.26 $\pm$ 0.43	0.12 $\pm$ 0.47
LDL cholesterol (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	2.81 $\pm$ 0.64	2.74 $\pm$ 0.55	2.70 $\pm$ 0.53
Postperiod	2.93 $\pm$ 0.59	2.93 $\pm$ 0.61	2.86 $\pm$ 0.55
Difference	0.12 $\pm$ 0.42	0.19 $\pm$ 0.37	0.16 $\pm$ 0.45
HDL cholesterol (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	1.18 $\pm$ 0.19	1.19 $\pm$ 0.17	1.22 $\pm$ 0.19
Postperiod*	1.30 $\pm$ 0.21	1.22 $\pm$ 0.21	1.28 $\pm$ 0.21
Difference†‡	0.12 $\pm$ 0.09	0.03 $\pm$ 0.10	0.06 $\pm$ 0.10
HDL <sub>2</sub> cholesterol (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	0.21 $\pm$ 0.13	0.19 $\pm$ 0.14	0.22 $\pm$ 0.13
Postperiod*	0.31 $\pm$ 0.16	0.27 $\pm$ 0.16	0.29 $\pm$ 0.20
Difference	0.10 $\pm$ 0.15	0.08 $\pm$ 0.10	0.06 $\pm$ 0.14
HDL <sub>3</sub> cholesterol (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	0.97 $\pm$ 0.16	1.00 $\pm$ 0.12	0.99 $\pm$ 0.11
Postperiod	0.98 $\pm$ 0.10	0.95 $\pm$ 0.10	0.99 $\pm$ 0.09
Difference‡	0.02 $\pm$ 0.13	-0.05 $\pm$ 0.13	0.00 $\pm$ 0.14
Triglycerides (mmol $\cdot$ L <sup>-1</sup> )			
Preperiod	1.07 $\pm$ 0.42	1.03 $\pm$ 0.44	1.04 $\pm$ 0.45
Postperiod	0.88 $\pm$ 0.30	0.96 $\pm$ 0.48	0.81 $\pm$ 0.39
Difference	-0.19 $\pm$ 0.22	-0.07 $\pm$ 0.34	-0.23 $\pm$ 0.25
Apo A-1 (g $\cdot$ L <sup>-1</sup> )			
Preperiod	1.90 $\pm$ 0.24	1.87 $\pm$ 0.22	1.94 $\pm$ 0.24
Postperiod‡	2.04 $\pm$ 0.32	1.96 $\pm$ 0.25	1.95 $\pm$ 0.27
Difference†	0.14 $\pm$ 0.35	0.07 $\pm$ 0.12	0.01 $\pm$ 0.24
Apo B-100 (g $\cdot$ L <sup>-1</sup> )			
Preperiod	0.74 $\pm$ 0.16	0.74 $\pm$ 0.18	0.71 $\pm$ 0.14
Postperiod	0.77 $\pm$ 0.18	0.81 $\pm$ 0.18	0.80 $\pm$ 0.21
Difference	0.03 $\pm$ 0.15	0.07 $\pm$ 0.12	0.09 $\pm$ 0.24

\*After adjustment for random effects of the subject's baseline diet,  $P = .043$  for a treatment effect for total HDL cholesterol and .0655 for HDL<sub>2</sub> cholesterol.

†After adjustment for random effects of the baseline diet,  $P = .0148$  for a treatment effect for the change in HDL cholesterol and .0803 for the change in apo A-1.

‡Significant carryover and/or period effects ( $P < .05$ ).

cholesterol and on the change in HDL cholesterol. HDL cholesterol was highest when subjects ate diet 12:0 + 14:0, although the significant carryover and period effects identified in the statistical analysis had an impact on the results (Table 3).  $P$  values for the treatment effect on the postperiod concentration of HDL<sub>2</sub> cholesterol ( $P = .0655$ ) and for the change in apo A-1 ( $P = .0803$ ) approached significance. Highest values for both occurred on diet 12:0 + 14:0. When the 14 subjects with the apo E3/3 phenotype were analyzed separately, the change in apo A-1 was significant ( $P = .02$ ). Dietary treatment did not affect serum concentrations of total cholesterol, LDL cholesterol, triglycerides, and apo B-100 in the subjects analyzed as a whole and stratified according to apo E phenotype. The response of serum LDL cholesterol appeared to be different in apo E3/3 and E3/2 groups (Fig 1), but one cannot generalize on the basis of the small apo E3/2 group, which by chance all followed the same diet rotation.

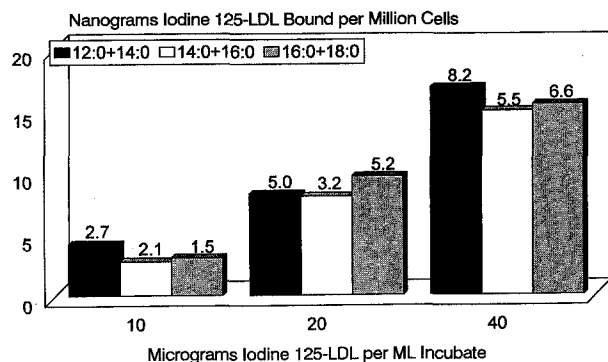


**Fig 1.** Change from baseline in serum LDL cholesterol concentrations in subjects stratified according to apo E phenotype. Apo E3/3 phenotype,  $n = 14$ ; apo E3/2 phenotype,  $n = 3$ . Numbers above each bar are standard deviations.

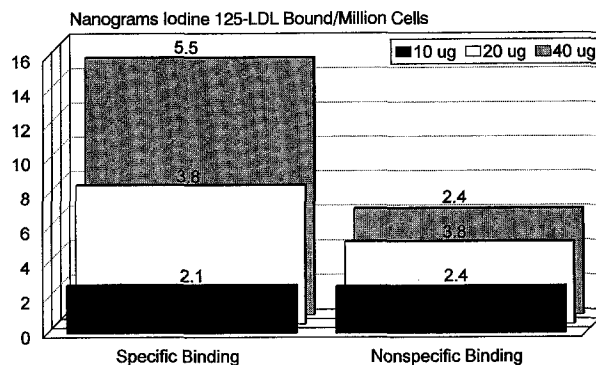
### LDL Degradation and Binding

LDL binding to the LDL receptor increased almost linearly as the concentration of labeled LDL increased in the in vitro incubate (Fig 2). Diet had no effect on the amount of LDL bound at any concentration of labeled LDL. Nonspecific binding increased threefold in the example shown in Fig 3 for diet 14:0 + 16:0 and less than twofold for the other diets as the amount of labeled LDL was increased while binding of LDL to LDL receptors increased almost eightfold. The relationship between specific and nonspecific binding was comparable to the one reported by Ho et al<sup>19</sup> for freshly isolated MNC.

Treatment had no effect on cholesterol biosynthesis and specific and nonspecific degradation in MNC when all subjects were included in the statistical analysis (Table 4). Receptor-mediated degradation of LDL increased 30%, on average, from baseline when subjects ate the 16:0 + 18:0 diet, and it decreased 9% on diet 12:0 + 14:0 and increased 6% on diet 14:0 + 16:0. However, when 14 subjects with the apo E3/3 phenotype were analyzed separately (Fig 4), the  $P$  value for a treatment effect on receptor-mediated degradation was .11 when random effects of baseline were adjusted statistically, and .03 when they were not. In the latter



**Fig 2.** Binding of <sup>125</sup>I-LDL to LDL receptors when MNC obtained from subjects eating three saturated-fat mixtures were incubated with different amounts of <sup>125</sup>I-LDL.  $n = 18$  for diets 12:0 + 14:0 and 14:0 + 16:0, and  $n = 17$  for diet 16:0 + 18:0. Numbers above each bar are standard deviations.



**Fig 3.** Specific and nonspecific binding of <sup>125</sup>I-LDL to MNC from subjects given the 14:0 + 16:0 fatty acid combination ( $n = 18$ ). Numbers above each bar are standard deviations.

analysis, the significant difference was between diets 12:0 + 14:0 and 16:0 + 18:0. Values for receptor-mediated degradation were not correlated with concentrations of serum total cholesterol or LDL cholesterol. Changes in these values between baseline and end of period also were not related.

### Platelet Aggregation

The dietary treatments had no significant effect on collagen-stimulated platelet aggregation in vitro in whole blood measured in impedance units or in terms of adenosine triphosphate secretion, an indirect measure of the potential for secondary aggregation. The  $P$  value for a treatment effect on changes in lag time in platelet aggregation was .09. Diet 12:0 + 14:0 increased lag time the most compared with baseline, by  $0.17 \pm 0.24$  minutes ( $n = 18$ ), in contrast to  $0.08 \pm 0.21$  (diet 14:0 + 16:0,  $n = 18$ ) and  $0.02 \pm 0.30$  (diet 16:0 + 18:0,  $n = 17$ ).

### DISCUSSION

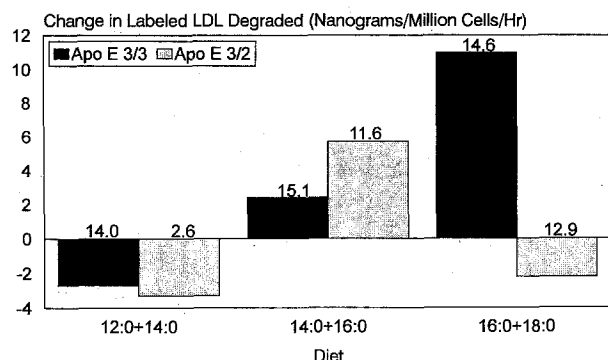
This was a controlled feeding study in which the amounts of total fat and the general types of fat given approached

**Table 4.** LDL Degradation and Cholesterol Biosynthesis (mean  $\pm$  SD) in MNC From Subjects Fed Three Saturated-Fat Mixtures

Variable	Diet		
	12:0 + 14:0 ( $n = 18$ )	14:0 + 16:0 ( $n = 18$ )	16:0 + 18:0 ( $n = 17$ )
<b>Cholesterol biosynthesis*</b>			
Preperiod	3.29 $\pm$ 1.89	2.41 $\pm$ 1.98	2.29 $\pm$ 1.93
Postperiod†	3.35 $\pm$ 2.13	2.71 $\pm$ 1.95	2.33 $\pm$ 1.35
Difference†	0.06 $\pm$ 2.93	0.30 $\pm$ 2.68	0.04 $\pm$ 2.62
<b>LDL degradation</b>			
<b>Specific*</b>			
Preperiod	32.8 $\pm$ 10.4	30.2 $\pm$ 12.8	26.7 $\pm$ 9.6
Postperiod	30.0 $\pm$ 10.4	32.1 $\pm$ 9.6	34.9 $\pm$ 11.2
Difference	-2.8 $\pm$ 12.2	1.9 $\pm$ 14.6	8.1 $\pm$ 14.4
<b>Nonspecific*</b>			
Preperiod	24.6 $\pm$ 11.1	24.4 $\pm$ 14.7	21.7 $\pm$ 10.1
Postperiod	22.4 $\pm$ 9.4	25.2 $\pm$ 13.6	21.4 $\pm$ 6.2
Difference	-2.2 $\pm$ 14.5	0.8 $\pm$ 16.7	-0.24 $\pm$ 11.1

\*Units for cholesterol biosynthesis are pmol  $\cdot$  million cells<sup>-1</sup>  $\cdot$  h<sup>-1</sup>. Units for LDL degradation are ng  $\cdot$  million cells<sup>-1</sup>  $\cdot$  h<sup>-1</sup>.

†Carryover and/or period effects were significant ( $P < .05$ ).



**Fig 4.** Change from baseline in receptor-mediated degradation of  $^{125}\text{I}$ -LDL by MNC isolated from subjects given three saturated-fat mixtures and stratified according to apo E phenotype ( $n = 14$  for E3/3 and  $n = 3$  for E3/2). Numbers above each bar are standard deviations.  $P = .03$  for a treatment effect for subjects with the apo 3/3 phenotype when random effects of baseline were not taken into account, and  $P = .11$  after statistical adjustment. Significant differences in the unadjusted data were actually between diets 12:0 + 14:0 and 14:0 + 16:0.

quantities available in the US diet.<sup>11</sup> In 1985, actual mean daily consumption by men 19 to 34 years of age was 40.2 g saturated fat, 41.8 g monounsaturated fat 20.6 g polyunsaturated fat, and 440 mg cholesterol.<sup>28</sup> The mean consumption of the men in this study was similar except for monounsaturated fat, which averaged 52 to 60 g (17 to 18 en% of the diets). Thus, the fatty acids we fed equaled the availability in the United States but were higher in unsaturated fat than is usually selected. The variables of interest in this study were saturated fatty acids, which were fed in equal combinations of two or three in the case of diet 12:0 + 14:0, since quantities of 16:0 approached those of 12:0 and 14:0. Amounts of 12:0, 14:0, and 18:0 in diets emphasizing these fatty acids were about as high as typically consumed in the United States. In the Bogalusa Heart Study, 14:0, 16:0, and 18:0 comprised 1.4, 7.3, and 3.3 en%, respectively, of the diet of 10-year-old children in 1981 to 1982.<sup>29</sup>

#### *Was the Combination of 16:0 and 18:0 Less Cholesterolemic Than the Other Combinations?*

No, according to our measurements of serum total and LDL cholesterol concentrations. However, less 18:0 was fed

in this study (5 en% v 15 to 17 en%) than in some previous studies<sup>3,30</sup> that found that diets with 12:0 + 14:0 or 16:0 increased blood LDL cholesterol 27% to 41% as compared with diets high in 18:0 and low in 16:0 (Table 5). Much lower or insignificant differences were observed<sup>2,29</sup> in comparisons of diets with butter (14:0 + 16:0) and cocoa butter, which provided a combination of 18:0 and 16:0 (~10 en% each). Equal amounts of 18:0 and 16:0 were fed in this study.

In all the studies summarized in Table 5, except one of ours,<sup>31</sup> researchers fed diets lower in polyunsaturated fat (<5 en%) and lower in dietary cholesterol (0 to 350 mg/d) than the diets fed in this study. Usually, less monounsaturated fat was also given. We theorize that the higher and relatively typical levels of unsaturated fat consumed by our subjects and the lower but typical levels of the test saturated fatty acids given in this study obliterated potential differences in the cholesterolemic response to the various saturated fatty acid combinations.

Previous assumptions made about the relative hypercholesterolemic effects of the different saturated fatty acids are being reexamined. Myristic acid was not consistently more hypercholesterolemic than palmitic acid in studies using semisynthetic diets (Table 5). Similarly, 16:0 increased LDL cholesterol only slightly (but significantly) more than did 12:0.<sup>4</sup> In our<sup>31</sup> previous two-part study in which male subjects consumed over 400 mg/d cholesterol and 4 to 8 en% polyunsaturated and 9 to 17 en% saturated fatty acids, serum LDL cholesterol levels were similar when diets high in the so-called hypercholesterolemic fatty acids (12:0, 14:0, and 16:0) were compared with diets high in presumably neutral or less digestible saturated fatty acids (8:0, 10:0, and 22:0). The results of this study are consistent with our previous study suggesting that the hypercholesterolemic effect of different fats cannot always be predicted by the total amount of 12:0 + 14:0 + 16:0 in the fats.

Interpretation of results is difficult when two or more fatty acids are varied in the diet simultaneously. If fatty acid B were the only cholesterolemic fatty acid among fatty acids A, B, and C, replacement of diet AB with diet BC might produce no change in serum cholesterol concentrations and might suggest that all three fatty acids were hypercholester-

**Table 5.** Comparison of Fatty Acid Effects on LDL and HDL in Human Feeding Studies

Reference	Fatty Acid Comparison	Difference in LDL Cholesterol (%)	Difference in HDL Cholesterol (%)	Dietary Cholesterol (mg/d)
Denke and Grundy <sup>4</sup>	12 (18 en%)† v 16 (17 en%)†	-6*	7	0
Denke and Grundy <sup>4</sup>	12 (18 en%)† v 18:1 (30 en%)†	12*	16	0
Wardlaw et al <sup>31</sup>	12 + 14 (17 en%)† v 8 + 10 + 22 (12 en%)†	-6	16*	> 400
Tholstrup et al <sup>30</sup>	12 + 14 (14 en%)† v 16 (17 en%)†	4	20*	~ 200
Tholstrup et al <sup>30</sup>	12 + 14 (14 en%)† v 18 (15 en%)†	41*	38*	~ 200
Sundram et al <sup>6</sup>	12 + 14 (7 en%)† v 16 (11 en%)†	13*	9*	200
Zock et al <sup>5</sup>	14 (11 en%)† v 16 (15 en%)†	4*	9*	~ 350
Tholstrup et al <sup>32</sup>	14 (16 en%)† v 16 (16 en%)†	-1	9*	~ 200
Wardlaw et al <sup>31</sup>	14 + 16 (15 en%)† v 8 + 10 + 22 (9 en%)‡	< 1	0	> 400
Kris-Etherton et al <sup>33</sup>	14 + 16 (13 en%)† v 16 + 18 (20 en%)†	9-15*	—	350
Bonanome and Grundy <sup>3</sup>	16 (18 en%)† v 18 (17 en%)†	27*	6	0

\*Difference was significant ( $P < .05$ ).

†Polyunsaturated fat provided up to 2 to 5 en% of diet.

‡Polyunsaturated fat provided 8 en% of diet.

olemic. However, it is almost impossible to change one fatty acid at a time by feeding natural fats and oils. In a more recent study in progress in which we feed women synthetic fats, less cholesterol (300 instead of 450 mg), higher levels of the test fatty acid ( $\sim 13$  en% rather than 5 en%), and less polyunsaturated fat (4 en% instead of 7 en%), 18:0 was neutral and 16:0 was at least as hypercholesterolemic as 14:0 and perhaps more so. Also, 16:0 seemed to downgrade the LDL receptor activity of MNC compared with 14:0 and 18:0. The results of our study in progress are consistent with the study described here.

In this study, despite similar cholesterolemic effects of the saturated fatty acid combinations, diet 16:0 + 18:0 appeared to increase receptor-mediated LDL degradation in MNC when compared with diets 12:0 + 14:0 and 14:0 + 16:0, but only when subjects with a common apo E phenotype (3/3) were compared. Of the three saturated-fat combinations, 16:0 + 18:0 produced the greatest increase in receptor-mediated LDL degradation by MNC in 10 of 13 apo E3/3 subjects, and no change or the least-negative change in two of four subjects with other phenotypes who consumed this combination.

In a previous crossover study of diets high in corn oil versus butter, we showed that the MNC model detected significant differences in receptor-mediated LDL degradation, as well as in binding of LDL to the LDL receptor.<sup>12</sup> Increases in receptor-mediated LDL degradation and binding to the receptor correlated ( $r = -.4$  and  $-.6$ , respectively) with decreases in serum concentrations of LDL cholesterol. Mean increases in receptor-mediated degradation and binding to the receptor ranged from 68% to 134% in subjects given corn oil. In this study of different saturated fatty acids, changes in LDL degradation and serum LDL concentrations were not correlated, possibly because degradation rate increased less on diet 16:0 + 18:0 than on the corn oil diet, and receptor-mediated degradation is only one of several variables influencing serum LDL cholesterol concentrations.<sup>16,34,35</sup> Another potential contributing factor, cholesterol biosynthesis, was not lower on diet 16:0 + 18:0 compared with the other diets, at least in the *in vitro* MNC system. In contrast to our comparison of polyunsaturated versus saturated fat, the increase in receptor-mediated LDL degradation in this study of different saturated fatty acids was not accompanied in apo E3/3 subjects by an increase in LDL binding to the LDL receptor, a variable that gives an indication of receptor number and binding affinity.

Several questions can be raised. First, was the enhancement of receptor-mediated LDL degradation suggested by our results in apo E3/3 subjects due to the higher levels of 18:0 in the diet or the lower levels of 14:0? Spady et al<sup>35</sup> in reviewing studies on blood LDL cholesterol regulation in several species, suggested the effect of 18:0 on LDL receptor activity is neutral compared with that of 12:0, 14:0, and 16:0 fed in diets providing cholesterol. Fernandez et al<sup>34</sup> have shown in guinea pigs that receptor number, as determined in an *in vitro* binding assay performed with hepatic membranes, was higher in animals fed a beef tallow

(16:0 + 18:0) diet with about 5 en% 18:0 rather than a palm oil-based diet with about 15 en% 16:0 or a palm kernel oil-based diet with about 6 en% 14:0 and 18 en% 12:0.

Second, does apo E phenotype influence receptor-mediated degradation of LDL? Apo E phenotype affects the binding of apo E to B/E receptors.<sup>36</sup> Alterations in apo E binding and lipoprotein metabolism induced by the apo E4 and E2 phenotypes could influence uptake of lipoprotein remnants, which might in turn downgrade or upgrade LDL receptors.<sup>36</sup> More research is needed with more subjects with the apo E4 and E2 phenotypes to identify potential interactions, if they exist, among apo E phenotype, saturated fatty acids, and receptor-mediated degradation of LDL.

Third, would our results have been more conclusive if the study had occurred over a longer period? It is possible that a new steady state of LDL metabolism was not established in 4 weeks in our subjects. However, our results with the corn oil-butter comparison<sup>12</sup> were readily apparent after 4 weeks of experimental feeding. The changes in LDL metabolism induced by different saturated fatty acid combinations superimposed on diets relatively high in cholesterol and unsaturated fat were much more subtle and possibly of relatively small significance for persons consuming a typical US diet. Conversely, a change in LDL receptor activity in MNC may be an early indication of potential subtle dietary effects that will be manifested after prolonged ingestion of the diet.

#### *Saturated Fatty Acid Combinations and HDL Cholesterol*

An interesting observation in this study was that diet 12:0 + 14:0 caused a twofold to fourfold ( $P < .05$ ) greater increase in serum HDL cholesterol during the 4-week experimental period than the other two diets. This is a common but little-noticed finding (Table 5) in comparisons of tropical oils and other fats in humans, and may be due more to effects of lauric acid than of myristic acid. The mechanisms responsible are not known, but may involve changes in apo A-1 metabolism, since diet 12:0 + 14:0 produced the greatest increase in this apolipoprotein. We examined variables associated with the reverse cholesterol transport system (data not shown) to determine if diet 12:0 + 14:0 affected them differently. Serum concentrations of cholesterol ester increased on all diets while free cholesterol decreased. Rates of cholesterol esterification (lecithin cholesterol acyltransferase activity) decreased. Changes in the rates of cholesterol esterification and in concentrations of HDL<sub>3</sub> cholesterol were positively associated ( $r = .33$ ,  $P = .02$ ). Changes in HDL<sub>2</sub> cholesterol were negatively correlated with posttreatment rates of cholesterol esterification ( $r = -.38$ ,  $P = .005$ ). However, the three saturated fatty acid combinations had similar effects on these parameters.

#### *Saturated Fatty Acid Combinations and Platelet Aggregation*

There was an indication that diet 12:0 + 14:0 increased the lag time for platelet aggregation. Little is known about the effect of different saturated fatty acids on the tendency

of blood to clot, another risk factor for coronary heart disease. In two recent studies, a diet high in myristic acid as opposed to palm oil caused a slight increase in factor VII coagulant<sup>32</sup> and a diet high in 18:0 reduced activity of this blood-clotting factor compared with diets with 16:0 and 12:0 + 14:0.<sup>30</sup>

In summary, three saturated fatty acid combinations (12:0 + 14:0, 14:0 + 16:0, and 16:0 + 18:0) fed at levels that might be selected in a typical US diet did not have different effects on serum total and LDL cholesterol concentrations in healthy young men. In human studies showing a large difference between effects of 18:0 and other saturated fatty acids, larger amounts of 18:0 were fed and

levels of 16:0 were considerably lower. Also, the saturated fatty acid combinations in this study, in contrast to other studies summarized in Table 5, were fed with relatively high levels of dietary cholesterol and unsaturated fat, which have the potential for downgrading and upgrading, respectively, LDL receptor activity. Despite its failure to produce lower serum LDL cholesterol, diet 16:0 + 18:0 appeared to enhance receptor-mediated LDL degradation, at least in subjects with a common apo E (3/3) phenotype. We conclude that this effect is due to 18:0, not 16:0. The 12:0 + 14:0 combination increased serum HDL cholesterol concentration, a relatively common finding in human feeding studies.

## REFERENCES

1. Hegsted DM, McGandy RB, Myers ML, et al: Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr* 17:281-295, 1965
2. Kris-Etherton PM, Derr J, Mitchell DC, et al: The role of fatty acid saturation on plasma lipids, lipoproteins, and apolipoproteins. I. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter, and milk chocolate on the plasma lipids of young men. *Metabolism* 42:121-129, 1993
3. Bonanome A, Grundy SM: Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N Engl J Med* 318:1244-1248, 1988
4. Denke MA, Grundy SM: Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. *Am J Clin Nutr* 56:895-898, 1992
5. Zock PL, de Vries JHM, Katan MB: Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. *Arterioscler Thromb* 14:567-575, 1994
6. Sundram K, Hayes KC, Siru OH: Dietary palmitic acid results in lower serum cholesterol than does a lauric-myristic acid combination in normolipemic humans. *Am J Clin Nutr* 59:841-846, 1994
7. Khosla P, Hayes KC: Dietary palmitic acid raises plasma LDL cholesterol relative to oleic acid only at a high intake of cholesterol. *Biochim Biophys Acta* 1210:13-22, 1993
8. Hayes KC, Pronczuk A, Lindsey SL, et al: Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. *Am J Clin Nutr* 53:491-498, 1991
9. Hayes KC, Khosla P: Dietary fatty acid thresholds and cholesterolemia. *FASEB J* 6:2600-2607, 1992
10. US Department of Agriculture: Composition of Foods. Fats and Oils. Handbook No. 8-4. Washington, DC, Science and Education Administration, 1979, pp 142
11. Marston R, Raper N: Nutrient content of the U.S. food supply. *Natl Food Rev* (Winter-Spring) 36:18-23, 1987
12. Park S, Snook JT: Does a diet high in corn oil lower LDL-cholesterol levels in women via an effect on LDL-receptor activity? *J Nutr Biochem* 6:88-96, 1995
13. Blair SN, Haskell WL, Ho P, et al: Assessment of habitual physical activity by a seven-day recall in a community survey and controlled experiments. *Am J Epidemiol* 122:794-804, 1985
14. Allain CC, Poon LS, Chan CSG, et al: Enzymatic determination of total serum cholesterol. *Clin Chem* 20:470-475, 1974
15. Warnick GR, Benderson J, Albers JJ: Dextran sulfate-Mg<sup>2+</sup> precipitation procedure for quantitation of high density lipoprotein cholesterol. *Clin Chem* 28:1379-1388, 1982
16. Fernandez ML, Lin ECK, McNamara DJ: Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation. *J Lipid Res* 33:97-109, 1992
17. Poapst M, Uffman K, Steiner G: The chromogenicity and quantitation of apo B-100 and apo B-48 of plasma lipoproteins on analytical SDS gel electrophoresis. *Atherosclerosis* 65:75-88, 1987
18. McFarlane AS: Efficient trace-labelling of proteins with iodine. *Nature* 173:53, 1958
19. Ho YK, Brown MS, Bilheimer DW, et al: Regulation of low density lipoprotein receptor activity in freshly isolated human lymphocytes. *J Clin Invest* 58:1465, 1976
20. Eriksson M, Berglund L, Rudling M, et al: Effects of estrogen on low density lipoprotein metabolism in males. Short-term and long-term studies during hormonal treatment of prostatic carcinoma. *J Clin Invest* 84:802-810, 1989
21. McNamara DJ, Clifford B, Botha A, et al: Sterol synthesis in vitro in freshly isolated blood mononuclear leukocytes from familial hypercholesterolemic patients treated with probucol. *Biochim Biophys Acta* 833:412-416, 1985
22. Channon KM, Clegg RJ, Bhatnagar D, et al: Investigation of lipid transfer in human serum leading to the development of an isotopic method for the determination of endogenous cholesterol esterification and transfer. *Atherosclerosis* 80:217-226, 1990
23. Sukhija PS, Palmquist DL: Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J Agric Food Chem* 36:1202-1206, 1988
24. Havekes LM, De Knijff P, Beisiegel U, et al: A rapid micro method for apolipoprotein E phenotyping directly in serum. *J Lipid Res* 28:455-462, 1987
25. Steinmetz A: Phenotyping of human apolipoprotein E from whole blood plasma by immunoblotting. *J Lipid Res* 28:1364-1370, 1987
26. Kwon JS, Snook JT, Wardlaw GM, et al: Effects of diets high in saturated fatty acids, canola oil, or safflower oil on platelet function, thromboxane B<sub>2</sub> formation and fatty acid composition of platelet phospholipids. *Am J Clin Nutr* 199:351-358, 1991
27. Kuksis A: Newer developments in determination of structure of glycerides and phosphoglycerides. *Prog Chem Fat Other Lipids* 12:5-163, 1972
28. US Department of Agriculture: Nationwide Food Consumption Survey. Continuing Survey of Food Intakes by Individuals. Men 19-50 Years, 1 Day, 1985. Report No. 85-3. Hyattsville, MD, Nutrition Monitoring Division, Human Nutrition Information Service, 1985
29. Cresanta JL, Farris RP, Croft JB, et al: Trends in fatty acid intakes of 10-year-old children, 1973-1982. *J Am Diet Assoc* 88:178-184, 1988
30. Tholstrup T, Marckmann P, Jespersen J, et al: Fat high in



stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am J Clin Nutr* 59:371-377, 1994

31. Wardlaw GM, Snook JT, Park S, et al: Relative effects on serum lipids and apolipoproteins in men of diets enriched in a saturated fat free of C12:0-16:0 (caprenin) compared to palm oil/palm kernel oil or butter. *Am J Clin Nutr* 61:535-542, 1995

32. Tholstrup T, Marckmann P, Jespersen J, et al: Effect on blood lipids, coagulation, and fibrinolysis of a fat high in myristic acid and a fat high in palmitic acid. *Am J Clin Nutr* 60:919-925, 1994

33. Kris-Etherton PM, Mustad VA: Chocolate feeding studies: A novel approach for evaluating the plasma lipid effects of stearic acid. *Am J Clin Nutr* 60:1029S-1036S, 1994

34. Fernandez ML, Lin ECK, McNamara DJ: Differential effects of saturated fatty acids on low density lipoprotein metabolism in the guinea pig. *J Lipid Res* 33:1833-1842, 1992

35. Spady DK, Woollett LA, Dietschy JM: Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. *Annu Rev Nutr* 13:355-381, 1993

36. Davignon J, Gregg RE, Sing CF: Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 81:1-21, 1988