Dietary Fat Saturation Modifies the Metabolism of LDL Subfractions in Guinea Pigs

Maria Luz Fernandez, Ghada Abdel-Fattah, Donald J. McNamara

The effects of dietary fat saturation on the metabolism of low-density lipoprotein (LDL) subfractions were measured in adult male guinea pigs fed semipurified diets containing 15% (wt/wt) corn oil (CO; 58% linoleic acid), lard (24% palmitic/14% stearic acid), or palm kernel oil (PK; 52% lauric/18% myristic acid). Animals fed the CO diet had lower plasma total cholesterol levels than guinea pigs fed the PK or lard diets (P<.01). Plasma LDL-1 (d=1.019 to 1.05 g/mL) concentrations were 3.5- and 2.4-fold higher in animals fed the PK diet compared with the CO and lard groups, respectively, while LDL-2 (d=1.05 to 1.09 g/mL) concentrations were not different among groups. For all dietary fat groups LDL-1 had a higher molecular weight and a larger diameter than LDL-2. LDL fractional catabolic rates (FCRs) varied, depending on both the diet and the LDL subfraction. Animals fed the polyunsaturated CO diet had a more rapid LDL FCR than animals from the other two groups (P<.01). Within the same diet group, LDL-2 exhibited a slower turnover rate than LDL-1 in animals fed the PK diet, while no differences in LDL subfraction FCR were found in the CO and lard groups. Animals fed the PK and lard diets did not exhibit significant modifications in the density distribution of LDL subfractions over a period of 33 hours. In contrast, animals fed the CO diet exhibited a shift of more buoyant to denser LDL particles, suggesting that differences in LDL intravascular processing are mediated by dietary fat saturation. In vitro LDL binding to hepatic membranes confirmed the in vivo data with an increased expression of apolipoprotein B/E receptors (Bm) in animals fed the CO diet (P<.01). Hepatic apolipoprotein B/E receptors exhibited less affinity for LDL-2 in the PK group, a result consistent with the less rapid turnover of LDL-2 in PK-fed animals. The results suggest that dietary fatty acids varying in saturation and composition have distinctive atherogenic potentials. The lowest plasma LDL cholesterol concentrations mediated by CO intake could in part be explained by induced changes in the composition and processing of LDL subfractions, resulting in faster LDL turnover rates in addition to increased expression of hepatic apolipoprotein B/E receptors. Similarly, the hypercholesterolemic effects of the lard and PK diets appear to be related to differences in intravascular processing of LDL subfractions, with no interconversion between LDL-1 and LDL-2 and that is accompanied by decreased expression of hepatic apo B/E receptors. In addition, PK-fed guinea pigs exhibited a decreased apolipoprotein B/E receptor affinity for LDL-2.

**KEY WORDS** • LDL subfractions • dietary fat saturation • plasma LDL distribution • fractional catabolic rates • apo B/E receptor • guinea pigs

Numerous studies have investigated the effects of dietary fatty acids on plasma low-density lipoprotein (LDL) transport to determine the mechanisms by which specific fatty acids alter plasma LDL levels. Studies of LDL kinetics have suggested various metabolic mechanisms by which polyunsaturated fat decreases plasma LDL levels, including more rapid LDL fractional catabolic rates (FCRs) in humans and animals or decreased LDL apolipoprotein (apo) B flux rates. The composition of saturated fat has been shown to influence LDL turnover rates in guinea pigs, in that the intake of longer-chain fatty acids, ie, stearic and palmitic, results in higher LDL FCR values than in animals fed the shorter-chain fatty acids laurate and myristate. Intake of palmitic acid has also been shown to lower plasma LDL apo B production rates in rhesus monkeys compared with dietary myristic and lauric acids.

The heterogeneity of LDL in humans is well documented; however, the metabolic implications of these variations in LDL size and composition and their potential role in atherogenesis are not clearly defined. The mechanisms that define LDL atherogenicity in relation to its particle size and composition differ, depending on the experimental system and design. Significant correlations have been found between an increased number of smaller, denser LDL particles and coronary heart disease. In contrast, studies in nonhuman primates have associated the presence of a large, buoyant, cholesteryl ester–rich LDL subpopulation with increased risk for arteriosclerosis. Similarly, studies in spontaneously hypercholesterolemic pigs have associated this metabolic disorder with increased numbers of more buoyant LDL particles. Kinetic studies have demonstrated that these buoyant particles are catabolized at a

Received February 12, 1993; revision accepted July 7, 1993.
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slower rate because of their defective interactions with the LDL receptor. Other studies have also shown that the LDL isolated at a lower density range is catabolized more slowly than denser particles. Dietary fat saturation has been shown to affect the density distribution of LDL in guinea pigs, in that animals fed polyunsaturated fat have an increased population of denser LDL particles than animals fed saturated or monounsaturated fat. Based on these observations and the involvement of LDL heterogeneity in atherosclerosis, the importance of understanding how these different LDL subfractions are metabolized is evident.

These studies were undertaken to test the hypothesis that the different populations of LDL (dense vs buoyant subfractions) in guinea pigs that result from intake of fatty acids varying in saturation and composition have different plasma residence times due to variations in composition and size, which alter their interactions with the apo B/E receptor. Since previous experiments in guinea pigs have shown significant variations in plasma LDL levels in response to intakes of semipurified diets containing 15% (wt/wt) corn oil (CO; 58% linoleic acid), lard (24% palmitic/14% stearic), and palm kernel oil (PK; 52% lauric/18% myristic), these diets were selected to compare the effects of polyunsaturated fat and two different sources of saturated fat: a typical saturated fat (lard) and a highly saturated fat that results in extreme hypercholesterolemia (PK).

Guinea pigs were chosen as the experimental animal model on the basis of their similarity to humans with respect to the plasma lipoprotein profile and LDL metabolism. Like humans, guinea pigs have a high LDL to high-density lipoprotein (HDL) ratio, are responsive to dietary fat saturation and composition in the absence of dietary cholesterol, and have an active plasma cholesterol ester transfer protein (CETP) that transfers cholesteryl ester between HDL and apo E-containing lipoproteins, characteristics that make the intravascular lipoprotein processing of guinea pigs similar to that in humans.

This study describes the compositional and metabolic characteristics of two LDL subfractions (LDL-1, 1.019 to 1.05 g/mL, and LDL-2, 1.05 to 1.09 g/mL) found in guinea pigs that are fed diets varying in dietary fat saturation and composition. The observed differences in FCR, apo B/E receptor interactions, and intravascular processing of these subfractions partly explain the differences in LDL levels when guinea pigs are fed CO-, lard-, or PK-containing diets.

Methods

Materials
[125I]NaI and [131I]NaI were purchased from New England Nuclear Research Products (Boston, Mass); enzymatic cholesterol kits, cholesterol esterase, and cholesterol oxidase were from Boehringer Mannheim (Indianapolis, Ind); the triglyceride enzymatic kit was from Sigma Chemical Co (St Louis, Mo); halothane was from Halocarbon (Hackensack, NJ); and Quickseal ultracentrifugation tubes were from Beckman Instruments (Palo Alto, Calif). Other materials used were from previously reported sources.

<table>
<thead>
<tr>
<th>TABLE 1. Composition of Semipurified Diets</th>
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</thead>
<tbody>
<tr>
<td>Component</td>
</tr>
<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>Protein (soy)</td>
</tr>
<tr>
<td>Fat (corn oil, lard, or palm kernel oil)</td>
</tr>
<tr>
<td>Carbohydrate (sucrose/starch)</td>
</tr>
<tr>
<td>Fiber (cellulose/guar gum)</td>
</tr>
<tr>
<td>Mineral mix*</td>
</tr>
<tr>
<td>Vitamin mix*</td>
</tr>
</tbody>
</table>

The diets provided 3.8 kcal/g of diet.
*Mineral and vitamin mixes were formulated to meet National Research Council-specified requirements for the guinea pig.

Diets

Diets were prepared and pelleted by Research Diets, Inc (New Brunswick, NJ). The three diets had identical compositions except for the fat source (Table 1), which consisted of 15% (wt/wt) CO, lard, or PK. Fatty acid compositions of the dietary fats were determined by gas chromatography as previously described and are presented in Table 2. Fat represented 35% of the caloric content of the diets, and cholesterol and plant sterol levels were adjusted to normalize all diets to the same level, ie, 0.13 mg/g cholesterol and 1.0 mg/g plant sterol, as previously described.

Animals

Male Hartley guinea pigs purchased from Sasco Sprague-Dawley (Omaha, Neb), weighing 250 to 300 g, were randomly assigned to one of the three dietary fat groups. After 4 weeks on the test diets, the animals were used for isolation of plasma LDL subfractions and hepatic membranes or for in vivo LDL subfraction kinetics studies. Preliminary studies have shown that this time is sufficient to establish a constant plasma cholesterol level and a metabolic steady-state condi-

<table>
<thead>
<tr>
<th>TABLE 2. Fatty Acid Composition of Semipurified Diets</th>
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</thead>
<tbody>
<tr>
<td>Fatty Acids</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>10:0</td>
</tr>
<tr>
<td>12:0</td>
</tr>
<tr>
<td>16:0</td>
</tr>
<tr>
<td>18:0</td>
</tr>
<tr>
<td>18:1 (cis)</td>
</tr>
<tr>
<td>18:1 (trans)</td>
</tr>
<tr>
<td>18:2</td>
</tr>
<tr>
<td>18:3</td>
</tr>
<tr>
<td>20:0</td>
</tr>
<tr>
<td>P/S*</td>
</tr>
</tbody>
</table>

All values are in percent, except P/S.
*Ratio of polyunsaturated to saturated fatty acids.
LDL Isolation and Characterization

Plasma samples were obtained by cardiac puncture of guinea pigs that were anesthetized with halothane vapor and exsanguinated by cardiac puncture. Guinea pigs involved in the kinetics studies were killed at the end of the turnover measurements by an excess of halothane vapor. All animals consumed equal amounts of the diet, and there were no significant differences in rates of weight gain or final body weights between groups (Table 3). All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines, and experimental protocols were approved by the University of Arizona Institutional Animal Care and Use Committee.

LDL Isolation and Characterization

Plasma samples were obtained by cardiac puncture of guinea pigs that were anesthetized with halothane vapor, with EDTA as an anticoagulant (1 mg/mL) and a mixture of aprotinin (0.5 mg/mL), 0.1 mg/mL NaN₃, and phenylmethylsulfonyl fluoride (0.1 mg/mL) to minimize changes in lipoprotein composition during isolation. Animals used for the in vitro measurements were anesthetized by halothane vapor and exsanguinated by cardiac puncture. Guinea pigs involved in the kinetics studies were killed at the end of the turnover measurements by an excess of halothane vapor. All animals consumed equal amounts of the diet, and there were no significant differences in rates of weight gain or final body weights between groups (Table 3). All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines, and experimental protocols were approved by the University of Arizona Institutional Animal Care and Use Committee.

Analytical Methods

Plasma total and lipoprotein cholesterol levels were determined by enzymatic analysis, and isolated LDL subfractions were analyzed for protein, triglyceride, phospholipid, and total and free cholesterol as previously described. Plasma triglycerides were measured in fasted animals by enzymatic analysis as previously described.

In Vitro LDL Binding

Pooled samples of LDL-1 or LDL-2 from each dietary fat group were radioiodinated to give a specific activity between 150 and 300 cpm/μg of LDL protein. Hepatic membranes (200 μg protein) were dialyzed against 0.9% NaCl and 1.5% bovine serum albumin and kept at 4°C overnight. After applying LDL samples (10 μg of protein), electrophoresis was performed at 4°C for 6 hours at 50 mA.

Metabolic Studies

Plasma LDL turnover kinetics of LDL subfractions was determined as described previously. Briefly, LDL-1 (d=1.019 to 1.05 g/mL) and LDL-2 (d=1.05 to 1.09 g/mL) were isolated from animals fed CO, lard, or PK diets. They were alternately radioiodinated with 125I-LDL-1 or 131I-LDL-2 (10 to 80 μg/mL) from the homologous diet for 2 hours at 37°C in the absence or presence of 1.5 mg/mL human LDL to determine total and nonspecific binding, respectively. After incubation, the membranes were pelleted and washed by centrifugation as previously described. The apo B/E receptor-mediated binding was determined by subtracting nonspecific from total binding, and the binding parameters K₄ and B₅₈ were determined from Woolf plots.

Metabolic Studies

Plasma LDL turnover kinetics of LDL subfractions was determined as described previously. Briefly, LDL-1 (d=1.019 to 1.05 g/mL) and LDL-2 (d=1.05 to 1.09 g/mL) were isolated from animals fed CO, lard, or PK diets. They were alternately radioiodinated with 125I and 131I in two experiments to test possible effects due to isotope differences. The radiolabeled LDLs (40 μg of each lipoprotein) were simultaneously injected into guinea pigs fed the homologous diet. Guinea pig plasma LDL turnover was determined over a period of 33 hours by measuring plasma LDL radioactivity at 10 minutes (zero time) and at 0.5, 1, 2.5, 5, 10, 21, 28, and 33 hours after injection of the radiolabeled LDLs. Blood samples were taken via an indwelling Silastic catheter inserted in the jugular vein. The amounts of blood taken varied according to the time points. At the beginning of the experiment, 1 mL of blood was taken to determine LDL density distribution before injection of the radiolabeled LDLs. Five hundred microliters of blood was taken at 0-, 0.5-, 2.5-, and 5-hour time points, and 800 or 1200 μL was taken at 10, 21, 28, and 33 hours, depending on the plasma radioactivity. Animals had access to their respective diets and water ad libitum during the 33 hours of the metabolic studies. Radioactivity was measured in a gamma counter (LKB Wallac Clinigamma).
using a dual $^{32}$S and $^{35}$S program. The JANA program (SCI Software, Lexington, Ky) was used to calculate FCR values, and the data were best fitted with a two-pool model as described by Matthews.24

Measurement of Plasma LDL Processing

Blood samples were collected over a period of 33 hours to determine the density distribution of plasma radioactivity at different time points. After separation from red blood cells, the volume was determined for each plasma sample and the final volume adjusted to 1.2 mL with a 1.006-g/mL density solution. Density was adjusted to 1.3 g/mL with solid potassium bromide, and the plasma solution was overlaid with 4 mL of a 1.006-g/mL density solution in cellulose propionate Quickseal tubes. Plasma samples were centrifuged in a vertical rotor (Beckman VTi 65.2) at 125,000g for 45 minutes to generate a density gradient. After centrifugation, 250-μL aliquots were taken in a total of 18 to 20 tubes. Density was measured by using a refractometer and converted to grams per milliliter as previously described.13 The radioactivity of each fraction was measured to determine the distribution of LDL subfractions over the time intervals of the turnover study. Peak densities of LDL subfractions were determined at time 0 (10 minutes after injection of the labeled LDL) to determine the distribution of LDL-1 and LDL-2 for all diet groups before intravascular processing.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to assess differences due to diet or LDL subfraction for the kinetic parameter FCR and for the composition of LDL. One-way ANOVA was used to assess differences in plasma and lipoprotein cholesterol levels, LDL composition and peak densities, LDL binding parameters ($K_a$ and $B_{max}$), and LDL turnover rates. Data are presented as mean±SD for the number of assays shown. Statistical analysis of the turnover kinetic data indicated that the data were best fitted with a two-pool model (JANA and PCNONLIN, SCI Software).

Results

Plasma Total and Lipoprotein Cholesterol

Significant differences due to the type of dietary fat were found in total plasma cholesterol levels. Animals fed the polyunsaturated CO diet had the lowest plasma cholesterol levels, lard-fed animals had an intermediate value, and animals fed saturated PK had the highest ($P<.01$; Table 3). No significant differences in plasma triglyceride concentrations or final body weights were observed for animals fed the three dietary fats (Table 3).

Analysis of the plasma lipoprotein cholesterol distribution demonstrated no differences among diets in VLDL+IDL, LDL-2, or HDL cholesterol levels. The differences were in the LDL-1 subfraction: animals fed the CO diet had 3.5- and 2.4-fold higher concentrations than guinea pigs in the CO and lard groups, respectively ($P<.01$; Table 4). Total LDL concentrations followed the same pattern as total cholesterol: highest for the PK-, intermediate for lard-, and lowest for CO-fed animals (data not shown; $P<.01$), a result consistent with previous observations.47

<table>
<thead>
<tr>
<th>Lipoprotein Cholesterol, mg/dL</th>
<th>Diet</th>
<th>VLDL+IDL</th>
<th>LDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>8±2</td>
<td>15±7*</td>
<td>25±4</td>
<td>9±1</td>
<td></td>
</tr>
<tr>
<td>Lard</td>
<td>10±4</td>
<td>23±12*</td>
<td>32±6</td>
<td>10±3</td>
<td></td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>11±6</td>
<td>52±151</td>
<td>35±12</td>
<td>9±2</td>
<td></td>
</tr>
</tbody>
</table>

VLDL indicates very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein. Data are presented as mean±SD for n=8.

Values in the same column with different superscripts are significantly different ($P<.01$) as determined by analysis of variance and the least-significant-difference test.

LDL Characteristics

Both diet and LDL subfraction were significantly related to the composition, calculated diameter, and calculated molecular weight of LDL (Table 5 and Fig 1). The number of cholesteryl ester molecules per LDL particle was lower in the animals fed the CO diet in the LDL-1 subfraction, whereas the number of molecules of free cholesterol was lower in animals fed the PK diet in the LDL-2 subfraction (Fig 1) ($P<.025$). The LDL-2 subfraction had a lower content of cholesteryl ester, triglyceride, and free cholesterol than the LDL-1 subfraction for all three dietary fat groups. These compositional differences resulted in a lower ratio of core to surface components, calculated diameter, and calculated molecular weight for this subfraction (Table 5). Significant differences due to dietary fat were noted for the peak densities of the LDL-1 subfraction. LDL isolated from animals fed the CO diet exhibited a higher peak density than in that from animals in the other two dietary groups, indicating that within the same density distribution, CO intake resulted in a larger proportion of smaller, denser LDLs. Electrophoresis of the LDL subfractions on nondenatured composite gels indicated significant differences in the rate of migration between LDL-1 and LDL-2 subfractions for the three dietary groups (Fig 2) as determined by $R_t$ values from laser densitometer scans of the gels (Molecular Dynamics, Sunnyvale, Calif) (Table 5). These values agree with the calculated molecular weight and LDL diameter for these particles; animals fed the PK diet were found to have the largest LDL-1 particles and CO-fed guinea pigs the smallest. A significant correlation ($r=.99$) was found between calculated molecular weight and the $R_t$ values determined from electrophoretic mobility in nondenaturing composite gels (data not shown).

<table>
<thead>
<tr>
<th>LDL Characteristics</th>
<th>Diet</th>
<th>LDL-1</th>
<th>LDL-2</th>
<th>LDL-1</th>
<th>LDL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated Diameter</td>
<td>Corn oil</td>
<td>3.0±0.2</td>
<td>3.5±0.2</td>
<td>3.0±0.2</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>Corn oil</td>
<td>1500±100</td>
<td>2000±100</td>
<td>1500±100</td>
<td>2000±100</td>
</tr>
</tbody>
</table>

Metabolism of LDL Subfractions: In Vivo Studies

FCR values were significantly affected by both the type of dietary fat fed to the recipient animals and the LDL subfraction used as a tracer (Table 6). Animals fed the CO diet had the fastest LDL turnover rate relative to animals fed the lard and PK diets ($P<.01$). LDL-1 and LDL-2 had similar turnover rates within the same diet group in animals fed the CO and lard diets; however, the LDL-2 subfraction had a slower FCR in animals fed the PK diet (Table 6 and Fig 3). CO- and lard-fed animals tended to have a faster LDL turnover.
TABLE 5. Composition and Size Characteristics of LDL Subfractions Isolated From Guinea Pigs Fed Semipurified Diets Containing 15% Corn Oil, Lard, or Palm Kernel Oil

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Corn Oil</th>
<th>Lard</th>
<th>Palm Kernel Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesteryl ester/protein</td>
<td>1.56±0.38§</td>
<td>1.99±0.24§</td>
<td>2.13±0.30§</td>
</tr>
<tr>
<td>Core/surface ratio</td>
<td>0.86±0.06§</td>
<td>1.03±0.13§</td>
<td>1.11±0.17§</td>
</tr>
<tr>
<td>Diameter, Å</td>
<td>183±105</td>
<td>205±23§</td>
<td>219±26§</td>
</tr>
<tr>
<td>MW, 10^-6</td>
<td>1.84±0.34</td>
<td>1.99±0.24</td>
<td>2.02±0.25</td>
</tr>
<tr>
<td>Peak density, g/mL</td>
<td>1.050±0.002§</td>
<td>1.046±0.005§</td>
<td>1.045±0.003§</td>
</tr>
<tr>
<td>R, values</td>
<td>0.334</td>
<td>0.315</td>
<td>0.297</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; MW, molecular weight. Values are presented as mean±SD for n=5.

Within the same diet, LDL-1 and LDL-2 were significantly different in all measured characteristics as determined by two-way analysis of variance (P<.001).

Core to surface ratio was determined by dividing the relative percentages of cholesteryl ester plus triglycerides by the relative percentage sum of protein, free cholesterol, and phospholipid.

R, values for LDL-1 and LDL-2 were determined from laser densitometer scans as described in "Methods."

Values in the same row with different superscripts are significantly different as determined by analysis of variance and the least-significant-difference test (P<.025).

Metabolism of LDL Subfractions: In Vitro Studies

The in vitro binding data were consistent with the in vivo observations, in that hepatic membranes isolated from the CO group had a higher expression of hepatic apo B/E receptors (Bmax) than the other two dietary fat groups (Table 7). No differences were found between LDL-1 and LDL-2 in each dietary group in terms of Bmax values (Table 7); however, the affinity of the receptor for LDL (Kd) was altered in animals fed the PK diet, in which a higher value (lower affinity) was observed when hepatic membranes were incubated with the LDL-2 subtraction. Fig 8 represents the saturation curves for the binding of LDL subfractions to hepatic membranes from animals fed the PK diet. While no differences were observed in the expression of hepatic apo B/E receptor number (Bmax), the affinity of the apo B/E receptor was greater (lower Kd value) for the LDL-1 than for the LDL-2 subtraction. These studies reaffirm the in vivo data: animals fed the PK diet had a lower FCR when LDL-2 was the radiolabeled tracer.

Significant correlations were found between the levels of LDL subtraction in each dietary group and the hepatic membrane apo B/E receptor Bmax values (Fig 9), indicating that the changes in expressed hepatic apo B/E receptors mediated by dietary fat are a significant determinant of plasma LDL levels, consistent with previous reports.4,7

Discussion

Dietary Fat Saturation and Composition and Size Characteristics of Plasma LDL Subfractions

LDL heterogeneity is an important determinant of risk for arteriosclerosis8,10 and can be used as a criterion for targeting individuals predisposed to coronary heart disease.25 In population-based studies the smaller, denser LDLs have been identified as atherogenic, while studies in nonhuman primates indicate that larger, buoyant LDL particles are indicative of increased risk for arteriosclerosis.11 The finding of larger, more buoy-
ant LDL particles in hypercholesterolemic pigs exhibiting a decreased LDL FCR,12,13 along with data that an increased risk for atherosclerotic lesions occurs in nonhuman primates that are fed diets resulting in larger LDL particles,26 suggests that LDL size has a significant impact on the metabolic behavior of LDL and the eventual atherosclerotic outcome.

The beneficial effects of polyunsaturated fat intake compared with saturated fat in decreasing the concentration of plasma LDL1 and the number of atherosclerotic lesions occurs in nonhuman primates26 are well established. Studies in guinea pigs that are fed diets varying in fat saturation and composition have demonstrated the presence of two distinct density populations of LDLs.15 Based on these observations and data on LDL heterogeneity in hypercholesterolemic pigs and nonhuman primates, the present studies were undertaken to test whether different LDL populations exhibit distinct metabolic characteristics that, in addition to dietary fat-induced changes in hepatic apo B/E receptor expression,4,7,15 contribute to differences in plasma LDL residence time in animals fed different dietary fats.4,7

In this study LDL-2 (d=1.05 to 1.09 g/mL) had a smaller size, calculated molecular weight, and diameter; had a higher electrophoretic mobility; and exhibited a reduced ratio of core to surface components than LDL-1 (d=1.019 to 1.05 g/mL). Similar to our data, results from studies in normolipidemic and hyperlipidemic individuals have shown that denser LDL subfractions have decreased triglyceride and a decreased cholesteryl ester to protein ratio compared with lighter LDL.27-29 In addition to shifts in LDL size, dietary fat saturation also affected some characteristics of the two LDL subpopulations in a specific manner. Animals fed the saturated PK diet had larger LDL-1 particles with a higher cholesteryl ester content, larger diameter, and reduced electrophoretic migration than LDL-1 from the other two dietary groups. Intake of polyunsaturated CO resulted in the smallest LDL-1 particles with reduced core components, a smaller diameter, and a higher electrophoretic Rv value. While the LDL subfractions from each diet group were isolated over the same

![FIG 1. Bar graphs of cholesteryl ester (CE), free cholesterol (FC), triglyceride (TG), and phospholipid (PL) composition of low-density lipoprotein (LDL) subfractions. The number of CE molecules per LDL-1 particle (upper graph) was lowest in animals fed the corn oil diet (P<.025). Animals fed the lard diet had LDL-1 particles with an intermediate value that was not different from the other two fat groups. The number of molecules of FC per LDL-2 particle (lower graph) was lower in LDL from animals fed the PK diet (P<.05) as determined by one-way analysis of variance. Two-way analysis of variance indicated that LDL-2 from all three diets contained less CE, FC, and TG than did LDL-1 (P<.01).](http://atvb.ahajournals.org/)

**TABLE 6. Fractional Catabolic Rates of LDL Subfractions of Guinea Pigs Fed Semipurified Diets Containing 15% Corn Oil, Lard, or Palm Kernel Oil**

<table>
<thead>
<tr>
<th>Diet, n</th>
<th>LDL-1</th>
<th>LDL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil (5)</td>
<td>0.094±0.016*</td>
<td>0.124±0.050*</td>
</tr>
<tr>
<td>Lard (5)</td>
<td>0.070±0.006†</td>
<td>0.085±0.018†</td>
</tr>
<tr>
<td>Palm kernel oil (6)</td>
<td>0.074±0.006†</td>
<td>0.055±0.006†</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; n, number of determinations. Values are presented as mean±SD for the number of assays shown.

Values in the same column with different superscripts are significantly different as determined by analysis of variance and the least-significant-difference test (P<.005).

*Significantly different from LDL-2 (P<.001).
density range, animals fed the CO diet had smaller LDL particles than lard- or PK-fed guinea pigs for both LDL subpopulations.

Since a higher LDL cholesteryl ester content has been related to increased coronary artery intimal area in the African green monkey, intake of PK would be predicted to increase atherogenic risk. The PK diet favored formation of larger LDL particles, since LDL-1 composed 60% of the total LDL in PK-fed animals while LDL-1 represented approximately 40% of total LDL in the CO and lard diets. Teng et al reported that patients with familial hypercholesterolemia had a higher percentage of LDL-1 than normal individuals or patients with hyperapobetalipoproteinemia, suggesting that elevated concentrations of plasma LDL result in more buoyant particles, consistent with our observations in guinea pigs fed the PK diet.

Plasma CETP plays an important role in determining the cholesteryl ester content of plasma LDL. Studies in the hamster have shown an increase in CETP activity with intake of saturated fat and a positive correlation between plasma cholesterol levels and CETP activity. These data suggest that in animals fed the PK diet with the highest plasma cholesterol concentrations and the highest number of CE molecules in LDL, plasma CETP activity would also be increased and the formation of the larger LDL-1 would be favored. Another possibility is that nascent VLDL from animals fed the PK diet has been found to have a higher concentration of cholesteryl ester than nascent VLDL from animals fed the lard and CO diets, suggesting that this cholesteryl ester–enriched VLDL from the PK group would lead to formation of larger LDL subpopulations, which would explain the higher percentage of LDL-1 in the PK-fed animals. Further studies are needed to clarify the role of dietary fat saturation in determining the activities of plasma lipid transfer proteins and the enzymes involved in the intravascular processing of lipoproteins.

**FIG 3.** Plasma disappearance curves of low-density lipoprotein (LDL)-1 and LDL-2 isolated from guinea pigs fed 15% corn oil (upper panel), lard (middle panel), or palm kernel oil (lower panel) and injected into animals fed the homologous diet. These values represent the mean±SD of five determinations for animals fed corn oil and lard and six determinations for animals fed the palm kernel oil diet. Identical amounts of LDL-1 (○) and LDL-2 (●) protein (40 μg) were injected into the guinea pigs. Plasma decay curves were analyzed as described in "Methods."

**FIG 4.** Scatterplots showing correlations between total plasma cholesterol levels and the fractional catabolic rate (FCR) of low-density lipoprotein (LDL)-1 (upper panel; P<.0001) and LDL-2 (lower panel; P<.001) of guinea pigs fed 15% corn oil (●), lard (○), or palm kernel oil (■) diets.

![Image_of_Fig_3](https://example.com/image3.png)

![Image_of_Fig_4](https://example.com/image4.png)
**Dietary Fat Saturation and Apo B/E Receptor Interactions With LDL Subfractions**

Similar to animals in previous studies, 47 guinea pigs fed the polyunsaturated CO diet had higher apo LDL FCR values than animals fed the lard or PK saturated fat diets, consistent with a higher number of hepatic apo B/E receptors. These data demonstrate that changes in LDL receptor expression are a primary mechanism by which dietary fat saturation modulates plasma LDL concentrations. Results of previous studies have also shown that dietary fat saturation affects LDL size and that smaller LDL particles are catabolized at a faster rate compared with larger LDL particles. Swinkels et al. 14 reported that guinea pigs injected with human LDL subfractions exhibited a faster FCR for the denser LDL-2, a finding consistent with the notion that smaller LDL particles are catabolized faster. In contrast, Luc and Chapman 34 found no differences in FCR between guinea pig LDL-1 and LDL-2 in guinea pigs fed a nonpurified diet; however, that diet resulted in LDL particles that were significantly smaller and denser than LDLs isolated from guinea pigs fed 15% fat diets 4 or human LDL.

When homologous LDL-1 and LDL-2 were simultaneously injected into guinea pigs, no significant differences in LDL turnover were observed in the CO and lard diet groups; however, there was a tendency for LDL-2 to be catabolized faster in both groups (P=.11). In contrast, the denser LDL-2 particle was catabolized at a significantly slower rate in animals fed the PK diet.
Intravascular processing of low-density lipoprotein (LDL)-1 and LDL-2 in guinea pigs fed semipurified diets containing 15% palm kernel oil. Guinea pigs were injected with 40 μg of LDL-1 (○) and LDL-2 (○). The x axis represents the densities from 1.02 to 1.1 g/mL, and the y axis represents the percent radioactivity in each fraction at specific time intervals. Density and radioactivity distributions were determined as described in “Methods.”

While the hepatic apo B/E receptor dissociation constant $K_d$ was not different when binding measurements used either LDL-1 or LDL-2 as the ligand with membranes from animals in the CO and lard groups, there was a significant increase in $K_d$ (ie, less receptor affinity) for LDL-2 with hepatic membranes from animals fed the PK diet. These results suggest that the slower FCR of LDL-2 in PK-fed animals could be due to a decreased affinity of the apo B/E receptor for this LDL subfraction. The major differences in composition of LDL-2 particles from PK-fed animals compared with LDL-2 from the other two dietary groups are a decrease in free cholesterol and a higher core to surface ratio and diameter compared with LDL-2 from the CO group. Such differences in composition and size could account for the decreased interaction of the LDL-2 particle from PK-fed animals with the hepatic apo B/E receptor. Consistent with this scenario, in vitro studies have shown alterations in the expression of apo B-100 epitopes of LDL that have been modified by incubation with VLDL and lipid transfer protein, and Nigon et al have reported differences in LDL subfraction binding affinity to the apo B/E receptor of U-935 cells that are related to the LDL cholesteryl ester to protein ratio. These data demonstrate that changes in LDL composition, resulting in smaller particles, can significantly affect receptor-ligand interactions and the rates of LDL catabolism.

**Table 7. Binding of LDL Subfractions to Guinea Pig Hepatic Membranes From Animals Fed Semipurified Diets Containing 15% Corn Oil, Lard, or Palm Kernel Oil**

<table>
<thead>
<tr>
<th>Diet</th>
<th>$B_{max}$ μg/mg</th>
<th>$K_d$ μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>5.07±0.53*</td>
<td>62±7</td>
</tr>
<tr>
<td>Lard</td>
<td>3.91±0.81</td>
<td>39±10</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>3.43±0.79</td>
<td>46±101</td>
</tr>
</tbody>
</table>

*Significantly different from lard and palm kernel oil as determined by analysis of variance and the least-significant-difference test ($P<.01$).

†Significantly different from LDL-2 ($P<.01$).

LDL indicates low-density lipoprotein. Values are presented as mean±SD for $n=4$ membrane preparations.
Dietary Fat Saturation and Intravascular Processing of LDL Subfractions

To define the intravascular processing of both LDL subfractions, plasma samples were taken over the 33 hours of the turnover studies, and the relative density distribution of the radioiodinated LDL subfractions was determined. Animals fed the saturated lard and PK diets did not exhibit significant changes in the density distribution profile of either LDL subfraction and maintained the initial density profile up to 33 hours. In contrast, animals fed the CO diet exhibited a shift of LDL-1 toward higher densities, which was evident as early as 2.5 hours after injection, with a complete shift of LDL-1 to LDL-2 occurring by 33 hours. In contrast, the LDL-2 subfraction maintained a constant density distribution over the same time period. Since animals fed the CO diet had a more rapid FCR of LDL than LDL from the CO group. Increased LDL apo B flux rates in PK-fed animals could explain differences in LDL-1 concentrations between PK- and lard-fed guinea pigs, consistent with previously reported effects on apo B flux rate for the total population of LDL. Another unexpected factor that contributes to the hypercholesterolemia observed in these animals is the slower FCR of LDL-2, possibly due to compositional changes that decrease the interaction with hepatic apo B/E receptors.

Several models of LDL kinetics have been described by Foster et al.68 to account for the kinetic effects due to LDL heterogeneity. One model, which could be applied to the data from animals fed PK and lard diets, has two distinct pools of LDL with independent apo B fluxes to the plasma compartment and only one pool that equilibrates with the extravascular compartment.35 In contrast, animals fed the CO diet appear to have some conversion of LDL-1 to LDL-2, indicating that another multicompartamental model might be more compatible with the findings in animals fed the polyunsaturated fat. Vega and Grundy27 have proposed a model in which one plasma compartment of LDL is the sole precursor of another plasma compartment (LDL-1 conversion to LDL-2), as was observed in guinea pigs fed the CO diet. In this model LDL-1 can also be catabolized independent of its conversion to LDL-2.

Dietary Fat Saturation and Regulation of Plasma LDL

From these studies we suggest that the lower plasma LDL concentrations observed in animals fed the CO diet can be explained by a number of interrelated factors: a significant increase in the expression of hepatic apo B/E receptors, a decrease in apo B flux,4 and a more rapid FCR of the smaller LDLs associated with CO intake. The observation that the intravascular processing of LDL in CO-fed animals favors the conversion of LDL-1 to LDL-2 suggests a trend for the formation of smaller LDLs, which presumably have increased turnover rates.4

Animals fed the lard diet did not exhibit intravascular conversion of larger to denser LDL particles over the time period measured, which along with decreased expression of hepatic apo B/E receptors, partially explains the lower FCR compared with CO-fed animals and the associated higher plasma LDL levels. Finally, animals fed the PK diet have a higher proportion of larger LDL particles that are catabolized more slowly than LDL from the CO group. Increased LDL apo B flux rates in PK-fed animals could explain differences in LDL-1 concentrations between PK- and lard-fed guinea pigs, consistent with previously reported effects on apo B flux rate for the total population of LDL. Another unexpected factor that contributes to the hypercholesterolemia observed in these animals is the slower FCR of LDL-2, possibly due to compositional changes that decrease the interaction with hepatic apo B/E receptors. Both of these alterations result in the elevated plasma LDL concentrations observed in PK-fed animals. These findings support our hypothesis that the increased plasma LDL concentrations in the PK and lard groups result in part from differences in LDL FCR and flux associated with alterations in the intravascular processing of LDL subfractions.

Acknowledgments

These studies were supported by a Grant-in-Aid from the Cattlemen's Beef Promotion and Research Board, administered in cooperation with the Beef Industry Council; by a Grant-in-Aid from the American Heart Association, Arizona Affiliate; and funds from the University of Arizona Agricultural Experimental Station. The authors express their appreciation to Dr Donald DeYoung, Ms Stephanie Cameron, and Ms Evangelina Patula from the University of Arizona Division of Animal Resources for performing surgery on the animals used in these studies and to Mr Henrik Steinberg for performing the nonnadenaturing gel electrophoresis.
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Dietary fat saturation modifies the metabolism of LDL subfractions in guinea pigs.
M L Fernandez, G Abdel-Fattah and D J McNamara

doi: 10.1161/01.ATV.13.10.1418

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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