Effect of Dietary Fat Saturation and Cholesterol on LDL Composition and Metabolism

In Vivo Studies of Receptor and Nonreceptor-mediated Catabolism of LDL in Cebus Monkeys


The mechanism(s) by which polyunsaturated fats reduce low density lipoprotein (LDL) cholesterol and apolipoprotein (apo) B were investigated in 20 Cebus monkeys (Cebus albifrons) fed diets containing corn oil or coconut oil as fat (31% of calories) with or without dietary cholesterol (0.1% by weight) for 3 to 10 years. Coconut-oil feeding compared to corn-oil feeding resulted in significant increases in levels of plasma total cholesterol (176%), very low density lipoprotein (VLDL)-LDL cholesterol (236%), high density lipoprotein (HDL) cholesterol (148%), apo B (78%), and apo A-I (112%). The addition of dietary cholesterol to corn oil compared to corn oil alone resulted in smaller, but significant, increases in levels of total cholesterol (44%), HDL cholesterol (40%), and apo A-I (33%). Although the increases in VLDL-LDL cholesterol were of similar magnitude (52%), they barely failed to reach statistical significance (p<0.08), while the changes in apo B levels were negligible. The addition of dietary cholesterol to coconut oil, compared to coconut oil alone, resulted in no significant changes in lipoprotein cholesterol or apoproteins, although levels of VLDL-LDL cholesterol and apo B values increased 22% and 16%, respectively. Although hepatic free cholesterol content was not altered by diet, coconut-oil compared to corn-oil feeding resulted in significant increases in hepatic cholesteryl esters (236%) and triglycerides (325%), the latter increasing still further when dietary cholesterol was added to coconut oil (563%). To further assess the effects of these dietary changes on LDL metabolism, radiolabeled normal and glucosylated LDL kinetics were performed. The production rate of LDL apo B was not altered by diet. With corn-oil feeding, 63% of LDL catabolism was via the receptor-mediated pathway. Coconut-oil compared to corn-oil feeding resulted in a 50% decrease in receptor-mediated LDL apo B fractional catabolic rate (FCR) and a 27% reduction in nonreceptor-mediated LDL apo B FCR. The addition of dietary cholesterol to corn oil, compared to corn oil alone, resulted in no significant effect on LDL apo B catabolism. The addition of dietary cholesterol to coconut oil, compared to coconut oil alone, was associated with no significant change in nonreceptor catabolism of LDL apo B but with a 58% decrease in receptor-mediated catabolism of LDL (p<0.059). The diet-induced alterations of LDL catabolism were significantly correlated with hepatic lipids, which were enriched in saturated fatty acids. These data indicate that the degree of dietary fatty acid saturation induced greater changes in plasma lipoprotein levels and LDL metabolism than did dietary cholesterol. The significant degree of correlation between LDL catabolism and the accumulation of hepatic lipids enriched in saturated fatty acids suggests that dietary modifications leading to alterations in membrane fatty acyl content may also influence cellular LDL metabolism. (Arteriosclerosis 10:119-128, January/February 1990)

Many experimental studies in humans and animals have demonstrated that diets enriched in polyunsaturated, compared to saturated, fatty acids lower plasma total cholesterol, and in particular, low density lipoprotein (LDL) cholesterol, although the mechanism(s) for this effect remain uncertain. A number of mechanisms have been postulated, including alterations: 1) fecal sterol excretion, 2) rates of de novo cholesterol synthesis, 3) intestinal cholesterol absorption, 4) the distribution of cholesterol between plasma and extrahepatic pools, and 5) rates of synthesis and catabolism of plasma lipoproteins. There have been a number of earlier studies...
that have demonstrated that the reductions in circulating levels of LDL with polyunsaturated fat feeding were associated with increases in fractional catabolic rate (FCR) \(^1\) or with decreases in production rate with no changes in FCR. \(^3\) More recent experimental animal studies indicate that increases in LDL catabolism may be a predominant factor. \(^4\) In addition, it is now apparent that, depending on the species, 60% to 80% of the LDL is catabolized by receptor-dependent mechanisms, \(^5\) and these pathways are influenced by the type of dietary fat and the level of dietary cholesterol. For example, Spady and Dietschy \(^6\) have demonstrated in the hamster that the degree of down-regulation of the LDL receptor by dietary cholesterol can be attenuated by the type of dietary triglycerides, with unsaturated triglycerides up-regulating LDL receptor activity compared to saturated triglyceride. The manner in which specific dietary fatty acids regulate plasma lipoprotein cholesterol levels is unknown. One possibility proposed by Spady and Dietschy \(^6\) and supported by the hepatic lipid fatty acid alterations described in the present study and the diet-induced membrane fatty acid changes described by us \(^8\) suggest that dietary fatty acids can induce changes in cellular membrane lipids that may influence certain metabolic properties, such as receptor-mediated uptake of lipoproteins. In the studies described here, we have chosen the cebus monkey, *Cebus albifrons*, for our study of the effects of dietary fat saturation at levels of dietary cholesterol equivalent to a human consumption of 550 mg/day on receptor- and nonreceptor-mediated catabolism of LDL. We have demonstrated that: 1) at concentrations of dietary cholesterol that approximate human consumption, the degree of dietary fat saturation may be more important than dietary cholesterol in regulating LDL metabolism, 2) the FCR of LDL apo B in monkeys fed unsaturated fat diets was significantly greater than in monkeys fed saturated fat-containing diets with no apparent effects on production rate, and 3) LDL receptor activity was negatively correlated with hepatic triglycerides that were enriched in saturated fatty acids. On the other hand, the coconut oil-induced suppression of LDL receptor activity was not consistently associated with an accumulation of hepatic cholesterol ester and not correlated with free cholesterol content. Thus, in addition to the possibility that dietary saturated fat and/or cholesterol may increase a putative hepatic intra-cellular regulatory pool of cholesterol that can suppress LDL receptor activity, we hypothesize that dietary modifications leading to alterations in membrane fatty acyl content may also influence cellular LDL metabolism.

### Methods

#### Animals and Diets

Twenty adult cebus monkeys (*Cebus albifrons*) between the ages of 5 and 10 years were fed semipurified diets containing 31% of calories as corn oil or coconut oil with or without 0.1% cholesterol (0.3 mg/kcal) for 3 to 10 years (Table 1). Fatty acid analyses of the two dietary fats have been reported elsewhere \(^9\); coconut oil, rather than butter or animal fat, was used because it does not contain any cholesterol, and its ingestion is associated with plasma LDL that is similar in composition to those produced by an average American diet. The animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the University of Lowell Research Foundation and the guidelines prepared by the Committee on Care in Use of Lab Animals of the Institute of Lab Animal Resources, National Research Council (DHEW publication no. 85-23, revised 1985).

#### Lipoprotein Isolation

After an overnight fast, monkeys were tranquilized with ketamine (Bristol Laboratories, Veterinary Products, Syracuse, NY), and blood samples were drawn from the femoral vein into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Plasma was separated at 2500 rpm for 20 minutes at 4°C; phenylmethylsulfonyl fluoride, \(2 \times 10^{-4} \text{M}\), and \(N\)-ethylmaleimide (0.125%, Sigma Chemical, St. Louis, MO) were added as proteolytic and lecithin cholesterol acyltransferase (LCAT) inhibitors, respectively. LDL (1.023 g/ml <d< 1.055 g/ml) were isolated by sequential ultracentrifugation \(^10\) at 10°C with a Beckman 70.1 Ti rotor and were dialyzed against 0.9% NaCl, 0.04% EDTA, and 0.01% NaN\(_3\), pH 7.0.

#### Analytical Methods

Total protein was estimated by the method of Lowry \(^12\) by using bovine serum albumin (BSA) as a standard with no correction for possible differences in chromogenicity. Cholesterol \(^13\) and triglycerides \(^14\) were quantified with enzymatic methods, and HDL cholesterol was estimated after heparin-Mn\(^{2+}\) precipitation \(^15\) of VLDL and LDL. Assays were standardized by participation in the Centers for Disease Control/National Heart, Lung and Blood Institute Standardization Program. Unesterified cholesterol was measured by omitting the cholesterol esterase from the enzymatic reagent, \(^13\) and phospholipids were quantitated by the method of Bartlett. \(^16\) Apo A-I and apo B were measured by radial immunodiffusion (RID) using anti-cesus apo A-I and LDL as we have previously described. \(^10\) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Weber and Osborne. \(^17\) Liver and adipose tissue samples were obtained by biopsy in anes-
DIET EFFECTS ON LDL METABOLISM Nicolosi et al. 121

Metabolic Studies

LDL was isolated and concentrated by sequential isopycnic ultracentrifugation at a density of 1.023 to 1.055 g/ml, was dialyzed extensively against 0.9% NaCl, and was divided into two portions. One portion was used to prepare glucosylated LDL [LDL<sub>glc</sub>] as described previously. In a typical experiment, 5 to 10 mg of LDL protein was incubated at 37°C for 48 hours in a phosphate-buffered saline (PBS) solution containing 200 mM of glucose and 30 mM of sodium cyanoborohydride. The second portion containing control or native LDL [LDL<sub>con</sub>] was prepared by incubating half of the original LDL preparation under identical conditions except for the omission of glucose. After each incubation, free glucose and cyanide were removed by extensive dialyses against PBS. LDL was radioiodinated with 125I iodine (or 131I iodine) by a modification of the method of MacFarlane. The specific activities of LDL<sub>glc</sub> and LDL<sub>con</sub> ranged from 50 to 100 cpm/ng of protein.

Preparation of Animals for Metabolic Studies

Monkeys were prepared for the metabolic studies as we have previously described. Briefly, monkeys were acclimated to metabolic restraining chairs before the study. This was accomplished over several weeks by introducing each monkey to the chair for short periods (less than 1 hour) and eventually up to 8 hours, the longest duration of chair-restraint to which each monkey was subjected. On the morning of the experiment, temporary venous catheters were implanted into the femoral and saphenous veins for isotope injection and blood sampling. After the first 8 hours, the catheters were removed, and the animals were returned to their respective cages. All subsequent blood samples at 24 to 96 hours were collected from unrestrained animals under anesthesia. Each morning the appropriate diets in liquid form were fed as a single bolus by orogastric tube to receive adequate caloric intake. In addition, all monkeys received an oral solution (0.5 to 1.0 ml) containing 0.5% l, and 1% KI (Lugol’s solution). After simultaneous injection of LDL<sub>glc</sub> and LDL<sub>con</sub> radiolabeled with 125I or 131I-iodine into each recipient monkey, 2 mls of blood were collected at appropriate times (10 minutes, 1, 3, 6, 8, 24, 48, and 72 hours) into EDTA-containing tubes. The total radioactivity was measured in plasma since preliminary data from the present study (not shown) and those from other laboratories have indicated that more than 95% of the label appears in apo B; therefore, the LDL apo B specific activity was considered equivalent to whole LDL specific activity. As a result, the plasma decay curve for this apolipoprotein possesses the same characteristics as the LDL decay curve. The plasma disappearance curve of native or glucosylated LDL apo B (Figure 1) was analyzed by using the general principles defined by Matthews. The slopes (b<sub>1</sub> and b<sub>2</sub>) and the intercepts (c<sub>1</sub> and c<sub>2</sub>) were calculated (as we have reported previously) by computer methods with a curve-peeling program that uses least-squares analyses.

![Figure 1. Plasma decay curve for low density lipoprotein (LDL) apo B after injection of native 125I-LDL (o) and glucosylated 131I-LDL (•). Each value represents the mean±standard deviation of 20 cebus monkeys fed diets containing either corn oil or coconut oil. The retarded rate of decay of glucosylated LDL by the nonreceptor pathway compared to that of native LDL by the receptor and nonreceptor pathways is shown.]

Statistical Analyses

All results are presented as the means±the standard deviations. A two-way analysis of variance was used to simultaneously test for the effect of dietary fat and cholesterol, and comparisons were evaluated by Newman-Keuls variant analysis and multivariate linear regression analysis. Pearson’s correlations were performed to test for associations between parameters.

Results

As expected, cebus monkeys fed the diet with saturated fat (coconut oil) compared to those fed the diet with polyunsaturated fat (corn oil) had significantly greater total cholesterol (176%), VLDL-LDL cholesterol (236%), and HDL cholesterol (148%) (Table 2). The addition of dietary cholesterol to corn oil compared to corn oil alone resulted in smaller, but significant, increases in plasma total cholesterol (44%), VLDL-LDL cholesterol (52%), and HDL cholesterol levels (40%). Unexpectedly, the coconut oil plus cholesterol group had plasma cholesterol levels similar to the coconut oil-fed animals because the 22% increase in VLDL-LDL cholesterol in the coconut oil plus cholesterol group was partially offset by a corresponding 13% decrease in HDL. Plasma triglyceride levels were not significantly influenced by dietary fat or cholesterol, although values were highest in the coconut oil plus cholesterol group (Table 2).

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Table 2. Effect of Dietary Fat Saturation and Cholesterol on Plasma Lipids, Lipoprotein Cholesterol, and Apolipoproteins

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Total* chol</th>
<th>HDL+* chol</th>
<th>VLDL+LDL+* chol</th>
<th>Total trig</th>
<th>Apo B*</th>
<th>Apo A-I*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>127±36</td>
<td>85±21</td>
<td>42±11</td>
<td>40±18</td>
<td>50±15</td>
<td>181±47</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>351±50</td>
<td>211±30</td>
<td>140±30</td>
<td>42±30</td>
<td>89±18</td>
<td>384±90</td>
</tr>
<tr>
<td>Corn oil+ cholesterol</td>
<td>183±12</td>
<td>119±14</td>
<td>64±8</td>
<td>45±3</td>
<td>54±4</td>
<td>242±13</td>
</tr>
<tr>
<td>Coconut oil+ cholesterol</td>
<td>356±56</td>
<td>184±37</td>
<td>172±38</td>
<td>58±11</td>
<td>103±22</td>
<td>343±67</td>
</tr>
</tbody>
</table>

Values are the means±the standard deviations for five animals in each diet group.

*Difference due to effect of dietary fat, at least p<0.05; tdifference due to effect of dietary cholesterol, at least p<0.05; tdifference due to effect of interaction between dietary fat and cholesterol, at least p<0.05.

Table 3. Effect of Dietary Fat Saturation and Cholesterol on Composition of Plasma Low Density Lipoprotein

<table>
<thead>
<tr>
<th>Distribution (%)</th>
<th>Ratios of constituents (wt/wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FC/PRO*</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.7±0.8</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>8.6±1.0</td>
</tr>
<tr>
<td>Corn oil+ cholesterol</td>
<td>7.5±0.6</td>
</tr>
<tr>
<td>Coconut oil+ cholesterol</td>
<td>6.7±0.6</td>
</tr>
</tbody>
</table>

The values are the means±the standard deviations for five monkeys in each diet group.

*Difference due to effect of dietary fat, at least p<0.05; tdifference due to effect of interaction between dietary fat and dietary cholesterol, at least p<0.05.

FC=free cholesterol, CE=cholesteryl ester, TG=triglyceride, PL=phospholipids, PRO=protein.

Table 4. Effect of Dietary Fat Saturation and Cholesterol on Fatty Acid Distribution of Plasma Low Density Lipoprotein Lipids

<table>
<thead>
<tr>
<th>Lipid class fatty acid</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
<th>Cholesteryl esters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Com</td>
<td>Coco</td>
<td>Com + chol</td>
</tr>
<tr>
<td>12:0*†</td>
<td>ND</td>
<td>0.5±0.1</td>
<td>ND</td>
</tr>
<tr>
<td>14:0*†</td>
<td>0.5±0.2</td>
<td>6.0±0.3</td>
<td>1.0±1.4</td>
</tr>
<tr>
<td>16:0†</td>
<td>29.9±3.6</td>
<td>28.0±1.9</td>
<td>28.2±4.4</td>
</tr>
<tr>
<td>18:0†</td>
<td>31.8±2.6</td>
<td>27.6±2.3</td>
<td>31.2±2.5</td>
</tr>
<tr>
<td>20:4§</td>
<td>8.9±0.2</td>
<td>18.5±1.8</td>
<td>10.4±0.7</td>
</tr>
<tr>
<td>22:1</td>
<td>23.8±2.9</td>
<td>15.7±1.3</td>
<td>27.5±4.3</td>
</tr>
<tr>
<td>20:4§</td>
<td>5.1±3.8</td>
<td>5.7±0.5</td>
<td>1.6±0.9</td>
</tr>
</tbody>
</table>

The values are the means±the standard deviations for five monkeys in each diet group.

*Difference in PL, TG, and CE due to dietary fat, at least p<0.05; tdifference due to effect of dietary fat, at least p<0.05; tdifference due to effect of dietary fat and dietary cholesterol, at least p<0.05.

**Corn**=corn oil, **Coco**=coconut oil, **chol**=cholesterol, **ND**=not detected.

Low Density Lipoprotein Lipid Composition

The LDL from animals fed coconut oil without cholesterol had relatively more free cholesterol than the LDL from corn oil-fed monkeys (Table 3). The constituent ratios indicate that the free cholesterol content of LDL from animals fed the coconut-oil diet was significantly higher than that from monkeys on the corn-oil diet (Table 3). The triglyceride/protein ratio indicated that the content of LDL triglyceride was influenced by the interaction of dietary fat and cholesterol.

Composition of Low Density Lipoprotein Esterified Fatty Acids

The nature of the fatty acids esterified to triglyceride differed substantially, while phospholipids were the least affected (Table 4). The triglyceride fatty acids generally reflected the diet composition with some notable exceptions. For example, although coconut oil contains approximately 49% lauric acid (12:0),10 the content of laurate esterified to LDL triglyceride was minimal. In contrast, myristate (14:0) was incorporated into all lipid classes in coconut oil-fed monkeys. Both LDL triglycerides and cholesteryl esters were enriched in palmitate (16:0) in coconut oil-fed monkeys compared to the corn-oil group. While neither diet contained significant amounts of stearate (18:0), this fatty acid accounted for approximately 30% of the phospholipid fatty acids. Although the triglyceride and cholesteryl ester fractions were not as enriched in stearate as the phospholipids, monkeys fed diets containing coconut oil had less stearate in these fractions.
As expected, the high concentration of laurate in the triglyceride fatty acids was influenced to the greatest extent by diet, while phospholipids were the least affected. The enrichment of the phospholipid and cholesteryl ester fraction by arachidonate (20:4) was significantly more oleate and less linoleate than did monkeys fed corn oil. The major effect of dietary fat saturation was on the levels of oleate (18:1) in the phospholipid and cholesteryl ester fraction and linoleate (18:2) in all three lipid fractions. Monkeys fed coconut oil had significantly more oleate and less linoleate than did animals fed corn oil (Table 3). The levels of linoleate esterified to all hepatic and adipose tissue lipid classes were dramatically reduced in monkeys fed coconut oil (Table 6).

**Hepatic Lipid Content**

Compared to monkeys fed corn oil, livers from monkeys fed coconut oil had significantly more cholesterol ester (263%) and triglycerides (325%) (Table 5). However, such differences in hepatic cholesterol ester content relative to corn oil were not observed in the coconut plus cholesterol group, although triglyceride content continued to increase (563%). No significant diet effects on hepatic free cholesterol content were observed.

**Composition of Liver and Adipose Tissue Esterified Fatty Acids**

Similar to the LDL lipid fatty acid composition, hepatic triglyceride fatty acids were influenced to the greatest extent by diet, while phospholipids were the least affected. As expected, the high concentration of laurate in the coconut oil diet resulted in substantial deposition of laurate in adipose tissue triglycerides (Table 6). Both liver and adipose tissue triglycerides were enriched in myristate. As in the LDL fatty acid data, hepatic cholesterol ester, and in particular, triglycerides, were strikingly enriched in palmitate. The content of oleate esterified to hepatic phospholipid and cholesteryl esters in monkeys fed coconut oil was increased while oleate levels of both hepatic and adipose tissue triglycerides were reduced compared to the levels in animals fed corn oil. The levels of linoleate esterified to all hepatic and adipose tissue lipid classes were dramatically reduced in monkeys fed coconut oil (Table 6).

**Apolipoprotein Concentration**

Plasma apo B and apo A-I levels were 78% and 112% higher, respectively, in monkeys fed the coconut-oil diet compared to those fed corn oil (Table 2). Addition of dietary cholesterol tended to influence apoprotein levels, but not all of these changes were statistically significant. For example, the addition of cholesterol to coconut oil increased plasma apo B concentration approximately 16%. The addition of cholesterol to corn oil caused a 33% increase in apo A-I with no effect on apo B levels. As with HDL, the addition of cholesterol to coconut oil reduced apo A-I (Table 2). Both 12.5% and 3.5% SDS-PAGE of the LDL apoproteins revealed only apo B-100 as the major apoprotein (gel not shown).

**Kinetic Analyses of Low Density Lipoprotein**

The plasma pools of apo B in saturated fat-fed monkeys with or without dietary cholesterol were 75% to 85% larger than in unsaturated fat-fed animals (Table 7). Figure 1 illustrates the predictable retarded clearance of glucosylated LDL taken up by nonreceptor pathways.

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**Table 5. Effect of Diet on Hepatic Lipid Content**

<table>
<thead>
<tr>
<th>Diet groups (n=4)</th>
<th>Hepatic lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free cholesterol</td>
</tr>
<tr>
<td>Corn oil</td>
<td>14±4</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>17±8 (21%)</td>
</tr>
<tr>
<td>Corn oil + cholesterol</td>
<td>15±4 (7%)</td>
</tr>
<tr>
<td>Coconut oil + cholesterol</td>
<td>17±4 (21%)</td>
</tr>
</tbody>
</table>

The results are expressed as μg/mg tissue protein, means±standard deviations (% change from corn-oil diet).

*Significantly different from corn, at least p<0.05; †significantly different from corn and corn+cholesterol, at least p<0.05.

**Table 6. Effect of Dietary Fat Saturation on Hepatic and Adipose Tissue Lipid Fatty Acids**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Cholesterol esters</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Adipose tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coco</td>
<td>Coco</td>
<td>Coco</td>
<td>Coco</td>
</tr>
<tr>
<td>12:0§</td>
<td>ND</td>
<td>0.2±0.2</td>
<td>2.6±0.6</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>14:0*</td>
<td>0.4±0.1</td>
<td>2.2±1.1</td>
<td>0.5±0.1</td>
<td>18.5±2.3</td>
</tr>
<tr>
<td>16:0†</td>
<td>23.8±2.9</td>
<td>39.8±5.4</td>
<td>57.9±4.8</td>
<td>19.1±2.8</td>
</tr>
<tr>
<td>18:0</td>
<td>9.9±1.9</td>
<td>10.1±1.8</td>
<td>15.1±2.6</td>
<td>7.6±3.2</td>
</tr>
<tr>
<td>18:1†</td>
<td>25.0±2.1</td>
<td>40.6±5.3</td>
<td>21.6±2.3</td>
<td>13.9±2.8</td>
</tr>
<tr>
<td>18:2†</td>
<td>34.0±4.2</td>
<td>6.7±1.1</td>
<td>32.6±3.4</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>20:4§</td>
<td>6.8±3.4</td>
<td>0.5±0.3</td>
<td>9.4±2.8</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

The values are the means±the standard deviations for four monkeys in each diet group.

*Difference in liver and adipose tissue TG, at least p<0.05; †difference in liver CE, TG, PL, and adipose tissue TG, at least p<0.05; □difference in liver CE, TG, and adipose tissue TG, at least p<0.05; §difference in liver CE and TG, at least p<0.05.

See the legends for Tables 3 and 4 for an explanation of abbreviations.
The values represent the means ± the standard deviations. The values were calculated based on biphasic exponential die-away of radiolabeled low density lipoprotein (LDL) apolipoprotein, assuming a metabolic steady state. Pool size was calculated as the plasma volume (4% of body weight) × plasma apoprotein concentration.

*Differs due to dietary fat, at least p < 0.05.
†Production rate = fractional catabolic rate of LDLapoB × apo B pool size.
LDLapoB = native LDL; LDLapoB = glucosylated LDL metabolized by nonreceptor pathways; LDLapoB = LDL metabolized by receptor pathways derived from the difference between LDLapoB and LDLapoB.

### Table 7. Correlation Coefficients of Low Density Lipoprotein Metabolism and Plasma and Hepatic Lipid Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LDLapoB*</th>
<th>LDLapoB*</th>
<th>LDLapoB*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol</td>
<td>-0.786 (p &lt; 0.001)</td>
<td>-0.790 (p &lt; 0.0001)</td>
<td>-0.732 (p &lt; 0.002)</td>
</tr>
<tr>
<td>Plasma triglyceride</td>
<td>-0.286 (NS)</td>
<td>-0.356 (NS)</td>
<td>-0.257 (NS)</td>
</tr>
<tr>
<td>Plasma apo B</td>
<td>-0.520 (p &lt; 0.05)</td>
<td>-0.576 (p &lt; 0.02)</td>
<td>-0.456 (p &lt; 0.08)</td>
</tr>
<tr>
<td>Liver total cholesterol</td>
<td>-0.559 (p &lt; 0.03)</td>
<td>-0.530 (p &lt; 0.04)</td>
<td>-0.537 (p &lt; 0.04)</td>
</tr>
<tr>
<td>Liver cholesterol ester</td>
<td>-0.571 (p &lt; 0.02)</td>
<td>-0.566 (p &lt; 0.02)</td>
<td>-0.541 (p &lt; 0.04)</td>
</tr>
<tr>
<td>Liver free cholesterol</td>
<td>-0.432 (NS)</td>
<td>-0.366 (NS)</td>
<td>-0.429 (NS)</td>
</tr>
<tr>
<td>Liver triglyceride</td>
<td>-0.667 (p &lt; 0.007)</td>
<td>-0.611 (p &lt; 0.01)</td>
<td>-0.641 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

LDLapoB = native low density lipoprotein (LDL) catabolized by receptor and nonreceptor pathways; LDLapoB = glucosylated LDL catabolized by nonreceptor pathways; LDLapoB = LDL catabolized by receptor-mediated pathways.

Compared to native LDL, the calculated production rates for LDL apo B were not influenced by diet. Receptor-mediated LDL catabolism was determined by measuring the FCR of glucosylated LDL and then subtracting this value from the FCR of native LDL to obtain the FCR of LDL via the receptor-mediated pathway. With corn-oil feeding, 63% of LDL catabolism was via the receptor-mediated pathway (Table 7). Coconut-oil feeding as compared to corn-oil feeding resulted in a 50% decrease in receptor-mediated LDL apo B FCR and a 27% reduction in nonreceptor-mediated LDL apo B FCR. The addition of dietary cholesterol to corn oil as compared to corn oil alone resulted in no significant effect on LDL apo B catabolism by either nonreceptor or receptor-mediated pathways (Table 7). The addition of dietary cholesterol to coconut oil as compared to coconut oil alone was associated with no change in nonreceptor catabolism of LDL apo B, but with a 58% decrease in receptor-mediated catabolism (p < 0.059).

### Correlations of Low Density Lipoprotein Metabolism and Lipid Parameters

Correlations between pathways of nonreceptor- and receptor-mediated catabolism of LDL and specific lipid parameters are shown in Table 8. As expected, receptor-mediated catabolism of LDL was negatively correlated with plasma total cholesterol (r = 0.732, p < 0.002). Surprisingly, nonreceptor-mediated catabolism of LDL was also negatively correlated with plasma cholesterol levels (r = 0.790, p < 0.0001). There were also negative correlations between LDL apo B and nonreceptor-mediated (r = 0.576, p < 0.02) and receptor-mediated (r = 0.456, p < 0.08) catabolism of LDL. While liver cholesterol ester content was negatively correlated with receptor-mediated (r = 0.541, p < 0.04) and nonreceptor-mediated (r = 0.566, p < 0.02) pathways of LDL catabolism, surprisingly, no such relationship existed for hepatic free cholesterol. Unexpectedly, liver triglyceride content was inversely correlated with both receptor (r = 0.641, p < 0.01) and nonreceptor (r = 0.611, p < 0.01) pathways of LDL catabolism.

### Discussion

These studies have demonstrated that, in the presence of dietary cholesterol levels that approximate human consumption, the degree of dietary fat saturation produces significant alterations in: 1) plasma lipoprotein cholesterol and apoprotein levels; 2) plasma LDL, hepatic, and adipose tissue fatty acyl content; 3) hepatic cholesterol ester and triglyceride accumulation; and 4) LDL catabolism via receptor-dependent and nonreceptor-dependent pathways. Dietary cholesterol at 0.1% of the diet (wt/wt) had smaller effects on LDL cholesterol and apo B levels compared to dietary fatty acids but, surpris-
tingly, even this level of dietary cholesterol when added to coconut oil was associated with reductions in HDL cho-
estroler and apo A-I, a finding more often attributed to feeding much higher levels of dietary cholesterol to nonhuman primates. It has been reported that the mechanism associated with the decline in HDL with high levels of cholesterol feeding is secondary to an enhanced fractional catabolic rate. We are currently investigating the metabolism of HDL apo A-I in these monkeys fed levels of dietary cholesterol that approximate human consumption.

In this study, we report only minimal diet-induced alterations in plasma LDL composition, suggesting that the observed elevations in lipoprotein cholesterol in these studies were due to increases in the number of LDL particles. This is in contrast to other nonhuman primate studies in which the feeding of excessive levels of dietary cholesterol was associated with changes in lipoprotein lipid and apoprotein composition. The enrichment of plasma LDL and of hepatic and adipose tissue lipids by the fatty acids that composed the diet treatments were, in general, predictable. The minimal deposition of laurate by plasma LDL and hepatic membranes may be the result of natural conservation of specific fatty acids or elongation of laurate to palmitate or stearate. This may account for the significant incorporation of palmitate by plasma LDL and hepatic lipids despite its paucity in the diet, implying a significant contribution from synthetic pathways involving active elongation. This suggests that differences may exist in those factors that regulate the contribution of exogenous versus endogenous sources of fatty acids in determining the fatty acid composition of various lipopro-
tins and cell membranes. The effect of dietary fat satu-
ration was most profound in the relative enrichment of LDL, and of hepatic and adipose tissue membranes by oleate and linoleate, with monkeys fed coconut oil having more of the former and monkeys fed corn oil, more of the latter.

Alterations in the fatty acid composition of LDL cho-
esteryl esters similar to those described in these studies may contribute to the atherogenic nature of LDL. For example, an increase in the content of saturated fatty acids elevates the transition temperature of LDL above the body temperature of the animal. Thus, the satu-
rated fatty acid-containing LDL would have liquid crystal-
line cores at body temperature, and as other studies have shown, cells in culture loaded with lipid crystalline cholesteryl esters after incubation with the saturated fatty acid-containing LDL have lower cholesterol efflux rates than do cells loaded with cholesteryl ester containing unsaturated fatty acids in the liquid state.

The failure to demonstrate any significant dietary satu-
rated fat or cholesterol-induced increase in hepatic free cholesterol content (Table 5) and the lack of any signifi-
cant correlation between hepatic free cholesterol content and receptor or nonreceptor pathways of LDL catabolism (Table 8) is surprising in view of the reported role that hepatic free cholesterol content plays in the regulation of LDL receptors, especially in familial hypercholestero-
lemia. On the other hand, at least two reports indicate that saturated fat compared to polyunsaturated fat feeding is not associated with increased hepatic choles-
terol content. Thus, in the studies by Spady and Dietschy, in which LDL receptor activity was reduced by coconut-oil plus cholesterol feeding, livers from hamsters fed high saturated fat and cholesterol-containing diets accumulated less hepatic cholesterol than did those fed polyunsaturated fat. Similarly, in the present study, total hepatic cholesterol content in monkeys fed corn oil or coconut oil plus cholesterol was virtually identical, and therefore we cannot explain the significant differences in LDL catabolism as a function of dietary fat saturation. However, since the small size of the liver biopsies taken for lipid quanti-
tation precluded the fractionation of hepatic tissue chole-
esteryl into membrane and cytoplasmic compartments (a caveat which also exists in the above-mentioned studies), it is possible that small regulatory pools of intracellular cholesterol, which could not be measured in this study, may have accumulated to a greater extent in saturated fat-fed monkeys in which LDL receptor activity was suppressed. Hepatic triglyceride content rose with increasing dietary fat saturation, and when cholesterol was added to coconut oil, these changes were negatively correlated with LDL catabolism by receptor and nonreceptor pathways (Tables 7 and 8). The mechanism(s) for this association remain to be determined.

One possible, indirect manner in which hepatic triglyceride stores enriched in saturated fatty acids may elevate circulating LDL levels is by enhancing the hepatic pro-
duction of cholesteryl ester-rich lipoprotein particles. For example, we have previously shown that coconut-oil feeding in monkeys increases the circulating levels of cholesteryl ester-rich VLDL, which was largely explained by increased hepatic production.

It has also been shown that up to 80% of cholesteryl ester-rich LDL in saturated fat-fed monkeys may be derived from the catabolism of VLDL. Since LDL enriched in saturated fatty acids are not metabolized as efficiently as those enriched in polyunsat-
urated fatty acids, this may partially explain the elevated LDL in saturated fat-fed monkeys.

The hepatic phospholipids were moderately enriched by dietary fatty acids when certain assumptions were made. Based on the early studies of Keys et al. and Hegsted et al., which reported the neutrality of stearate compared to other saturates and the recent studies of Bonanome and Grundy, which reported stearate's lipid-lowering characteristics compared to other saturates, stearate was excluded as a hypercholesterolemic satu-
rated fatty acid from the comparisons. Saturates (C12 to C16) were then compared to polyunsaturates (C18:2, C20:4) of the hepatic phospholipid fraction of animals fed the different diets. Thus, the hepatic phospholipid fractions in the monkeys fed corn oil were enriched in polyunsaturates (39%) compared to that of monkeys fed coconut oil, while in the latter, the fractions were enriched in saturates (39%). These fatty acid alterations are also reflected by the differences in the polyunsaturated/saturated (P/S) ratio of the hepatic phospholipid fatty acids, i.e., 1.91 vs. 0.71 for the monkeys fed corn oil and the monkeys fed coconut oil.

The metabolic significance of the relative incorporation of predominantly saturated versus polyunsaturated fatty
acids in liver was not studied at the cellular level in the present communication, but it has been previously documented that a similar degree of diet-induced fatty acid enrichment seen in the hepatic phospholipids reported in this article resulted in alterations in membrane fluidity of mononuclear cells that were significantly correlated with the extent of LDL degradation. Thus, the finding that mononuclear cell metabolism of LDL reflects hepatic patterns, coupled with the observation that the liver is responsible for up to 90% of receptor-mediated catabolism of LDL, raises the possibility that the fatty acid changes observed in the livers of the monkeys studied may contribute to the diet-associated alterations in LDL metabolism.

The manner in which fatty acid changes can influence the LDL receptor is not known, but alterations in LDL receptor conformation that induce a larger ligand affinity or an obligate functional requirement for specific fatty acyl moieties in the LDL receptor lipid domain are possibilities that have been previously raised. There were no significant effects of dietary fat or cholesterol on LDL apo B production rate, although our investigations did not measure VLDL apo B production but only apo B production in the LDL density region.

The increase in the FCR of LDL apo B from monkeys fed polyunsaturated fat compared to monkeys fed saturated fat is in agreement with other studies. This finding was totally dependent on the diet composition of the recipient monkey, because the diet effect of the donor LDL was not significant, as we have noted (unpublished data) and others have reported, possibly due to the low levels of dietary cholesterol or to the lack of diet-induced alteration of LDL composition. This is in contrast to our recent observations where the donor LDL had a significant influence on LDL degradation. This may be due to the fact that in our in vitro mononuclear cell culture system, the donor LDL was the sole source of lipoprotein, whereas in the in vivo studies reported here, donor LDL represented only a trace amount of the LDL circulating in the recipient monkey. In the normocholesterolemic monkeys fed corn oil, the receptor-mediated pathway accounted for 63% of the total LDL catabolized, which is in agreement with other experimental and some human studies of individuals with normal serum cholesterol values. Compared to corn oil feeding, coconut-oil feeding was associated with a 50% reduction in receptor-mediated catabolism of LDL in the liver, a finding similar to the reports of Spady and Dietzsch. However, one significant difference between our findings and their reports is that only in hamsters fed dietary cholesterol did polyunsaturated fat feeding attenuate the down-regulation of LDL receptor activity. Also, in contrast to our studies, in which the polyunsaturated fat-fed monkeys without cholesterol up-regulated LDL receptor activity compared to coconut-oil feeding, the hypocholesterolemia in hamsters fed corn oil was associated with a decreased production rate. However, this decrease in the production rate of LDL, or conversion of VLDL to LDL, may have been in response to an increase in the activity of LDL receptor-mediated uptake of precursors of LDL, i.e., VLDL and VLDL remnants, so that less of these were converted to LDL. The addition of dietary cholesterol to coconut oil compared to coconut oil alone resulted in a 58% reduction in the receptor-mediated catabolism of LDL apo B (p < 0.059). Unexpectedly, the type of dietary fat also influenced nonreceptor, and presumably lipoprotein, uptake by endocytosis. We hypothesize that coconut-oil feeding causes cellular membranes to be tightly packed and highly ordered by the predominantly saturated fatty acid nature of the coconut-oil diet, while corn-oil feeding is associated with cellular membranes that are more fluid and less organized with lipid bilayers which are loosely packed, reflecting the nature of the unsaturated fatty acids in corn oil. Thus, the greater spatial distances between the membrane lipid bilayers in the monkeys fed corn oil may hypothetically permit LDL to enter by nonreceptor pathways via endocytosis. An alternative explanation for the effect of diet on the nonreceptor uptake of LDL could be that glucosylated LDL may be partially taken up by LDL receptors in vivo, although numerous in vitro studies have shown that reductive glucosylation of LDL is irreversible and completely blocks receptor-mediated LDL uptake. In monkeys fed oils with less divergent degrees of dietary fat unsaturation, saturated fatty acids feeding down-regulates the receptor-mediated catabolism of LDL without influencing nonreceptor pathways.

Although responsiveness to cholesterol or dietary fat does vary among individuals and species, our data are consistent with human studies, which indicate that dietary fat saturation compared to dietary cholesterol appears to be a more important factor in the regulation of LDL concentration. In conclusion, the precise mechanism for the increase in LDL receptor activity upon exchanging dietary unsaturated fatty acids for saturated fatty acids remains elusive. Although changes in the concentration of a metabolically active hepatic pool of intracellular cholesterol, which were not investigated in this study, can influence LDL receptor activity, our studies also suggest that dietary fatty acids can induce significant alterations in plasma LDL and hepatic lipid fatty acyl content and that these fatty acid changes may be associated with alterations in membrane structure, which can concomitantly influence cellular metabolism of LDL.

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DIET EFFECTS ON LDL METABOLISM Nicolosi et al.


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