Differential effects of saturated fatty acids on low density lipoprotein metabolism in the guinea pig

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Abstract Studies have shown that dietary fat saturation affects guinea pig plasma low density lipoprotein (LDL) levels by altering both LDL receptor-mediated catabolism and flux rates of LDL (Fernandez et al. 1992. *J. Lipid Res.* **33:** 97-109). The present studies investigated whether saturated fatty acids of varying chain lengths have differential effects on LDL metabolism. Guinea pigs were fed 15% (w/w, 35% calories) fat diets containing either palm kernel oil (PK), 52% lauric acid/18% myristic acid; palm oil (PO), 43% palmitic acid/4% stearic acid; or beef tallow (BT), 23% palmitic acid/14% stearic acid. Plasma LDL cholesterol levels were significantly higher for animals fed the PK diet ($P < 0.001$) with values of 83 ± 19 (n = 12), 53 ± 8 (n = 12) and 44 ± 16 (n = 10) mg/dl for PK, PO, and BT diets, respectively. The relative percentage composition of LDL was modified by fat type; however, LDL diameters and peak densities were not different between diets, indicating no effect of saturated fatty acid composition on LDL size. ApoB/E receptor-mediated LDL fractional catabolic rates (FCR) were significantly lower in animals fed the PK diet ($P < 0.01$) and LDL apoB flux rates were reduced ($P < 0.01$) in animals fed the BT diet. A correlation was found between plasma LDL levels and receptor-mediated LDL catabolism ($r = -0.66$, $P < 0.01$). A higher apoB/E receptor number (B_{max}), determined by in vitro LDL binding to guinea pig hepatic membranes, was observed for animals fed BT versus PK or PO diets and B_{max} values were significantly correlated with plasma LDL levels ($r = -0.776$, $P < 0.001$). These results indicate that saturated fatty acids of varying chain length have differential effects on hepatic apoB/E receptor expression and on LDL apoB flux rates which in part account for differences in plasma LDL cholesterol levels of guinea pigs fed these saturated fats.—**Fernandez, M. L., E. C. K. Lin, and D. J. McNamara.** Differential effects of saturated fatty acids on low density lipoprotein metabolism in the guinea pig. *J. Lipid Res.* 1992. **33:** 1833-1842.

Supplementary key words apoB/E receptor • apoLDL fractional catabolic rate • dietary fatty acids • apoLDL flux rates

High dietary intakes of saturated fatty acids have been implicated as a causative factor in the high incidence of hypercholesterolemia and increased risk for atherosclerosis and cardiovascular disease which exist in many Western cultures (1). While this generalization was originally considered applicable to all saturated fatty acids varying in chain length from 10 to 18 carbons, recent data indicate that individual saturated fatty acids can have specific, and in some cases opposite, effects on plasma cholesterol levels (2-8). Bonanome and Grundy (2) reported that stearic acid (C18:0) had a hypocholesterolemic effect in normolipidemic subjects when compared to palmitic acid (C16:0). Metabolic ward studies in normolipidemic individuals demonstrated that butter fat intake increased low density lipoprotein (LDL) levels compared to intake of beef tallow or cocoa butter, two fats with a high stearic acid content (3). In addition, cocoa butter when compared to corn, palm kernel, or coconut oil did not exhibit hyperlipidemic properties in rats and this was attributed in part to an inhibitory effect of cocoa butter on cholesterol absorption (4, 5). Woollett, Spady, and Dietschy (8) reported that lauric, myristic, and palmitic acids increased plasma LDL cholesterol levels in hamsters compared to animals fed stearic acid by decreasing LDL apoB/E receptor activity and increasing LDL production rates.

Studies by Hegsted et al. (6) indicated that of the various saturated fatty acids in the diet, the most hypercholesterolemic was myristic acid followed by palmitic acid; stearic acid was found to be hypocholesterolemic. It has been reported that intake of palmitic acid has a neutral effect on plasma LDL levels in nonhuman primates when compared to myristic (C14:0) + lauric (C12:0) acids and to be slightly hypercholesterolemic as compared to linoleic acid (C18:2) (7). Moreover, when compared to myristic and lauric acids, intake of palmitic acid increased apoB/E receptor mRNA levels in hamsters (9) and lowered plasma LDL apoB pool sizes by decreasing LDL

Abbreviations: LDL, low density lipoprotein; PK, palm kernel oil; PO, palm oil; BT, beef tallow; FCR, fractional catabolic rate; PL, phospholipid; CE, cholesteryl ester; TG, triglyceride; FC, free cholesterol; PRO, protein.

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TABLE 1. Composition of test diets

Component	Weight (%)	Calories (%)
Soy protein	22.4	23.0
Fat (beef tallow, palm oil or palm kernel oil)	15.1	35.1
Carbohydrates (sucrose/corn starch)	39.6	41.9
Fiber (cellulose/guar gum)	13.6	
Mineral mix[a]	8.2	
Vitamin mix[a]	1.1	
Caloric density (kcal/g)	3.8	

[a] Mineral and vitamin mixes were formulated to meet NRC specified requirements for the guinea pig.

apoB production rates in rhesus monkeys (10). The data from these studies suggest that saturated fatty acids have different potentials for modifying plasma cholesterol levels, especially the atherogenic LDL, due to differential effects on parameters of LDL metabolism.

In order to gain a better understanding of the mechanisms by which different saturated fatty acids affect plasma LDL levels, the effects of dietary palm kernel oil (PK) (52% C12:0 + 18% C14:0), palm oil (43% C16:0 + 4% C18:0), and beef tallow (BT) (23% C16:0 + 14% C18:0) on LDL metabolism were evaluated in the guinea pig. These studies were carried out to test the hypothesis that saturated fatty acids of varying chain length have differential effects on LDL flux and receptor-mediated catabolism that account for the reported variations in plasma LDL cholesterol levels. As guinea pigs carry the majority of plasma cholesterol in LDL and are responsive to changes in dietary fat saturation in the absence of dietary cholesterol (11–13), this animal model was selected for studies of the effects of saturated fatty acid chain length on plasma LDL levels and metabolism.

EXPERIMENTAL PROCEDURES

Materials

$Na^{125}I$ and $Na^{131}I$ were purchased from New England Nuclear Research Products (Boston, MA); enzymatic cholesterol kits, cholesterol esterase, and cholesterol oxidase from Boehringer Mannheim (Indianapolis, IN); halothane from Halocarbon (Hackensack, NJ); Quickseal ultracentrifugation tubes from Beckman Instruments (Palo Alto, CA). Other materials were obtained from sources previously reported (11–13).

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% (w/w) fat, either palm kernel oil (PK), palm oil (PO), or beef tallow (BT). The fatty acid compositions of the dietary fats presented in **Table 2** were determined by gas–liquid chromatography (11). Fat represented 35% of the total caloric content of the diets, and cholesterol and plant sterols were adjusted to normalize all diets to the same level of 0.13 mg/g cholesterol and 1.0 mg/g plant sterol as previously described (10, 11).

Animals

Male Hartley guinea pigs purchased from Sasco Sprague-Dawley (Omaha, NE), weighing between 250 and 300 g, were randomly assigned to one of the three dietary fat groups. After 4 weeks on the test diets, animals were used either for isolation of plasma LDL and hepatic membranes or for LDL turnover studies. Preliminary studies have shown that this length of time is sufficient to establish a constant plasma cholesterol level and a metabolic steady state condition (11–13). Animals were anesthetized with halothane vapors and exsanguinated by cardiac puncture. Animals involved in the kinetic studies

TABLE 2. Fatty acid composition of diets

	Dietary Fat		
Fatty Acid	Palm Kernel Oil	Palm Oil	Beef Tallow
		%	
8:0	0.4		
10:0	2.5		
12:0	52.4		
14:0	18.0	1.5	2.8
14:1			0.7
16:0	8.5	43.2	23.3
16:1		0.1	3.9
18:0	14.0	4.1	14.1
18:1	1.4	39.8	47.0
18:1 (*trans*)	2.7		
18:2	0.3	9.7	1.8
18:3		0.2	0.1
20:0	0.2	0.3	
20:1		0.1	0.3
20:2		0.3	
P/S ratio[a]	0.02	0.20	0.04

[a] Ratio of polyunsaturated to saturated fatty acids.

TABLE 3. Final body weights and plasma total and LDL cholesterol levels of guinea pigs fed 15% (w/w) palm kernel oil, palm oil, and beef tallow semi-purified diets

Dietary Fat (n)	Body Weight	Cholesterol[1]	
		Total	LDL
	g	*mg/dl*	
Palm kernel oil (12)	585 ± 20	106 ± 25[a]	83 ± 19[a]
Palm oil (12)	555 ± 47	71 ± 12[b]	53 ± 8[b]
Beef tallow (12)	582 ± 33	56 ± 18[b]	44 ± 16[b]

Values are presented as mean ± SD for (n) determinations.
[1]Values in the same column with different superscript are significantly different ($P < 0.001$) as determined by ANOVA and least significant difference test.

were killed at the end of the turnover studies with an excess of halothane vapors. All animals consumed equal amounts of diet and there were no significant differences in the rates of weight gain or final body weights among groups (**Table 3**). All animal experiments were conducted in accordance with U.S. Public Health Service/U.S. 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 under halothane vapors, using EDTA as an anticoagulant (1 mg/ml) and a mixture of aprotinin (0.5 mg/ml), NaN_3 (0.1 mg/ml), and PMSF (0.1 mg/ml) to minimize changes in lipoprotein composition during isolation. Plasma lipoproteins were isolated by adjusting the plasma density to 1.25 g/ml with KBr followed by centrifugation for 36 h at 125,000 *g* at 15°C in a Beckman Ti-50 rotor. The isolated lipoprotein fraction was adjusted to a density of 1.3 g/ml with KBr, and a 10-ml volume was overlayered with 30 ml of d 1.006 g/ml density solution in a Quickseal centrifugation tube. Centrifugation was performed in a VC-53 vertical rotor for 3 h at 100,000 *g* at 10°C to generate a density gradient fractionation of the lipoproteins, and the lipoprotein profile was determined by measuring the cholesterol concentrations and refractive index of the isolated fractions as previously described (11). LDL used for the kinetic studies was isolated in a density range of 1.02 to 1.09 g/ml, dialyzed against 0.9% NaCl and 0.01% EDTA for 24 h, and concentrated to 1.5 to 2 mg/ml protein. Guinea pig plasma LDL apoB was quantitated as the difference between total LDL protein and soluble protein after isopropanol precipitation (14).

LDL particle diameters were calculated from the core to surface volume ratio according to Van Heek and Zilversmit (15) using the formula:

$$r = \frac{1.093(TG) + 1.044(CE)}{[0.968(FC) + 0.97\ (PL) + 0.705\ (PRO)]} \times 3 \times 21.5\ \text{Å}$$

and $d = 2r + 2(21.5)$

where r = radius of the particle, d = diameter; PL = phospholipid mass; TG = triglyceride mass; CE = cholesteryl ester mass; FC = free cholesterol mass, and PRO = protein mass; 21.5 Å is the assumed thickness of the surface layer of the lipoprotein; 1.093, 1.044, 0.968, 0.97, and 0.705 are the corresponding specific volumes of PL, TG, CE. FC, and PRO, respectively, as previously reported (13).

Human LDL was isolated from plasma samples of normolipidemic volunteers by sequential ultracentrifugation between densities 1.019 and 1.063 g/ml and washed at d 1.063 g/ml. Methylated human LDL was prepared as described by Weisgraber, Innerarity, and Mahley (16). In vitro competition studies for ^{125}I-labeled LDL binding to guinea pig hepatic membranes were carried out at 37°C to verify the efficacy of the methylation procedure as previously reported (13). Iodination of lipoproteins was performed according to the method of Goldstein, Basu, and Brown (17). Radiolabeled LDL was used within 2 to 4 days of preparation to minimize potential changes due to radiation oxidation (18).

Analytical methods

Plasma total and lipoprotein cholesterol levels were determined by enzymatic analysis and isolated LDL separated at $1.019 < d < 1.09$ g/ml was analyzed for protein, triglyceride, phospholipid, total and free cholesterol by previously described methods (11-13). This density range for LDL isolation was based on density distributions of the plasma lipoproteins as determined by density gradient fractionation using a vertical rotor (11).

Fatty acid analysis

Total lipids were extracted from guinea pig hepatic membranes (19) and triglycerides and phospholipids were separated by thin-layer chromatography using a solvent system of N-hexane–diethyl ether–acetic acid–methanol 90:20:2:3 (vol/vol). Phospholipid fatty acids were converted to fatty acid methyl esters (FAME) by base-catalyzed transesterification with sodium methoxide. FAME were determined by gas chromatography using a Packard gas chromatograph (Model 438A, Chromopack, Raritan, NJ) equipped with a Supelco SP-2380 (Bellefonte, PA) wide-bore capillary column, 30 m × 0.53 mm, using temperature programming with an initial temperature of 90°C and a final temperature of 240°C. Helium was the carrier gas at a flow rate of 5 cc/min. Peaks were identified by comparison of retention times with standards.

The saturation index (SI) values of membrane phospholipid fatty acids from PK, PO, and BT diet groups were calculated (20) as:

$$\text{S.I.} = \frac{\Sigma[(\%\text{ unsaturated fatty acids}) (\text{number of double bonds})]}{\%\text{ saturated fatty acids}}$$

In vitro LDL binding

Pooled samples of LDL from each dietary fat group were radioiodinated by the iodine monochloride method (17) to give a specific activity between 200 and 300 cpm/ng protein. Hepatic membranes (200 μg) isolated as previously described (11) were incubated with ^{125}I-labeled LDL (5-60 μg/ml) from the homologous diet for 2 h at 37°C in the presence or absence of 1 mg/ml of human LDL to determine apoB/E receptor mediated binding. After incubation, membranes were pelleted and washed by centrifugation as previously described (12) and the binding parameters K_d and B_{max} were determined from Woolf plots (21).

Metabolic studies

Plasma LDL turnover kinetics were measured as described previously (13). LDL isolated from animals fed 15% PK, PO, or BT diets was radioiodinated and injected into animals fed the same diet to determine FCR and flux rates in a completely autologous system. Guinea pig plasma LDL kinetics were measured over a period of 33 h by measuring plasma LDL radioactivity at 10 min (zero time) and at 0.5, 1, 2, 3.5, 5, 10, 15, 20, 24, 28, and 33 h after injection of the radiolabeled LDL. Samples were obtained by taking 300-μl blood samples via an indwelling catheter of silastic tubing, I.D. 0.64 mm (Dow-Corning, Corning NY) inserted in the jugular. FCR values were calculated using a two-pool model as described by Mathews (22). Receptor-independent LDL turnover was measured by simultaneously injecting ^{131}I-labeled methylated human LDL and determining its catabolism as previously reported (13). ApoLDL flux rates were calculated by multiplying LDL apoB pool size (mg/kg) by FCR (h^{-1}).

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess differences in plasma and lipoprotein cholesterol levels, LDL composition and peak densities, fatty acid composition of hepatic membranes, K_d and B_{max} values, and the metabolic parameters of apoLDL pool size, FCR, flux rates, receptor-independent and receptor-mediated plasma LDL turnover of animals fed PK, PO, and BT diets using the GBSTAT program (Dynamic Microsystems, Inc. Silver Springs, MD). Multiple regression analysis was used to determine significant correlations. Data are presented as mean ± SD for the number of animals tested.

Model selection

The two-pool Mathews model (22), which assumes that LDL catabolism occurs only from the plasma compartment, was used for calculating plasma apoLDL turnover. As previously reported (13), this model was selected assuming that LDLs from a given diet are kinetically homogeneous and have the same probability of following a given metabolic pathway. This model is the most commonly used for apoLDL kinetics in both clinical (23, 24) and animal studies (10, 13, 25) and therefore, for purposes of comparison, it is the most appropriate model for data analysis in the present study. Statistical analysis of the turnover kinetic data indicated that the data were best fitted using a two-exponential model (JANA and PCNONLIN, SCI Software, Lexington, KY).

RESULTS

Plasma cholesterol levels and LDL size and composition

Dietary saturated fatty acids had no significant effects on rates of body weight gain or final body weights (Table 3). Plasma total cholesterol levels were 1.5- and 1.9-fold higher in guinea pigs fed the PK diet than in animals fed the PO or BT diets (Table 3). As has been previously reported for guinea pigs (11-13), changes in plasma total cholesterol levels mediated by dietary fatty acids occur mainly in LDL and a parallel increase in plasma LDL was observed in animals fed the PK diet compared to the other two dietary fat groups (Table 3). Animals from the PO group had slightly higher total and LDL cholesterol levels than animals from the BT group; however, these values were not significantly different by ANOVA (Table 3).

TABLE 4. Composition and peak densities of LDL isolated from guinea pigs fed 15% (w/w) palm kernel oil, palm oil, and beef tallow semi-purified diets

	Dietary Fat[1]		
LDL	Palm Kernel Oil	Palm Oil	Beef Tallow
	%		
Composition			
Cholesteryl ester	45.2 ± 3.3[a]	41.9 ± 2.7[b]	41.7 ± 3.7[b]
Free cholesterol	6.9 ± 2.1	7.0 ± 1.8	6.3 ± 1.7
Triacylglycerol	7.0 ± 1.5[a]	8.0 ± 1.8[a]	10.9 ± 2.1[b]
Protein	18.2 ± 3.3[a]	24.9 ± 2.4[b]	21.8 ± 4.9[b]
Phospholipid	22.4 ± 3.1	18.6 ± 5.9	20.9 ± 5.4
Peak density (g/ml)	1.048 ± 0.003	1.043 ± 0.003	1.048 ± 0.007
Diameter (Å)	214 ± 23	205 ± 25	216 ± 25

Data are presented as mean ± SD for n = 10 for composition and diameter values and n = 6 for peak density values.

[1]Values in the same row with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA and the least significant difference test.

Differences in dietary fatty acid composition not only affected plasma LDL concentrations but also resulted in changes in LDL composition. The relative percentage of LDL triacylglycerol was higher in the BT diet group (**Table 4**). The percentage of LDL protein was lowest whereas the proportion of LDL cholesteryl ester was highest in guinea pigs fed the PK diet ($P < 0.05$) (Table 4). These compositional differences did not affect LDL size as judged by similar LDL peak densities and calculated diameters for the three dietary fat groups (Table 4). Higher levels of cholesteryl ester in LDL have been related to larger LDL particles (13). Although a higher percentage of cholesteryl ester was observed in LDL isolated from the PK group, the content of triacylglycerol, which is also a core component of LDL and makes a contribution to LDL diameter (15), was lower in this dietary fat group; therefore, no differences in LDL diameter were found among dietary fat groups (Table 4).

Fatty acid composition of hepatic membranes

Dietary saturated fatty acid composition significantly affected the fatty acid profile of guinea pig hepatic membrane phospholipids (**Table 5**). Major differences were found in the percentages of 14:0, 18:1, 18:2, and 22:6 while 16:0 and 18:0 remained relatively constant in the three dietary groups. Differences included the finding that 14:0 was present only in animals fed the PK diet; animals fed the BT diet had a lower percentage of 18:2 than animals fed PO and PK diets ($P < 0.05$); 18:1 content was highest in the BT, intermediate in PO, and lowest in the PK diet group; and 22:6 was higher in the BT fed animals (Table 5). Although the saturation index values for membrane phospholipid fatty acids were not significantly different among the dietary fat groups, PK-fed animals had a lower value consistent with a higher percentage of saturated and lower percentage of mono- and polyunsaturated fatty acids (Table 5). A significant negative correlation was found between plasma LDL cholesterol levels and saturation index values of hepatic membrane phospholipid fatty acids in these animals ($r = -0.63$, n = 9, $P < 0.05$, data not shown).

TABLE 5. Fatty acid composition of hepatic membrane phospholipids of guinea pigs fed 15% (w/w) palm kernel oil, palm oil, and beef tallow semi-purified diets

Fatty Acids	Dietary Fat[1]		
	Palm Kernel Oil	Palm Oil	Beef Tallow
	%		
14:0	1.1 ± 0.3		
16:0	12.0 ± 1.5	11.7 ± 1.0	10.3 ± 2.3
16:1	1.2 ± 0.3		1.5 ± 0.3
18:0	33.9 ± 1.9	28.2 ± 2.6	30.7 ± 1.8
18:1	11.1 ± 0.9[a]	17.6 ± 0.8[b]	21.1 ± 1.1[c]
18:2	32.4 ± 1.4[a]	31.7 ± 0.4[a]	27.5 ± 0.8[b]
18:3	1.0 ± 0.5	0.4 ± 0.2	1.3 ± 0.6
20:2	0.7 ± 0.1	0.5 ± 0.1	
20:3	0.6 ± 0.1	0.6 ± 0.2	
20:4	3.8 ± 0.4	4.4 ± 0.8	4.1 ± 0.5
22:6	0.3 ± 0.1[a]	0.6 ± 0.1[a]	2.1 ± 1.0[b]
Saturation index	2.12 ± 0.08	2.62 ± 0.31	2.67 ± 0.41

Data are presented as mean ± SD for n = 3.
[1]Values in the same row with different superscripts are significantly different as determined by ANOVA and the least significant difference test ($P < 0.001$).

TABLE 6. Equilibrium parameters of apoB/E receptor-mediated binding of LDL to hepatic membranes from guinea pigs fed 15% (w/w) palm kernel oil, palm oil, and beef tallow semi-purified diets

Dietary Fat (n)	K_d	B_{max}[1]
	μg/ml	μg/mg
Palm kernel oil (6)	37 ± 12	2.11 ± 0.40[a]
Palm oil (5)	46 ± 8	2.40 ± 0.30[a]
Beef tallow (5)	44 ± 4	3.71 ± 0.39[b]

Values are presented as mean ± SD for (n) determinations.
[1]Values in the same column with different superscripts are significantly different ($P < 0.02$) as determined by ANOVA and the least significant difference test.

LDL binding to hepatic membranes

Dietary saturated fat composition had significant effects on apoB/E receptor-mediated binding of LDL to hepatic membranes. Animals fed the BT diet had 1.5- and 1.8-fold higher expression of hepatic membrane apoB/E receptors (B_{max}) than the PO and PK fat groups, respectively (**Table 6**). Animals fed PO and PK diets had similar B_{max} values. The affinity of the apoB/E receptor for LDL (K_d) was not modified by saturated fat composition (Table 5). A significant correlation ($r = -0.776$, $P < 0.001$) was observed between plasma LDL cholesterol levels and B_{max} values for all dietary fat groups (**Fig. 1**). Saturation curves of LDL binding to hepatic membranes from BT, PO, and PK diet groups are presented in **Fig. 2.** The inset presents the Woolf plots used to determine K_d and B_{max} values. Animals from the BT diet group had higher LDL binding at all concentrations of labeled LDL consistent with the higher expression of apoB/E receptors (B_{max}) by hepatic membranes from this dietary fat group. PO- and PK-fed animals had similar values of ligand binding to hepatic membrane apoB/E receptor (Fig. 2).

Plasma LDL kinetics

Differences in dietary saturated fatty acid composition resulted in unique effects on plasma LDL turnover in that animals fed the PK diet had a slower plasma LDL disappearance than animals fed PO and BT diets (**Fig. 3**). Plasma LDL turnover rates were similar for the PO and BT dietary groups. LDL apoB pool sizes were

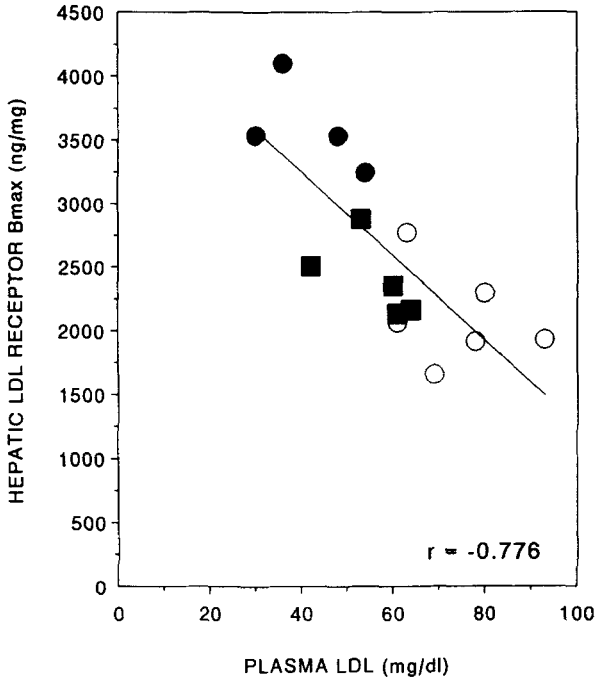

Fig. 1. Correlation between plasma LDL cholesterol levels (mg/dl) and hepatic membrane apoB/E receptor number (B_{max}) (μg LDL protein bound per mg membrane protein) of guinea pigs fed 15% (w/w) beef tallow (●), palm oil (■), and palm kernel oil (○) semi-purified diets. ($r = -0.776$, $P < 0.001$).

significantly modified by dietary saturated fat composition with BT-fed animals having an LDL apoB pool size 35 and 50% smaller than PO- and PK-fed guinea pigs respectively (**Table 7**). This reduction in LDL apoB pool size is in part explained by a significant decrease ($P < 0.01$) in LDL apoB flux rates in animals fed the BT diet (Table 7). Due to individual variations within diet groups, no significant differences were found for the total FCR values of LDL; however, receptor-mediated LDL FCR values were significantly lower in guinea pigs fed the PK diet (Table 7). No differences in receptor-mediated LDL FCR were observed between PO- and BT-fed animals. Receptor-independent FCR values, measured by the plasma disappearance of methylated human LDL, were not different among groups (Table 7). **Fig. 4** presents graphs of total and receptor-independent LDL turnover in animals fed PK (top panel), PO (middle panel), and BT (bottom panel) diets. As shown in Fig. 4, receptor-mediated LDL turnover, obtained by subtracting receptor-independent from total LDL turnover, was higher in PO- and BT-fed animals compared to the PK diet group. A significant correlation ($r = -0.66$, n = 14, $P < 0.01$) was found between receptor-mediated FCR and plasma LDL cholesterol levels for all three dietary fat groups (**Fig. 5**).

DISCUSSION

Saturated dietary fat and plasma LDL levels and composition

Clinical (2, 3) and animal studies (7–10) have shown that dietary saturated fat of varying chain lengths has differential effects on plasma lipoprotein levels. Studies have demonstrated that the shorter chain saturated fatty acids lauric and myristic are hypercholesterolemic compared to stearic acid (2–6, 8). However, some controversy exists regarding palmitic acid in that studies in humans demonstrated that palmitic acid is hypercholesterolemic relative to lauric acid (6), while it has been reported that in non-human primates lauric and myristic are hypercholesterolemic compared to palmitic acid (7).

Although several mechanisms have been proposed to account for the changes in plasma LDL levels resulting from intake of the different saturated fatty acids (4–10), one unanswered question relates to the effects of stearic,

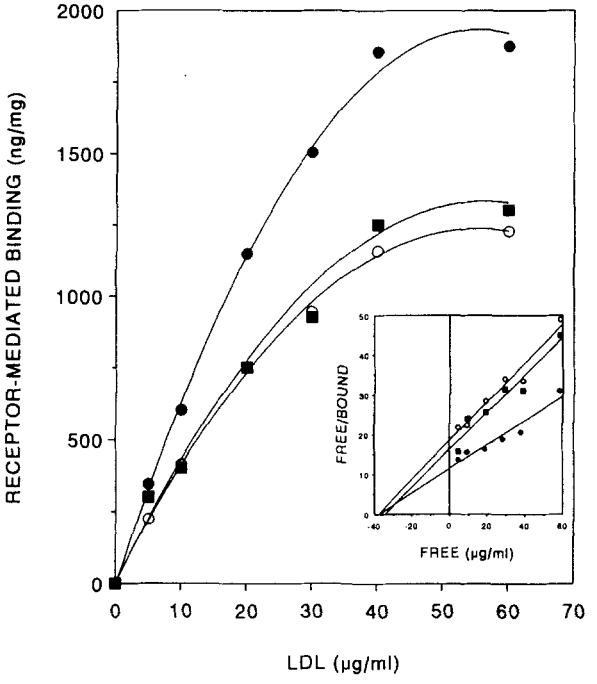

Fig. 2. ApoB/E receptor-mediated binding of ^{125}I-labeled LDL to hepatic membranes of guinea pigs fed different saturated fatty acids. LDL was isolated from guinea pigs fed 15% (w/w) beef tallow (●), palm oil (■), and palm kernel oil (○) semi-purified diets and incubated with hepatic membranes from the homologous dietary group. Receptor-mediated binding was obtained by subtracting nonspecific from total binding as described in Experimental Procedures. Each curve represents the mean of n = 6 membrane binding experiments for the PK group and n = 5 for the PO and BT groups. The inset presents Woolf plots used for calculation of the affinity constant (K_d) (μg/ml) and receptor number (B_{max}) (μg/mg). K_d values were: 37, 34, and 38 μg/ml and B_{max} values were: 2.09, 2.12, and 3.30 μg/mg for animals fed PK, PO, and BT diets, respectively.

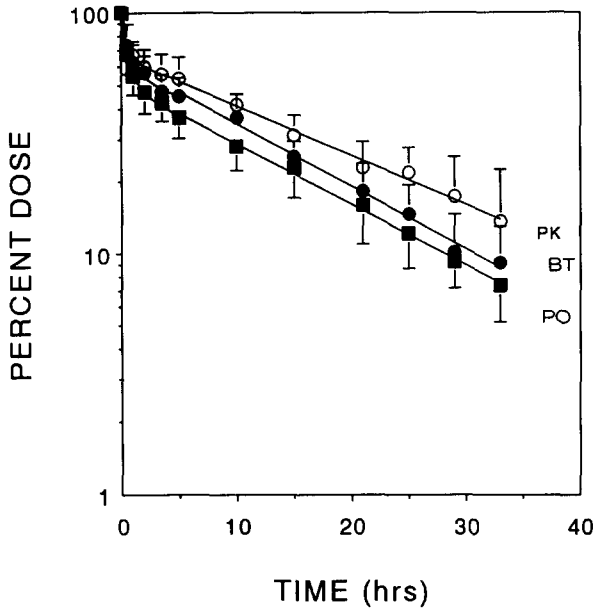

Fig. 3. Plasma decay curves of LDL isolated from animals fed 15% beef tallow (●), palm oil (■), and palm kernel oil (○) diets and injected into guinea pigs fed the homologous diet. Identical amounts of LDL protein were injected and the plasma decay curves were analyzed as described in Experimental Procedures. Each curve represents the mean ± SD of a minimum of four determinations.

palmitic, and lauric + myristic on receptor-mediated LDL catabolism and apoLDL flux rates. The present studies were designed to evaluate the differential effects of these fatty acids on LDL metabolism using an "LDL" animal, the guinea pig. Changes in LDL metabolism similar to those reported for humans have been demonstrated in this animal model in response to changes in dietary fat quality and quantity in the absence of dietary cholesterol (13, 23, 24).

The present studies have shown that different saturated fatty acids have unique effects on plasma LDL levels and metabolism. Guinea pigs fed the PK diet had the highest levels of LDL cholesterol consistent with reports that lauric + myristic acids are more hypercholesterolemic than palmitic and stearic acids (3, 7). A possible explanation for the observed differences in plasma LDL cholesterol levels among the three dietary fat groups could relate to the higher oleic acid content of the BT and PO diets compared to the PK diet (Table 2). However, previous studies in guinea pigs have demonstrated that intake of olive oil, which has 75% oleic acid, has a hypercholesterolemic effect in guinea pigs compared to intake of polyunsaturated fat. Intake of olive oil results in plasma LDL levels similar to those of animals fed a 15% lard (11, 12), 15% beef tallow, or 15% palm oil diets.

Changes in LDL composition and size mediated by dietary fat saturation have been shown to affect in vivo LDL turnover rates (13). Compositional changes in LDL were observed in response to differences in the type of saturated fat and these changes did not affect LDL core to surface ratio since LDL from the three dietary fat groups had similar sizes as determined by peak densities and LDL calculated diameters (Table 4). Previous LDL turnover studies indicated that when animals were injected with radiolabeled LDL from the homologous diet, differences in apoB/E receptor expression and apoLDL flux rates were the primary determinants of LDL turnover relative to the effect of LDL compositional differences (13).

Saturated dietary fat and hepatic apoB/E receptor expression

These studies demonstrate that expression of hepatic apoB/E receptors is affected by the type of dietary fatty acids and that the changes in receptor expression are related to changes in plasma LDL levels. Whether determined in vitro, from LDL binding to hepatic membranes, or in vivo, by analysis of receptor-mediated LDL FCR, significant relationships were found between indices of apoB/E receptor expression and plasma LDL levels (Figs. 1 and 5). Animals fed the PK diet had the lowest expression of apoB/E receptor, suggesting that intake of lauric and myristic acids had a negative effect on hepatic LDL

TABLE 7. Kinetic parameters of autologous plasma LDL turnover in guinea pigs fed 15% m(w/w) palm kernel oil, palm oil, or beef tallow semi-purified diets

	Dietary Fat[1]		
LDL Kinetic Parameters	Palm Kernel Oil	Palm Oil	Beef Tallow
Pool size (mg/kg)[2]	22.3 ± 7.1[a]	17.3 ± 2.6[a,b]	11.3 ± 2.8[b]
Flux (mg/kg-h)	1.53 ± 0.26[a]	1.86 ± 0.38[a]	1.04 ± 0.31[b]
FCR (h^{-1})			
Total	0.073 ± 0.016	0.105 ± 0.022	0.095 ± 0.027
Receptor-mediated	0.031 ± 0.006[a]	0.062 ± 0.020[b]	0.060 ± 0.025[b]
Receptor-independent	0.042 ± 0.013	0.047 ± 0.008	0.036 ± 0.008

Values are presented as mean ± SD for n = 5 animals fed PO and PK diets and n = 4 animals fed the BT diet.
[1]Values in the same row with different superscripts are significantly different ($P < 0.001$) as determined by ANOVA and the least significant difference test.
[2]Pool size was calculated as the plasma volume (4.5% of body weight) × plasma apoLDL concentration.

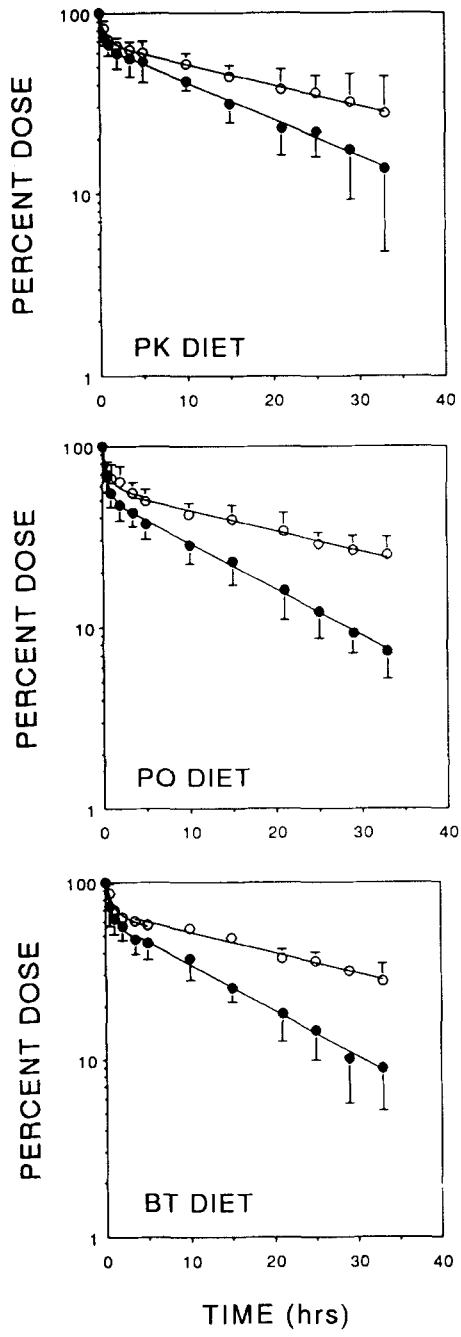

Fig. 4. Plasma decay curves of homologous LDL (●) and human methylated LDL (○) injected into guinea pigs fed 15% palm kernel oil (top panel), palm oil (middle panel), and beef tallow (bottom panel) semi-purified diets to determine total and receptor-independent LDL turnover. Each curve represents the mean ± SD of a minimum of n = 4 determinations.

receptors resulting in higher plasma LDL levels. Previous studies of the effects of dietary fat saturation on LDL metabolism indicated a significant correlation between in vivo and in vitro measures of receptor expression (13). The present studies did not exhibit a similar finding in that BT-fed animals expressed a higher hepatic receptor number than PO-fed animals, when determined in vitro, while both groups (BT and PO) had similar receptor-mediated LDL FCR values measured in vivo (Tables 6 and 7). However, a significant correlation was found between plasma LDL levels and both hepatic LDL receptor B_{max} and receptor-mediated LDL FCR for all dietary groups (Figs. 1 and 5). One possible explanation for this apparent inconsistency could be the variation in plasma LDL concentrations of guinea pigs fed the PO diet. PO-fed animals used in the LDL kinetic studies had a lower mean plasma LDL level (49 ± 8 mg/dl, n = 5) than animals used for in vitro LDL binding studies (59 ± 9 mg/dl, n = 6). While these values are not significantly different, the observation is consistent with the thesis that hepatic apoB/E receptor expression is a major determinant of plasma LDL levels. An alternative explanation for the observed differences between the in vivo (whole animal) and in vitro (hepatic) measures of LDL receptor expression could be that guinea pigs fed the PO diet have increased rates of receptor-mediated LDL catabolism by extrahepatic tissues relative to the other dietary groups, a hypothesis consistent with the finding that animals fed the PO diet had lower levels of hepatic cholesterol than those fed either the BT or PK diets (M. L. Fernandez and D. J. McNamara, unpublished observations).

Saturated dietary fat and LDL turnover

Receptor-mediated LDL FCR values were significantly correlated with plasma LDL levels; however, animals fed the PK diet exhibited a somewhat stronger correlation between receptor-mediated FCR and plasma LDL levels than animals fed the other two dietary fats (Fig. 5). This

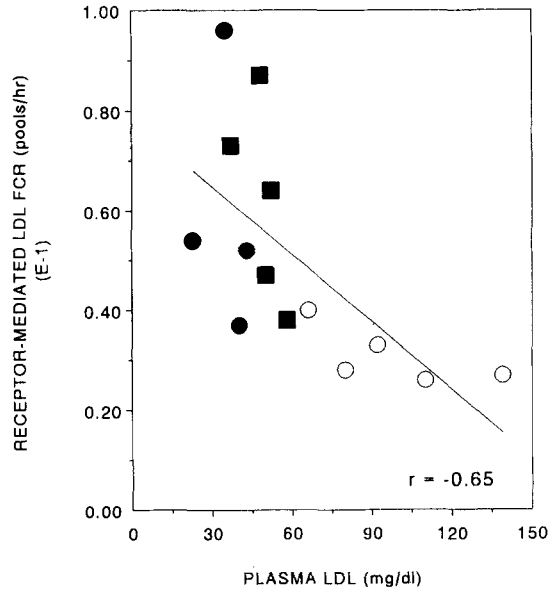

Fig. 5. Correlation between plasma LDL cholesterol levels and apoB/E receptor-mediated LDL turnover for guinea pigs fed 15% beef tallow (●), palm oil (■), and palm kernel oil (○) semi-purified diets ($r = -0.66$, $P < 0.01$).

suggests that other factors, including LDL apoB flux rates and intravascular processing, could make a significant contribution to the plasma LDL levels in animals fed BT and PO diets.

It has been speculated that dietary intake of polyunsaturated fat increases the fluidity of hepatic membranes and facilitates recycling of receptors in coated pits, resulting in an increased expression of membrane apoB/E receptors (26). Although fatty acid composition of hepatic membranes was significantly affected by the type of dietary saturated fat, SI values were not different among diet groups, suggesting that changes in membrane fluidity are not a major determinant of effects on LDL turnover rates when animals are fed different types of saturated fat.

In addition to the observed differences in apoB/E receptor expression, LDL apoB flux rates were found to be a significant factor accounting for differences in plasma LDL levels among the dietary fat groups. Previous studies of the effect of dietary fat saturation on LDL metabolism in guinea pigs noted that both a decrease in LDL apoB flux rates and an increase in receptor-mediated FCR contributed to the polyunsaturated fat-mediated decrease in the plasma LDL apoB pool size (13). Similar to these findings, Woollett et al. (27) reported that in hamsters substitution of saturated fat by polyunsaturated fat resulted in a significant increase in LDL receptor activity and a reduction in LDL production.

In the present studies the different saturated fats had specific effects on both FCR and flux rates of LDL apoB that account for the differences in plasma LDL levels in animals fed PK, PO, and BT diets. As the BT diet contains higher levels of stearic acid and has a lower palmitic acid content relative to the PO diet, the data indicate that stearic has a less stimulatory effect on LDL apoB flux rates than palmitic acid. Consistent with these effects of stearic acid, LDL apoB flux rates for the BT group (1.04 ± 0.31 mg/kg-h) are not different from values previously reported for guinea pigs fed a 15% (w/w) lard diet, which has an almost identical content of palmitic and stearic acids (13).

Limitations of study and new questions

Guinea pigs, similar to humans, carry the majority of their plasma cholesterol in LDL which makes the guinea pig a unique rodent model for study of dietary and drug effects on plasma LDL turnover (13, 28, 29). In addition to the fact that the LDL:HDL ratio is between 2.0 and 6.0, depending upon the dietary fat (12), guinea pigs are similar to humans in that the plasma LDL responds to changes in dietary fat quality in the absence of dietary cholesterol (30). The guinea pig also has an active plasma cholesteryl ester transfer (31) and the combination of these metabolic characteristics make this animal a suitable model for these studies.

While use of the Mathews model to analyze LDL turnover is the most appropriate in terms of comparison to other studies, a possible limitation is the assumption that LDL particles within each dietary fat group are kinetically homogeneous. The peak density profiles obtained for LDL from all diet groups indicate two major subpopulations of LDL that could have different compositions, as has been observed for LDLs isolated from corn oil-, olive oil- and lard-fed guinea pigs (M. L. Fernandez and D. J. McNamara, unpublished observations) and possibly be kinetically heterogeneous. Further studies are needed to determine whether the two major subpopulations of LDL have different in vivo turnover rates.

Another limitation of the present study is the high percentage of saturated fat in the PK diet compared to the PO and BT diets which could be a confounding variable when comparing the effects of the different saturated fatty acids on the measured LDL parameters. This consideration raises the possibility that the reduced expression of apoB/E receptors in animals fed the PK diet could be due to the low intake of monounsaturated fat compared to the PO and BT diets, along with decreased intakes of stearic and palmitic acids. As previous studies have demonstrated that intake of olive oil results in similar LDL metabolic responses to intake of lard (12, 13), it remains unclear what contribution oleic acid intake makes to the observed differences in LDL metabolism mediated by the PK, PO, and BT diets.

The apoB/E receptor expression and LDL apoB flux rates were affected in a distinctive manner characteristic of each type of saturated fat. Animals fed the PK diet had the lowest receptor-mediated LDL FCR values and higher apoLDL flux rates relative to animals fed the BT diet, resulting in higher plasma LDL levels compared to the other two dietary groups. Animals fed the PO diet had similar receptor-mediated FCR values and higher LDL apoB flux rates than animals from the BT group, which accounts in part for the slightly higher plasma LDL levels in the PO group. Two questions can be raised from these observations that require additional studies. *a)* Are the differences in receptor-mediated LDL FCR explained by differences in saturated fatty acid chain length or by the higher content of oleic acid in the BT and PO diets compared to the PK diet? *b)* Are the observed differences in apoLDL flux rates due to differences in the rate of VLDL apoB production, the conversion of VLDL to LDL, or the rate of LDL direct synthesis and secretion? Khosla and Hayes (10) reported that in the rhesus monkey diets high in lauric and myristic acids increase direct hepatic LDL production which contributes to increased plasma LDL levels. Studies measuring VLDL kinetics in animals fed BT, PO, and PK diets are needed to answer this question.

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