Effect of Dietary Fat Saturation and Cholesterol on Low Density Lipoprotein Degradation by Mononuclear Cells of Cebus Monkeys

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The mechanism by which dietary unsaturated fatty acids lower low density lipoprotein (LDL) cholesterol is unknown. Unsaturated fatty acids incorporated into the cell membrane can increase membrane fluidity and, as a result, dramatically alter membrane-dependent cell functions. Therefore, we examined the effect of long-term dietary consumption of corn oil and coconut oil with and without cholesterol in amounts equivalent to those of a typical Western diet on the degradation of human LDL by peripheral blood mononuclear cells in Cebus albifrons monkeys. Cellular LDL degradation was dramatically enhanced in the mononuclear cells isolated from animals fed corn oil in comparison with those from animals fed coconut oil. The addition of cholesterol to the diets resulted in a slight attenuation of LDL degradation in the corn oil group while no effect was noted in the coconut oil group. Crossover LDL binding and degradation experiments with LDL isolated from animals fed corn oil diets and coconut oil diets demonstrated increased binding and degradation of LDL in mononuclear cells from animals fed corn oil diets. Enhanced mononuclear cell LDL degradation was accompanied by increased cellular cis-unsaturated fatty acyl content, increased membrane fluidity, and decreased plasma cholesterol. Increased cellular cis-unsaturated fatty acyl content with its concomitant increase in membrane fluidity mirrored the dietary lipid profile of the host animal. A linear relationship was observed between cellular LDL degradation and both cellular cis-unsaturated fatty acyl content and membrane fluidity. These observations parallel results noted in whole-animal LDL catabolic studies with these same animals described elsewhere. These data suggest a novel mechanism by which dietary unsaturated fatty acids exert their LDL-lowering effect.

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Epidemiologic studies have linked diets rich in unsaturated fatty acids to a reduction in low density lipoprotein (LDL) cholesterol, but the molecular mechanism by which this phenomenon occurs remains poorly understood. A number of possible mechanisms have been suggested, including modification in: 1) de novo cholesterol synthesis, 2) cholesterol absorption from the small intestine, 3) physicochemical properties of the LDL surface coat or core, 4) cholesterol distribution between plasma and various intrahepatic pools, 5) fecal sterol or cholesterol excretion, 6) partitioning of cholesterol among “cellular” pools, 7) apolipoprotein metabolism, and 8) rates of LDL metabolism. Recent human and animal studies strongly support increased LDL metabolism as a major factor. The actual cellular mechanism by which LDL clearance is enhanced, however, remains unknown.

Alterations in the bulk physical properties of plasma membranes can produce changes in membrane-dependent cellular functions. Dietary fatty acids readily incorporate into cell membranes and can modify the physical properties of these membranes. Increasing cis-unsaturated fatty acyl composition increases membrane fluidity (or decreases microviscosity and order), while increasing the saturated fatty acyl composition decreases membrane fluidity. Similarly, increasing the cholesterol content of membranes decreases membrane fluidity and can produce perturbations in cellular functions. For example, capping of surface immunoglobulins in lymphocytes, platelet aggregability, erythropoiesis by macrophages, and receptor binding of β-adrenergic, serotoninergic, or opiate ligands in liver cells are modulated by changes in membrane lipid content and fluidity.

We hypothesized that the reduction in LDL cholesterol produced by dietary unsaturated fatty acids is the result of their direct effect on membrane-mediated LDL clearance. Previous work by this laboratory has demonstrated that incorporation of cis-unsaturated fatty acids into peripheral blood mononuclear cells by brief exposure to free fatty acid enhances cellular clearance of LDL by increasing LDL...
uptake and degradation. The present study was undertaken to investigate the effect of long-term dietary supplementation with unsaturated fatty acids in the form of corn oil or with saturated fatty acids in the form of coconut oil with and without added cholesterol on mononuclear cell LDL degradation in Cebus albifrons monkeys. Animals maintained on diets rich in unsaturated fatty acids are known to have increased hepatic fractional catabolic rates for LDL. Thus, in the ex vivo setting we studied LDL degradation, membrane fluidity, and fatty acyl content in peripheral blood mononuclear cells as functional equivalents of hepatocytes. Our results indicate that dietary unsaturated fatty acids enhance cellular LDL degradation and that this effect is accompanied by cellular enrichment in these fatty acids and decreased membrane microviscosity.

**Methods**

**Materials**

Na-125I was purchased from Amersham, Arlington Heights, IL. Sepacell-MN was purchased from Sepratech, Oklahoma City, OK. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was obtained from Sigma Chemical, St. Louis, MO. High-performance liquid chromatography-grade hexane, isopropanol, petroleum ether, methanol, acetyl chloride, and chloroform were obtained from Aldrich Chemical, Milwaukee, WI. Hepatidoceneate and fatty acyl methyl ester gas chromatography standards were purchased from NuChek Prep, Elysian, MN. All other chemicals used were reagent grade or better.

**Animals and Diets**

Adult Cebus monkeys (Cebus albifrons) between the ages of 5 and 10 years were fed semi-purified diets containing 31% of calories as corn oil or coconut oil, with or without 0.1% cholesterol, for 3 to 10 years. Details of the diet compositions, as well as the fatty acid analyses, have been reported elsewhere. Plasma cholesterol values were measured from freshly isolated serum obtained from the same blood specimen from which mononuclear cells were isolated.

**Low Density Lipoprotein Preparation and Iodination**

Human LDL (density 1.006 to 1.063 g/ml) and monkey LDL (1.023 to 1.095 g/ml) were isolated from plasma by sequential ultracentrifugation in 50 mM tris-[hydroxymethyl]aminomethane (Tris), 5 mM disodium ethylenediaminetetraacetate (EDTA), pH 7.4.17 Potassium bromide was used to adjust the solvent densities. The LDL was then sequentially dialedyzed with three changes of 50 mM Tris, pH 7.4. The total protein content was determined by the method of Lowry et al.18 LDL cholesterol was measured with a colorimetric method using cholesterol oxidase, peroxidase, 4-hydroxybenzozate, and 4-amino-phenazone. Polyacrylamide gel electrophoresis (4.3%) was performed to ensure the purity of the LDL. LDL prepared in this manner typically had a cholesterol-to-total protein mass ratio of 1.3 to 1.7 for human LDL, 2.5 for LDL from monkeys fed coconut oil, and 1.2 for LDL from monkeys fed corn oil. LDL was radioiodinated by the iodine monochloride method of McFarlane, as modified by Langer and colleagues and Sheperd and coworkers. Iodinated in this fashion, LDL had a specific activity of 300 to 800 cpm/ng protein.

**Mononuclear Cell Preparation**

Whole blood was obtained from fasting monkeys and was anticoagulated with 100 U/ml heparin. Mononuclear cells were prepared by mixing 20 ml of whole blood with 20 ml of Sepacell-MN solution. The suspension was then centrifuged at 1200 g for 20 minutes at 27°C. The mononuclear cells were harvested from the Sepacell-MN meniscus. The cells were washed twice with Hanks' balanced salt solution containing 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4 (Hanks'-HEPES). Cellular concentrations were determined with a Coulter Counter, Model ZM, equipped with a 50 μm aperture tube. Cell counts ranged from 1 x 10⁸ cells/ml to 5 x 10⁸ cells/ml. Cell viability was determined by Trypan blue exclusion and was routinely greater than 95%. Mononuclear cell samples prepared in this fashion were comprised of 72% lymphocytes, 22% monocytes, and 4% neutrophils. The samples were maintained at room temperature for up to 30 minutes before use.

**Low Density Lipoprotein Degradation**

The amount of human 125I-LDL degraded by monkey mononuclear cells was determined according to published methods. An aliquot of freshly isolated mononuclear cells in Hanks'-HEPES (pH 7.4) was incubated with human 125I-LDL (80 μg/ml) in the presence or absence of a 20-fold excess of unlabeled human LDL at 37°C for 4 hours. Each assay was performed in the presence of 10 mM CaCl₂. After incubation, an aliquot was removed from each assay to determine the total counts. The samples were treated with 20% trichloroacetic acid to precipitate the degraded, protein-bound iodotyrosine. The precipitate was sedimented by centrifugation at 14 000 g for 3 minutes. The supernatant was counted to determine the amount of acid-soluble, ether-insoluble, radiolabeled material generated by the cells and released into the aqueous phase. Degradation values are expressed as specific degradation, i.e., the difference in degradation in the presence and absence of a 20-fold excess unlabeled LDL. Degradation is expressed as micrograms of 125I-LDL protein degraded per 10⁸ cells/4 hours.

**Monkey Low Density Lipoprotein Crossover Binding and Degradation**

Monkey mononuclear cells were incubated with monkey 125I-LDL in the presence or absence of a 20-fold excess of unlabeled monkey LDL at 4°C for 6 hours to determine specific binding. Each assay was carried out in the presence of 10 mM CaCl₂. All reagents were chilled to 4°C before use. A range of monkey LDL concentrations from 10 μg/ml to 80 μg/ml was used to generate binding isotherms. After incubation, an aliquot from each vial was removed to determine total counts. Surface-bound LDL was separated from free LDL by centrifuging an aliquot of cells through a 0.15 M metrizamide cushion at 14 000 g for
3 minutes at 4°C. The cell pellets were isolated by amputating the tip of the microfuge tube and counting the radioactivity in a gamma counter. The total and bound counts were measured, and specifically bound counts were expressed as micrograms of LDL protein bound per 10^6 cells. LDL degradation was performed as described above.

LDL binding and degradation were performed by using LDL isolated from monkeys fed corn oil and, again, with LDL isolated from monkeys fed coconut oil. Mononuclear cells from both the corn oil- and coconut oil-diet groups were used in this LDL crossover study.

Membrane Fluidity Measurements

Freshly isolated mononuclear cells or plasma membrane vesicles were incubated with DPH.27,28 at a concentration of 1 μM for 1 hour at 25°C, after which the suspension was washed twice with Hank's-HEPES buffer, pH 7.4. Fluorescence measurements were performed with a Spex Fluorolog-2 spectrofluorimeter (Spex Industries, Edison, NJ) equipped with a thermostat-controlled cell holder and a polarization accessory. The steady-state fluorescence polarization was measured at 37°C by excitation at 380 nm and recording the emission at 430 nm. The polarization of fluorescence emission was calculated from the equation:

\[ P = \frac{I_v - G_{hv}}{I_v + G_{hh}} \]

where \( P \) is polarization, \( I \) is the fluorescence intensity, the first and second subscripts refer to the plane of polarization (v=vertical, h=horizontal), and \( G = l_v/l_h \).

Determination of Cellular Cholesterol and Fatty Acyl Content

Mononuclear cells were extracted with hexane-isopropanol (3:2, vol/vol).29 The samples were divided into two aliquots. A heptadecanoate internal standard was added to one aliquot of lipid extract. The fatty acyl groups were transesterified with methanolic-HCl and extracted into petroleum ether. The petroleum ether was then evaporated under nitrogen gas, and the lipids were re-solubilized in chloroform. Separation and identification of the fatty acid methyl esters were performed by gas-liquid chromatography by using a Chrompack CP Sil 88 capillary column in a Hewlett-Packard 5890A gas chromatograph with an automated injector, flame ionization detector, and Hewlett-Packard 3393 integrator. Separation was performed in the isothermal mode at 210°C with an injection volume of 5 μl, helium as the carrier gas, and a split ratio of 60:1. Fatty acid methyl ester gas chromatography standards were used to determine retention times.

The total cellular cholesterol content of the second aliquot was then determined by an enzymatic chromogenic system by using 4-hydroxybenzoate.19 Cholesterol ester/total cholesterol ratio was determined by using the p-hydroxyphenylacetic acid method of Gamble and coworkers.20

Determination of Plasma Membrane Fatty Acyl Content

Pooled mononuclear cell plasma membranes were prepared at 4°C by established methods.13 Mononuclear cell suspensions were sedimented at 200 g for 15 minutes and washed twice in 0.9% NaCl. Monocyes were resuspended in 15 ml of lysis medium (1 mM NaHCO_3, 0.5 mM CaCl_2, pH 7.4) and disrupted in a homogenizer (25 strokes). The cell lysate was diluted to 50 ml and centrifuged at 500 g for 20 minutes. The supernatant was removed and saved. The pellet was resuspended in 25 ml of lysis medium and was rehomogenized. This suspension was then centrifuged at 500 g for 20 minutes, and the supernatant was removed. The two supernatants were combined and sedimented at 12500 g for 20 minutes. The pellet was resuspended in 10 ml lysis medium and was mixed with 30 ml of 53% sucrose prepared in lysis medium to yield a final concentration of 40% sucrose. Then 20 ml of the 40% sucrose was layered under 15 ml of a 30% sucrose solution. This sucrose step gradient was centrifuged at 55000 g for 4 hours with a Ti70 rotor in a Beckman LB-70 ultracentrifuge. After centrifugation, the 10 ml fraction just above the interface was removed from the 30% sucrose layer and was diluted with 25 ml of lysis medium. This solution was then sedimented at 45000 g for 1 hour. The pellet was extracted with hexane-isopropanol (3:2, vol/vol), and determination of the fatty acyl content was performed as described above. This method results in less than 5% contamination with endoplasmic reticular and mitochondrial membrane.13

Statistical Analysis

All results are presented as averages±standard deviations of the means. Analysis of variance, the Newman-Keuls variant analysis, and multivariate linear regression analysis were used for the comparisons.

Results

Effect of Diet on Plasma Cholesterol

Animals maintained on a corn oil-enriched diet without cholesterol (n=5) had an average plasma cholesterol of 139±26 mg/dl; in contrast, animals fed coconut oil-enriched diets without cholesterol (n=4) had an average plasma cholesterol of 342±44 mg/dl (p < 0.01, corn oil vs. coconut oil). The addition of cholesterol to the corn oil diet (n=6) resulted in an average plasma cholesterol of 158±23 mg/dl, while the addition of cholesterol to the coconut oil diet (n=5) led to an average plasma cholesterol of 291±61 mg/dl (p < 0.05, corn oil and cholesterol vs. coconut oil and cholesterol). A comparison of plasma cholesterol values within the corn oil groups and within the coconut oil groups showed that the addition of cholesterol to the diets did not affect the plasma cholesterol in a statistically significant manner.

Effect of Diet on Mononuclear Cell Fatty Acyl Content

The effect of diet on the unsaturated-saturated fatty acyl ratio (U/S) for nonhuman primate mononuclear cells.
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Table 1. Cellular Unsaturated/Saturated Fatty Acyl Ratios

<table>
<thead>
<tr>
<th>Diet</th>
<th>Corn oil (n=5)</th>
<th>Corn oil+cholesterol (n=6)</th>
<th>Coconut oil (n=4)</th>
<th>Coconut oil+cholesterol (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.9±0.8</td>
<td>3.5±1.0</td>
<td>15.9±0.6</td>
<td>12.6±1.4</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.6±0.3</td>
<td>5.3±1.5</td>
<td>25.1±5.2</td>
<td>20.2±2.9</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.5±0.3</td>
<td>4.0±2.9</td>
<td>17.7±4.5</td>
<td>22.2±3.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.2±1.6</td>
<td>7.5±0.8</td>
<td>19.5±6.2</td>
<td>23.0±3.0</td>
</tr>
</tbody>
</table>

The values are averages±SD. The experiments were performed as described in the Methods section.

Table 2. Mononuclear Cell Fatty Acyl Content in Monkey Diet Groups

<table>
<thead>
<tr>
<th>Diet</th>
<th>C12:0</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C20:0</th>
<th>C18:2</th>
<th>C20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (n=5)</td>
<td>0.9±0.8</td>
<td>3.5±1.0</td>
<td>15.9±0.6</td>
<td>12.6±1.4</td>
<td>42.1±1.9</td>
<td>11.2±4.1</td>
<td></td>
</tr>
<tr>
<td>Coconut oil (n=4)</td>
<td>1.6±0.3</td>
<td>5.3±1.5</td>
<td>25.1±5.2</td>
<td>20.2±2.9</td>
<td>20.6±1.5</td>
<td>16.7±4.8</td>
<td></td>
</tr>
<tr>
<td>Corn oil+cholesterol (n=6)</td>
<td>1.5±0.3</td>
<td>4.0±2.9</td>
<td>17.7±4.5</td>
<td>22.2±3.7</td>
<td>19.1±1.2</td>
<td>17.7±3.5</td>
<td></td>
</tr>
<tr>
<td>Coconut oil+cholesterol (n=6)</td>
<td>2.2±1.6</td>
<td>7.5±0.8</td>
<td>19.5±6.2</td>
<td>23.0±3.0</td>
<td>2.8±1.5</td>
<td>19.2±2.2</td>
<td></td>
</tr>
</tbody>
</table>

The values are averages±SD and are given as mole percents. The experiments were performed as described in the Methods section. For a discussion of the significant differences between and among groups, see the Results section.

Table 3. Mononuclear Cell Cholesterol Content

<table>
<thead>
<tr>
<th>Diet</th>
<th>Corn oil (n=4)</th>
<th>Corn oil+cholesterol (n=5)</th>
<th>Coconut oil (n=4)</th>
<th>Coconut oil+cholesterol (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>1.25±0.34</td>
<td>1.94±0.36</td>
<td>2.43±0.53</td>
<td>2.52±0.56</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>0.35±0.09</td>
<td>0.68±0.14</td>
<td>0.80±0.17</td>
<td>0.98±0.21</td>
</tr>
</tbody>
</table>

The values are averages±SD and are given as μg/10⁶ cells. The experiments were performed as described in the Methods section. For a discussion of the significant differences between and among groups, see the Results section.

is shown in Table 1. Animals fed a diet enriched with corn oil alone exhibited the highest U/S, 1.93±0.07, while those fed a diet enriched in coconut oil had a U/S of 0.95±0.04 (p<0.01). The addition of cholesterol to a corn oil-enriched diet decreased the U/S to 1.12±0.17, while the addition of cholesterol to a coconut oil-enriched diet resulted in a U/S of 0.83±0.09 (p<0.01). Cells from corn oil-fed animals without dietary cholesterol had a 72% greater U/S than those given cholesterol supplementation (1.93±0.07 vs. 1.12±0.17, p<0.01). In contrast, cells from animals fed coconut oil with or without cholesterol had U/S ratios that were not statistically different from one another (0.95±0.04 vs. 0.83±0.09).

The effect of diet on the fatty acyl profiles of monkey mononuclear cells is shown in Table 2. The corn oil diet resulted in a 152% increase in C18:2 (p<0.01) with a concomitant 39% decrease in C18:1 (p<0.01), a 32% decrease in C18:0 (p<0.01), and a 37% decrease in C16:0 (p<0.01) in comparison to the coconut oil diet. In the diet groups with cholesterol, corn oil-fed animals again exhibited increased cellular C18:2 content when compared to animals fed coconut oil. Animals maintained on corn oil diets with cholesterol had 100% more cellular C18:2 than those fed coconut oil with cholesterol (p<0.01). The contents of C18:0, C18:1, and C16:0 were identical in the two diet groups fed cholesterol, while the cellular C14:0 content was 88% greater in the diet group fed coconut oil with cholesterol (p<0.05). Comparison of the corn oil diet groups showed that dietary supplementation with cholesterol was accompanied by a 37% decrease in cellular C18:2 (p<0.01), a 40% increase in cellular C18:1 (p<0.01), and a 61% increase in cellular C18:0 (p<0.01). Cells from animals fed coconut oil with or without cholesterol showed no significant differences in specific cellular fatty acyl content.

Effect of Diet on Cellular Cholesterol and Cholesteryl Ester Content

The effect of diet on total cellular cholesterol and cholesteryl ester content is shown in Table 3. Mononuclear cells isolated from animals maintained on a corn oil-enriched diet had an average cellular cholesterol content of 1.25±0.34 μg/10⁶ cells; in contrast, animals fed coconut oil-enriched diets without cholesterol had a higher average mononuclear cell cholesterol content of 2.43±0.53 μg/10⁶ cells (p<0.05). Addition of cholesterol to the corn oil diet resulted in an average mononuclear cell cholesterol content of 1.94±0.36 μg/10⁶ cells, while addition of cholesterol to the coconut oil diet resulted in an average mononuclear cell cholesterol content of 2.52±0.56 μg/10⁶ cells. Comparison of mononuclear cell cholesterol values with the corn oil group shows that the addition of cholesterol to the diet resulted in a 55% increase in mononuclear cell cholesterol content (p<0.05). Addition of cholesterol to the coconut oil diet did not affect the cellular cholesterol content in a statistically significant manner.

Animals fed a corn oil-enriched diet had an average cholesteryl ester content of 0.35±0.09 μg/10⁶ cells, while addition of cholesteryl to the coconut oil diet resulted in an average cholesteryl ester content of 0.88±0.14 μg/10⁶ cells, while addition of cholesterol to the coconut oil diet resulted in an average cholesteryl ester content of
The addition of cholesterol to the coconut oil diet resulted in an increase in mononuclear cell DPH fluorescence polarization, which did not achieve statistical significance, compared with that in cells from animals fed coconut oil without cholesterol. DPH fluorescence polarization was significantly different for monkeys fed corn oil and cholesterol compared with those fed coconut oil and cholesterol.

DPH fluorescence polarization for plasma membrane vesicles prepared from pooled mononuclear cells followed the same pattern as that seen with the whole-cell preparations. The membrane vesicles from corn oil-fed animals had the lowest fluorescence polarization values followed by vesicles from the animals fed corn oil with cholesterol. The vesicles from coconut oil-fed animals had the greatest fluorescence polarization values, and the addition of cholesterol to the coconut oil diet did not alter these values.

Effect of Diet on Low Density Lipoprotein Degradation

The effect of diet on monkey mononuclear cell degradation of LDL is shown in Figure 1. Animals fed a diet enriched with corn oil degraded 13.6 ± 3.6 ng LDL protein/10^6 cells/4 hours, while animals fed a diet enriched with coconut oil degraded 2.5 ± 0.6 ng LDL protein/10^6 cells/4 hours (p<0.01). A corn oil-enriched diet to which cholesterol was added was associated with LDL degradation of 9.6 ± 3.6 ng/10^6 cells/4 hours, while a coconut oil-enriched diet with cholesterol resulted in LDL degradation of 2.7 ± 1.0 ng/10^6 cells/4 hours (p<0.01). Comparison of diet groups without cholesterol showed a 5.5-fold greater degradation of LDL in the corn oil-fed animals than in those fed coconut oil (p<0.01). Similarly, in the diet groups given supplemental cholesterol, the corn oil-fed monkeys exhibited 3.6-fold greater mononuclear cell LDL degradation than those fed coconut oil (p<0.01). Degradation of LDL was identical in both coconut oil-diet groups (i.e., with and without cholesterol). Cells from the corn oil and cholesterol group degraded LDL 70% as well as those from animals fed corn oil alone (p<0.01).

The relationship between LDL degradation and plasma cholesterol is shown in Figure 2 for all diet groups. Cellular
Effect of Diet on Monkey Low Density Lipoprotein Crossover Binding and Degradation

The effect of diet on the specific binding of monkey LDL by mononuclear cells is shown in Figure 5. The estimated binding constants derived from the binding isotherms are shown in Table 6. The dissociation constant was increased by 43% in mononuclear cells isolated from animals fed a coconut oil diet compared to that in cells from animals fed a diet of corn oil. This increase occurred regardless of the source of the LDL ligand, that is, plasma from corn oil-fed monkeys or coconut oil-fed monkeys. In addition, maximal binding of either type of LDL ligand was dramatically decreased in mononuclear cells from animals fed coconut oil. The source of the LDL ligand did not affect the affinity...
of the ligand for its receptor. However, LDL isolated from animals fed coconut-oil diets had markedly decreased maximal binding to mononuclear cells isolated from either corn oil- or coconut oil-fed animals compared to the binding of LDL isolated from corn oil-fed animals.

The results of LDL degradation crossover studies are summarized in Table 7. These results parallel those found in LDL binding experiments. LDL isolated from the plasma of animals maintained on coconut oil diets was degraded 2.1-fold more efficiently by mononuclear cells from animals fed a corn oil diet than those from coconut oil-fed animals (p<0.05). Similarly, LDL isolated from animals fed corn oil-supplemented diets was degraded 2.4-fold more efficiently by mononuclear cells isolated from corn oil-fed animals than coconut oil-fed animals, although the difference was not statistically significant. Mononuclear cells from both coconut oil-fed animals and corn oil-fed animals degraded LDL isolated from corn oil-fed animals better, on average, than LDL from coconut oil-fed animals. Although this comparison was not statistically significant, a trend is apparent.

**Discussion**

In a number of whole-animal studies, the relationships between the major dietary lipids, whole-liver LDL metabolism, and plasma levels of LDL cholesterol have been investigated. From these studies, it is clear that feeding unsaturated fatty acids enhances hepatic receptor-dependent LDL uptake and decreases the concentration of plasma LDL cholesterol, while dietary saturated fatty acids suppress hepatic receptor-dependent LDL uptake and increase levels of plasma LDL. Similarly, feeding cholesterol alone also suppresses hepatic LDL uptake and increases plasma LDL cholesterol. The detrimental effects of feeding cholesterol or saturated fatty acids can be reversed by returning the experimental animal to a diet low in these lipids or to one rich in unsaturated fatty acids. The molecular and cellular correlates of these whole-animal and whole-organ studies are largely unknown.

Since cellular clearance of LDL is primarily mediated by the classic LDL receptor, a deficiency or dysfunction of this receptor leads to elevated circulating levels of LDL and, consequently, can promote atherogenesis. A number of steps in the processing of receptor-bound LDL can be defective. Investigators have described receptors with decreased LDL affinity, alterations in receptor clustering in coated pits, changes in endosome production, and reduced rates of LDL degradation in lysosomes. All of these processes occur within the plasma membrane milieu and, therefore, could be affected by the lipid composition of the membrane and the lipid content of the host animal’s diet.

Using peripheral blood mononuclear cells isolated from Cebus monkeys, we have demonstrated increased LDL degradation in cells from animals fed corn oil compared to cells from animals fed coconut oil. With the addition of cholesterol to the diets, LDL degradation was slightly attenuated in the cells from corn oil-fed animals but essentially did not change in the cells from coconut oil-fed animals. Again, even in the presence of dietary cholesterol, the corn oil-fed animals demonstrated greatly enhanced cellular LDL degradation. Both plasma cholesterol values and total cellular cholesterol content paralleled the decremental changes in cellular LDL degradation as a function of the host animal’s maintenance diet. These observations in an ex vivo isolated cell system parallel findings in whole-animal LDL metabolism studies performed in these same monkeys described in a companion article.

Dietary cholesterol played a relatively minor role in the regulation of cellular LDL degradation in this study. The addition of cholesterol to both the corn oil and the coconut oil diets resulted in decreased membrane levels of C18:2 and increased levels of C18:0 and C18:1. Other investigators have also noted these changes in fatty acid levels with dietary cholesterol supplementation. Huang and coworkers have suggested the decreased activity of the desaturase enzyme in cholesterol-fed animals as a potential explanation. No significant effect was seen in U/S, membrane fluidity, cellular LDL degradation, total cellular cholesterol, or individual cellular fatty acyl components in mononuclear cells isolated from the coconut oil-fed groups. Cholesterol supplementation of corn oil-fed animals resulted in a small decrease in U/S, membrane fluidity, LDL degradation, total cellular cholesterol, and cellular content of linoleate. The explanation for these fluidity findings may be that fatty acyl packing in membranes of mononuclear cells from coconut oil-fed animals is highly ordered, reflecting the highly saturated fatty acyl content of coconut oil. The membrane order may already be near its maximal limit; hence, the addition of dietary cholesterol does not further increase the order of the membrane, possibly as a result of homeoviscous adaptation. Conversely, corn oil-fed ani-

Table 6. Analysis of Low Density Lipoprotein Binding Crossover

<table>
<thead>
<tr>
<th></th>
<th>Com LDL Corn MNC</th>
<th>Com LDL Coconut MNC</th>
<th>Coconut LDL Corn MNC</th>
<th>Coconut LDL Coconut MNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated apparent K_D (µg/ml)</td>
<td>20.0</td>
<td>28.0</td>
<td>22.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Bmax (ng/10^6 cells)</td>
<td>13.9</td>
<td>4.8</td>
<td>3.8</td>
<td>0.97</td>
</tr>
</tbody>
</table>

LDL = low density lipoprotein, MNC = mononuclear cell, K_D = dissociation constant, Bmax = maximum binding.

Table 7. Low Density Lipoprotein Degradation Cross-over Study

<table>
<thead>
<tr>
<th>MNC source</th>
<th>LDL source</th>
<th>Corn (n=4)</th>
<th>Coconut (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>29.6±26.5</td>
<td>10.4±3.8</td>
<td></td>
</tr>
<tr>
<td>Coconut</td>
<td>12.3±8.5</td>
<td>5.0±3.3</td>
<td></td>
</tr>
</tbody>
</table>

The units are ng LDL protein/10^6 cells/4 hours. The values are averages±SD.

LDL = low density lipoprotein, MNC = mononuclear cell.

For a discussion of the significant differences between and among groups, see the Results section.
mals have cellular membranes that are highly disordered, reflecting the cis-unsaturated fatty acyl content of corn oil. The addition of cholesterol to this diet, in contrast, results in increased membrane order as a result of an increased cholesterol/phospholipid ratio and, hence, a relative decrease in membrane fluidity. However, these changes associated with dietary cholesterol supplementation are small compared with the differences in mononuclear cell LDL degradation, plasma cholesterol, membrane fluidity, and U/S caused by modifying the dietary fatty acid composition. These observations are particularly pertinent since these animals are fed a diet equivalent in cholesterol content to that consumed daily by the average adult in American society: 550 mg. Certainly, in human studies, it has been noted that the elevation in serum cholesterol that occurs with isocaloric dietary substitution of saturated fatty acids for unsaturated fatty acids is much greater than that seen with dietary supplementation with cholesterol.48

The effect of diet on the LDL particle itself and its binding and degradation were addressed in the crossover study with LDL from monkeys fed corn or coconut oil-supplemented diets. Again, the mononuclear cells from corn-oil-fed animals degraded LDL more efficiently than did mononuclear cells from coconut oil-fed animals. Within each diet group, LDL isolated from corn-oil-fed animals was bound and degraded more efficiently than was LDL from coconut-oil-fed animals. This points to the possibility that dietary fats may also attenuate LDL metabolism as a result of changes in the LDL particle, although the primary and most significant effect in this study appears to be on receptor function. Interestingly, the Scatchard plots of the binding data demonstrate both increased affinity and increased maximum binding capacity of the LDL receptor when expressed on the surface of mononuclear cells from corn-oil-fed animals. This occurred with LDL from both corn-oil-fed animals and coconut-oil-fed animals. This observation suggests that a simple increase in LDL receptor number is not the only explanation for the degradation data. In addition, a more basic change in the LDL receptor structure or conformation that increases affinity for its ligand is occurring.

In receptor systems such as the platelet thrombin receptor,46 the acetylcholine receptor,47 and the β-adrenergic receptor,18 changes in membrane lipid composition and fluidity result in an alteration in receptor affinity, receptor number, or coupled receptor function. With particular relevance to this study, our group has previously shown that LDL uptake and degradation is enhanced in human peripheral blood mononuclear cells when membrane cis-unsaturated fatty acyl content is enhanced by brief ex vivo incubation with free fatty acids.19 A number of hypotheses have been proposed to explain these observations. It has been suggested that cryptic functional sites or cryptic receptors exist as reservoirs for the adjustment of function in response to perturbations in membrane fluidity.48 Others have suggested requirements for specific lipids within the receptor lipid annulus for optimal receptor or enzyme function.49 Based upon theoretical analyses, it has been implied that receptor turnover rate is directly proportional to membrane fluidity.48 Finally, the Optimal Fluidity Hypothesis suggests that the oVert maximal velocity of receptor or enzyme function may possess a peak value at a specific membrane fluidity.48 The precise role of any of these potential mechanisms is currently unknown.

In this study, mononuclear cell LDL degradation varied in a linear manner with both cellular U/S ratio and membrane fluidity. LDL receptor binding affinity and maximal binding capacity were enhanced in mononuclear cells with increased membrane fluidity and cellular U/S. Both membrane fluidity and cellular U/S reflected the dietary lipid content of the host animals' maintenance diet. While the specific role of membrane fluidity and annular lipids in LDL receptor function are unknown, the possibilities include fluidity-induced changes in LDL receptor conformation toward a conformational state with greater ligand affinity or an absolute functional requirement for specific fatty acyl moieties in the LDL receptor lipid annulus. The effect of dietary cis-unsaturated fatty acids on cellular LDL metabolism in the context of the plasma membrane lipid microenvironment warrants further study. It remains to be seen whether enzymes involved in cellular cholesterol homeostasis, such as HMG-CoA reductase or acyl-CoA cholesterol acyl transferase, can also be affected by alterations in the membrane milieu either directly or through alterations in second messenger signal transduction.

This study demonstrates that cellular LDL metabolism can be dramatically altered by dietary modifications of both membrane fluidity and fatty acyl content. Dietary unsaturated fatty acids are readily incorporated into the mononuclear cell membrane, and cellular LDL binding and degradation are greatly enhanced in association with membrane enrichment with these fatty acids. Our results suggest a novel mechanism by which dietary fatty acids can alter LDL catabolism.

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