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Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation[1]

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Abstract Dietary fat saturation has been shown to affect hepatic apoB/E receptor expression and to modify low density lipoprotein (LDL) composition and density in guinea pigs. The current studies were designed to investigate the independent and interactive effects of dietary fat saturation alterations in apoB/E receptor expression and LDL composition on in vivo LDL turnover kinetics, both receptor-mediated and receptor-independent. Guinea pigs were fed semi-purified diets containing 15% fat, either polyunsaturated corn oil (CO), monounsaturated olive oil (OL), or saturated lard, and injected with radioiodinated LDL isolated from animals fed the homologous diet. Blood samples were obtained over 33 h to determine apoLDL fractional catabolic rates (FCR) and flux rates. Compared to animals fed OL- or lard-based diets, intake of the CO-based diet resulted in a 50% decrease in LDL apoB pool size associated with a twofold increase in receptor-mediated FCR ($P < 0.001$) and a 28% decrease in flux rate ($P < 0.05$). Maximal LDL binding capacity of hepatic apoB/E receptors, determined in vitro, was twofold higher for animals fed the CO-based diet compared to guinea pigs fed the OL- and lard-based diets ($P < 0.01$). There was a significant correlation between hepatic apoB/E receptor number and in vivo receptor-mediated LDL FCR ($r = 0.987$). Significant differences in LDL turnover were related to the source of LDL. When injected into animals fed a nonpurified commercial diet, the smaller, cholesteryl ester-depleted LDL isolated from animals fed the CO-based diet had a twofold higher FCR compared to larger LDLs from guinea pigs fed the OL- and lard-based diets, which had similar turnover rates. When LDL from animals fed the commercial diet was radiolabeled and injected into animals fed the three types of dietary fat, significant differences in LDL turnover were observed in the order CO > lard > OL, suggesting that intravascular processing and tissue uptake of the smaller LDL from animals fed the commercial diet varies depending on the dietary fat saturation fed to the recipient animals. These studies demonstrate that guinea pigs fed polyunsaturated fat diets lower plasma LDL levels in part by an increase in apoB/E receptor-mediated fractional LDL turnover and a decrease in apoLDL flux. In addition, fat saturation alters LDL composition and size which independently affect LDL turnover rates in vivo.—**Fernandez, M. L., E. C. K. Lin, and D. J. McNamara.** Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation. *J. Lipid Res.* 1992. **33:** 97–109.

Supplementary key words apoB/E receptor • low density lipoprotein • apoLDL fractional catabolic rate • dietary fat saturation • apoLDL production rate

Low density lipoprotein (LDL) constitutes the major plasma lipoprotein cholesterol fraction in humans, and numerous studies have demonstrated that increased levels of plasma LDL cholesterol are related to increased risk for cardiovascular disease (1). In vivo metabolic studies have shown that plasma LDL levels are regulated by the balance between rates of LDL production and of catabolism by both receptor-mediated and nonspecific tissue uptake of circulating LDL (2).

Dietary intake of polyunsaturated fat (PUFA) has been shown to decrease plasma LDL cholesterol levels compared to saturated fat (SFA); however, clinical studies have been inconsistent in determining the mechanisms by which dietary PUFA lowers plasma LDL cholesterol levels (3). Turner, Le, and Brown (4) and Cortese et al. (5) reported lower LDL flux rates in both normal and hyperlipidemic subjects fed a PUFA-rich diet compared to an SFA-rich diet. In contrast, Shepherd et al. (6) found that PUFA in the diet lowers plasma LDL levels by increasing LDL apolipoprotein B (apoB) fractional catabolic rates (FCR) compared to intake of an SFA diet. These authors (6) attributed part of the difference in LDL apoB FCR to

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; OL, olive oil; CO, corn oil; FCR, fractional catabolic rate; PR, production rate; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MONO, monounsaturated fatty acids.

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changes in LDL composition induced by dietary fat saturation. Changes in LDL composition mediated by dietary fat saturation have been related to changes in LDL metabolism by cultured fibroblasts in that LDL derived from subjects fed SFA had decreased binding, internalization, and degradation by fibroblasts relative to LDL from subjects fed a PUFA diet (7).

Spady and Dietschy (8) reported that apoB/E receptor-mediated LDL clearance was increased in hamsters fed a PUFA diet compared to animals fed SFA or monounsaturated (MONO) fat diets. In vivo LDL kinetics studies in Cebus monkeys have shown a 70% increase of LDL uptake when animals were fed a corn oil-(PUFA) versus a coconut oil (SFA)-based diet (9). Crossover studies in mononuclear cells isolated from Cebus monkeys demonstrated that LDL isolated from animals fed the PUFA diet had higher rates of degradation than LDL isolated from animals fed the SFA diet (10). A higher apoB LDL fractional catabolic rate and lower apoB production rate were reported for squirrel monkeys consuming safflower oil relative to animals fed a butter-based diet (11).

Studies in guinea pigs have demonstrated changes in LDL peak densities and chemical composition mediated by dietary fat saturation (12, 13). Animals fed PUFA diets had higher LDL peak densities and less LDL cholesteryl ester than animals fed MONO or SFA diets. In addition, an increase in LDL binding to hepatic apoB/E receptors of guinea pigs fed PUFA as compared to animals fed MONO or SFA diets has been reported (12, 13). While various studies indicate that intake of PUFA results in increased receptor-mediated LDL catabolism, it is not clear whether this is due to increased expression of apoB/E receptors, to changes in LDL composition related to differences in receptor interaction with LDL, changes in LDL flux rates, or to interactive effects of these variables.

The present studies were designed to test the hypothesis that the reported differences in hepatic apoB/E receptor number and in LDL composition and density mediated by dietary fat saturation (12, 13) have independent effects on in vivo LDL kinetics. In addition, since no previous reports exist that correlate in vitro LDL binding and in vivo kinetics, the data were analyzed to determine the relationship of dietary fat effects on in vivo apoB/E receptor-mediated LDL turnover and hepatic LDL receptor number measured in vitro. The guinea pig was chosen as the animal model for these studies because, like humans, guinea pigs transport plasma cholesterol mainly in LDL.

TABLE 1. Composition of diets

Component	Weight	Calories
	%	%
Protein (soy)	22.4	23.0
Fat (CO, OL, or lard)	15.1	35.1
Carbohydrate (sucrose/starch)	39.6	41.9
Fiber (cellulose/guar gum)	13.6	
Mineral mix[a]	8.2	
Vitamin mix[a]	1.1	
kcal/g	3.8	

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

EXPERIMENTAL PROCEDURES

Materials

^{125}INa and ^{131}INa were purchased from NEN Research Products (Boston, MA); enzymatic cholesterol assay kits, cholesterol esterase, and cholesterol oxidase from Boehringer Mannheim (Indianapolis, IN), halothane from Halocarbon (Hackensack, NJ), and Quickseal ultracentrifugation tubes from Beckman Instruments (Palo Alto, CA). Other materials were obtained from previously reported sources (12, 13).

Diets

Diets were prepared and pelleted by Research Diets Inc. (New Brunswick, NJ) as previously described (12, 13). All diets had identical compositions except for the fat source (**Table 1**) which was 15% corn oil (CO), high in PUFA; olive oil (OL), high in MONO; or lard, high in SFA. The fatty acid compositions of the dietary fats presented in **Table 2** were determined by gas–liquid chromatography as previously reported (12). Fat represented 35% of the total caloric content of the diets and the required amounts of cholesterol and plant sterols were added to each diet to assure equivalent levels; the cholesterol content was 0.013 mg/g of diet. Guinea pig commercial nonpurified diet was obtained from Teklad (Madison, WI) and is reported to be, by weight, 17.9% protein (mainly soy-

TABLE 2. Fatty acid composition of diets

Fatty Acids	Dietary Fat		
	Corn Oil	Olive Oil	Lard
	%	%	%
14:0	0.2	0.0	1.6
16:0	10.0	8.8	24.0
16:1	0.2	0.6	3.1
16:2	9.3	0.1	0.3
18:0	1.8	3.2	13.6
18:1	22.2	78.5	42.3
18:2	48.4	7.3	10.7
18:3	1.1	0.9	1.2
20:0	6.0	0.4	2.4
P/S ratio	3.11	0.63	0.29

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bean meal), 1.9% vegetable fat, 49.3% carbohydrates, and 14% fiber (mainly alfalfa) with a caloric density of 2.9 kcal/g.

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 for isolation of LDL 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 (12–14). Animals were anesthetized by halothane vapors and exsanguinated by cardiac puncture. Animals involved in the kinetic studies were killed by 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 weights between groups (**Table 3**).

LDL isolation and characterization

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 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 (15). The lipoprotein profile was determined by measuring cholesterol in the isolated fractions, and density values were determined by measurement of the refractive index (16). LDL used for 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 (12, 13).

TABLE 3. Final body weights and plasma total and LDL cholesterol levels of guinea pigs fed corn oil, olive oil, and lard diets

Diet (n)	Body Weight	Cholesterol	
		Total	LDL
	g	*mg/dl*	
Corn oil (16)	642 ± 31^a	45.8 ± 7.9^a	25.3 ± 4.4^a
Olive oil (14)	622 ± 41^a	71.5 ± 12.9^b	48.9 ± 9.9^b
Lard (13)	651 ± 51^a	68.1 ± 9.7^b	43.9 ± 6.9^b

Values are presented as mean ± SD for (n) determinations. Values in the same column with different superscripts are significantly different as determined by ANOVA ($P < 0.0001$).

LDL cholesterol determinations were based on the plasma total cholesterol concentration for each guinea pig and regression lines for the relationship between total and LDL cholesterol concentrations for animals fed the three dietary fats as calculated from previous experiments (12, 13, 17). Guinea pig plasma apoB LDL was quantitated as the difference between total LDL protein and soluble protein after isopropanol precipitation (18).

LDL particle diameters were calculated from the core to surface volume ratio according to Van Heek and Zilversmit (19) by use of the following 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 as previously reported (20).

Human LDL was isolated by sequential ultracentrifugation between densities 1.019 to 1.063 g/ml and washed at d 1.063 g/ml. Methylated human LDL was prepared as described by Weisgraber, Innerarity, and Mahley (21). 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 in blocking receptor recognition. While human LDL is an effective competitor for guinea pig ^{125}I-labeled LDL binding to hepatic membranes (22), methylated human LDL exhibited no competition, confirming the specificity of the methylation procedure. Iodination of lipoproteins was performed according to the method of Goldstein, Basu, and Brown (23). Radiolabeled LDL were used within 2–4 days of preparation to minimize potential changes due to radiation oxidation (24).

Analytical methods

Plasma total and lipoprotein cholesterol levels were determined by enzymatic analysis (25) and isolated LDL was analyzed for protein (26), triglyceride (27), total and free cholesterol (28), and phospholipid (29) as previously described (13).

In vitro LDL binding

Hepatic membranes were isolated from animals fed PUFA, MONO, and SFA diets (12, 13) and incubated at 37°C for 2 h with ^{125}I-labeled LDL isolated from animals fed the homologous diet. After incubation, membranes were pelleted and washed by ultra-

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centrifugation and binding was determined as previously described (12, 13). To determine receptor number (B_{max}), and affinity (K_d), hepatic membranes (200 μg) were incubated with ^{125}I-labeled LDL over a range of 5 to 40 μg of LDL protein/ml in the presence or absence of 1 mg/ml of unlabeled human LDL. Previous studies have demonstrated that at 37°C, human LDL is an effective competitor for guinea pig LDL binding to guinea pig hepatic apoB/E receptors (22). B_{max} and K_d were calculated from Woolf plots by plotting free LDL (μg/ml) versus free/bound [(μg/ml)/ (ng/mg)] LDL protein (30).

Metabolic studies

Plasma LDL turnover kinetics were determined as described by Witztum et al. (31). FCR values were calculated using a two-pool model as described by Matthews (32). For all studies, guinea pig LDL kinetics were measured over a period of 33 h by sampling plasma LDL radioactivity at 10 min (zero time) and at 0.5, 1, 1.5, 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 filasting tubing, I.D. 0.64 mm (Dow-Corning, Corning, NY) inserted in the jugular. In the first set of experiments, LDL isolated from animals fed 15% CO-, OL-, and lard-based diets were radiolabeled with ^{125}I and injected into animals fed the homologous diets to determine FCR and production rates (PR) in a completely autologous system. Receptor-independent LDL turnover was measured by injecting either ^{125}I-labeled or ^{131}I-labeled methylated human LDL (9). As observed in other reports (30), no differences were found for the measured FCR between the two isotopes.

In the second set of experiments, LDL isolated from animals fed CO, OL, and lard diets were radiolabeled with ^{125}I and injected into animals fed a commercial diet to determine whether LDL composition and size differences due to dietary fat saturation (12, 13) affected plasma LDL kinetics. Receptor-independent LDL turnover in chow-fed animals was determined by measuring the disappearance of radiolabeled methylated human LDL over the same 33-h time period.

Finally, a third set of experiments was carried out to determine the effect of dietary fat saturation on intravascular processing and receptor-mediated turnover of LDL in guinea pigs injected with the same LDL particle. For that purpose, LDL isolated from animals fed a commercial diet was radiolabeled and injected into animals fed CO-, OL-, and lard-based diets and LDL kinetics were determined.

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess differences in plasma cholesterol levels, pool size, FCR, PR, and the metabolic parameters K_d and B_{max} of animals fed the three dietary fats. The least significant difference test was used to evaluate differences between means (33). Multiple regression analysis was used to determine significant correlations. Data are presented as the mean ± SD for the number of animals tested.

Model selection

The selected model for measuring plasma LDL turnover was the two-pool model or the Mathews model which assumes that the plasma LDL exchanges with a second compartment which is extravascular (32). This model was selected assuming that LDL particles from a given diet are kinetically homogeneous (i.e., all LDL particles have the same probability of following the different metabolic pathways) and that LDL catabolism occurs only from the plasma compartment.

Other models used to measure LDL kinetics assume that the population of plasma LDLs are kinetically heterogenous and establish the presence of two or more plasma LDL apoB compartments which have different metabolic fates (34). Other studies measure plasma and urinary radioactivity and fractional catabolic rates are determined by calculating the daily (24 h) urinary iodine radioactivity divided by the mean plasma concentration of labeled iodine during the same time period (34). The determination of urinary radioactivity (loss of labeled LDL from the plasma pool) is necessary in order to test other models that assume LDL is kinetically heterogeneous. Since urine radioactivity must be measured during plasma sampling in the course of the experiment, the testing of other models that assume LDL is kinetically heterogeneous could not be determined in this experimental design.

The two-pool or Mathews model is the most routinely used for apoB LDL kinetics in both clinical (3–6) and animal (9, 11, 31, 35, 36) studies; therefore, for purposes of comparison, this model was selected for data analysis in this study.

LDL isolated from the various diets exhibit LDL subfractions that differ in composition (Fernandez, M. L., and D. J. McNamara, unpublished observations), therefore the possibility of kinetically heterogenous plasma LDL particles cannot be excluded. However, Luc and Chapman (35) measured plasma LDL turnover using two different LDL subfractions isolated from guinea pigs fed a nonpurified diet and

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found no significant differences in FCR, suggesting that while LDL subfractions differ in composition, they are kinetically homogeneous (35).

RESULTS

Effects of dietary fat saturation on plasma cholesterol levels and LDL size and composition

No significant differences in body weight gain or final body weights were observed for guinea pigs fed the three dietary fats (Table 3). Plasma cholesterol levels were 34% lower in CO-fed animals compared to animals fed either the OL or lard diets (Table 3). As previous reports have demonstrated (12, 13), the hypocholesterolemic effect of the CO diet was due to a significant reduction of plasma LDL cholesterol concentrations (Table 3). Animals fed the non-purified diet had an average weight of 845 g and plasma total and LDL cholesterol values were 31 ± 12 and 17 ± 7 mg/dl (n = 10), respectively. As previously reported, dietary fat saturation affected both the composition and peak densities of LDL (12, 13). LDL isolated from animals fed PUFA diets had a higher peak density compared to LDL from animals fed MONO and SFA diets ($P < 0.001$), consistent with CO-fed guinea pigs having smaller LDL particles (**Table 4**). Animals fed the PUFA diet had a lower percentage of LDL cholesteryl ester and higher percentage of phospholipids than LDL from animals fed MONO or SFA diets. These compositional changes resulted in a lower core (cholesteryl ester and triglyceride) to surface (protein, free cholesterol, and phospholipids) ratio ($P < 0.05$) for LDL isolated from CO-fed animals compared to LDL from OL- or lard-fed guinea pigs (Table 4). LDL isolated from animals fed a commercial nonpurified diet had significantly higher peak densities and lower core to surface ratios (Table 4) indicating that LDL isolated from guinea pigs fed the 1.9% fat nonpurified diet are smaller than LDL isolated from animals fed the semi-purified 15% fat diets. Calculation of LDL diameters indicated that LDL from guinea pigs fed the nonpurified diet or on the corn oil-based diets were 25% smaller than LDL isolated from animals fed OL- and lard-based diets ($P < 0.001$) (Table 4).

TABLE 4. Composition and size parameters of LDL isolated from corn oil, olive oil, lard and chow-fed guinea pigs

LDL Source (n)	Peak Density	Core/Surface Ratio	Calculated Diameter
	g/ml		*Å*
Chow (6)	1.068 ± 0.003[a]	0.72 ± 0.13[a]	159 ± 21[a]
Corn Oil (4)	1.054 ± 0.003[b]	0.84 ± 0.10[a]	174 ± 17[a]
Olive Oil (4)	1.045 ± 0.002[c]	1.15 ± 0.21[b]	222 ± 17[b]
Lard (4)	1.047 ± 0.002[c]	1.19 ± 0.18[b]	227 ± 26[b]
P value	< 0.0001	< 0.001	< 0.005

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

Effects of dietary fat saturation on LDL binding to hepatic membranes

Previous studies of LDL binding to hepatic membranes have shown that membranes of CO-fed guinea pigs bound significantly more LDL at 4°C than membranes from animals on the OL and lard diets (13). Similar data were obtained in these studies where the LDL receptor number (B_{max}) of isolated membranes was significantly higher ($P < 0.01$) for guinea pigs fed the CO diet than for animals fed OL and lard diets when binding assays were performed at 37°C. Animals fed MONO and SFA diets had similar receptor numbers. B_{max} values were 1796 ± 335 (n = 3) versus 647 ± 225 (n=3) and 976 ± 261 (n = 3) ng bound/mg of membrane protein for animals fed CO, OL, and lard diets, respectively. As previously reported (12, 13), the affinity of the receptor for LDL was not significantly affected by dietary fat saturation with K_d values of 35 ± 13 (n = 3), 20 ± 8 (n = 3), and 26 ± 12 (n = 3) μg/ml for guinea pigs fed PUFA, MONO, and SFA diets, respectively.

Effects of dietary fat saturation on plasma LDL kinetics

Dietary fat saturation significantly affected LDL turnover kinetics when the source of injected LDL was from a homologous source (i.e., animals fed the same diet). As shown in **Fig. 1**, animals fed PUFA diets had more rapid plasma LDL disappearance rates than guinea pigs fed SFA or MONO diets. Animals fed MONO and SFA diets had virtually identical plasma LDL disappearance curves for homologous LDL (Fig. 1).

Dietary fat saturation significantly affected plasma LDL apoB pool sizes, flux rates, and FCR (**Table 5**). Animals fed PUFA diets had significantly smaller LDL apoB pool sizes than animals fed MONO or SFA diets, which were related to a 33% increase in the FCR ($P < 0.001$) and a 28% decrease in flux rates ($P < 0.05$) compared to animals fed MONO or SFA diets. ApoB pool size from the three dietary fats could be predicted from the equation:

$$\text{LDL apoB pool size (mg/kg)} = 9.803\ \text{PR (mg/kg}\cdot\text{h)} - 110.62\ \text{FCR (pools/h)} + 11.70.$$

Since plasma LDL turnover occurs by both receptor-mediated and receptor-independent processes,

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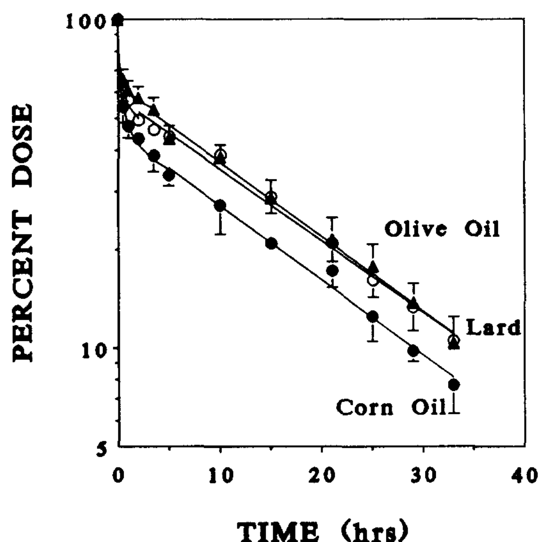

Fig. 1. Plasma decay curves of LDL isolated from animals fed 15% corn oil (●) olive oil (▲), and lard (○) diets and injected into guinea pigs fed the homologous diet. Identical amounts of LDL protein were injected in the guinea pigs and the plasma decay curves were analyzed as described in Experimental Procedures. Each curve represents the mean ± SD of four determinations.

studies were carried out to determine the effects of dietary fat saturation on receptor-independent LDL turnover. Initial studies were carried out measuring the turnover rate of methylated human LDL in guinea pigs fed a commercial diet (**Fig. 2**). Receptor-independent LDL turnover was 0.035 ± 0.002 (n = 3) pools/h which accounted for 29% of total LDL turnover. Similar values for receptor-independent LDL turnover have been reported by other investigators for guinea pigs fed commercial nonpurified diets (31, 35). In contrast, receptor-independent LDL turnover was higher in animals fed the 15% CO diet (Fig. 2). Animals fed CO-, OL-, and lard-based diets had virtually identical values for receptor-independent LDL turnover with an average FCR of 0.057 ± 0.009 (n=8) pools/h. Nonreceptor-mediated LDL catabolism accounted for 50% of total LDL turnover in animals fed the PUFA diet and 70% in animals fed MONO or SFA diets.

A significant relationship was observed between LDL receptor B_{max} values, determined from in vitro LDL binding studies, and in vivo receptor-mediated LDL FCR. The data presented in **Fig. 3** demonstrate that apoB/E receptor expression, whether determined from in vitro LDL binding or from in vivo receptor-mediated FCR, was significantly higher in animals fed the CO diet ($P < 0.01$). OL- and lard-fed guinea pigs had similar B_{max} values and receptor-mediated FCRs.

TABLE 5. LDL metabolic parameters in guinea pigs fed corn oil, olive oil, and lard diets determined by injecting radiolabeled autologous LDL

Diet (n)	Pool Size[1]	FCR	Flux Rates
	mg/kg	*pools/hr*	*mg/hr · kg*
Corn oil (4)	7.6 ± 1.0[a]	0.112 ± 0.009[a]	0.88 ± 0.07[a]
Olive oil (4)	15.0 ± 2.5[b]	0.082 ± 0.009[b]	1.24 ± 0.30[b]
Lard (4)	13.8 ± 1.0[b]	0.086 ± 0.006[b]	1.20 ± 0.15[b]
P value	< 0.001	< 0.001	< 0.05

Values are presented as mean ± SD for (n) determinations. Values in the same column with different superscripts are significantly different as determined by ANOVA.
[1]Pool size was calculated as the plasma volume (4.5% of body weight) x plasma apoLDL concentration.

Plasma LDL kinetics: effects of LDL source

Since homologous LDL was used to determine plasma LDL kinetics in guinea pigs fed the various fat diets, the question arises whether observed differences in FCR due to dietary fat saturation are solely due to differences in LDL-receptor expression or also involve effects related to differences in LDL composition and size. Two additional sets of experiments were performed to address this question.

To investigate potential effects of dietary fat-mediated changes in LDL composition and size on LDL turnover, LDL isolated from animals fed the three dietary fats were radiolabeled with ^{125}I and injected into guinea pigs fed a commercial nonpurified diet. As shown in **Fig. 4**, plasma LDL turnover was more rapid for LDL isolated from guinea pigs fed the CO-based diet compared to LDL obtained from animals fed either the OL- or lard-based diets. FCR values were significantly higher for ^{125}I-labeled LDL from CO-fed animals than for ^{125}I-labeled LDL derived from OL- or lard-fed guinea pigs (**Table 6**). Analysis of the $t_{1/2}$ of the α and β pools indicated that the $t_{1/2}$ of the β accounted for the observed differences in FCR. Animals fed the PUFA diet had a significantly shorter β pool $t_{1/2}$ than animals fed MONO or SFA diets ($P < 0.05$). The α pool exhibited similar half-life in all dietary fat groups and was not significantly different among diets (Table 6).

Plasma LDL kinetics: effects of dietary fat

In order to determine the effects of dietary fat saturation on LDL kinetics in the absence of differences in LDL composition and size, LDL isolated from animals fed a commercial nonpurified diet was radiolabeled and injected into guinea pigs fed PUFA, MONO, and SFA diets. Plasma LDL turnover was highest in animals fed the CO diet, intermediate in animals fed lard, and slowest in animals fed the OL diet (**Fig. 5**). A comparison of FCR values obtained for the three dietary fat groups is shown in **Table 7**.

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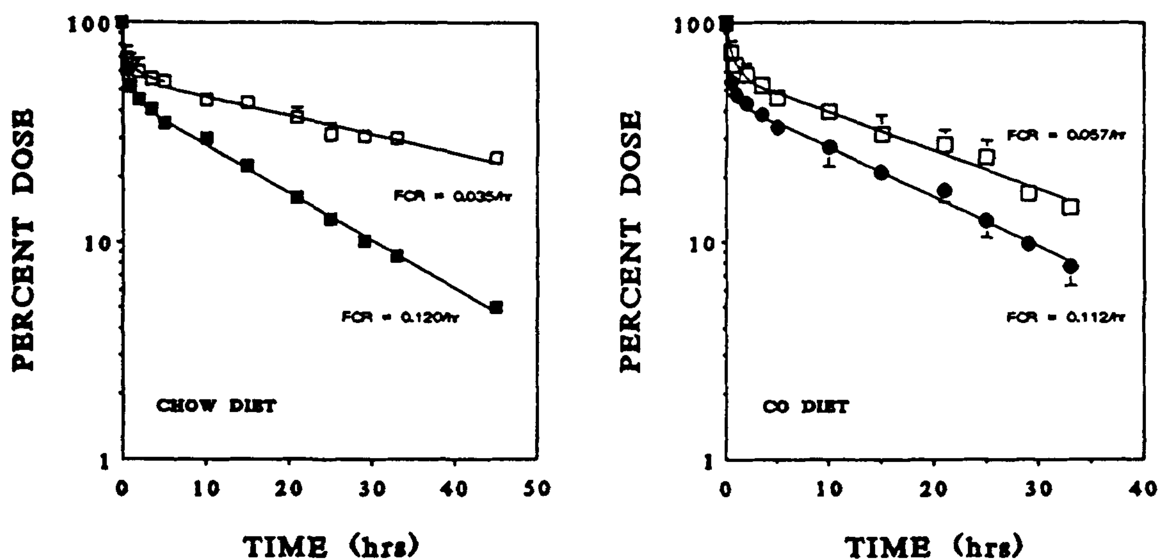

Fig. 2. Plasma decay curves of homologous (■, ●) and human methylated LDL (□) injected into guinea pigs fed a commercial nonpurified diet (left panel) and animals fed a 15% corn oil diet (right panel). Composition of the diets are presented in Experimental Procedures and in Table 1. Native LDL curve in left panel represents the mean of two determinations for guinea pig LDL and of three determinations for human methylated LDL. Curves in right panel represent the mean ± SD of four determinations each.

FCR values for animals fed the commercial nonpurified diet were found to be similar to reported values (35, 36).

Differences in FCR were observed when animals were injected with homologous LDL compared to LDL isolated from guinea pigs fed the commercial diet. In the case of CO- and lard-fed guinea pigs, FCR values were higher when the animals were injected with the smaller LDL isolated from the animals fed the commercial nonpurified diet (Table 7). However, no differences in FCR values, relative to the LDL source, were observed for OL-fed guinea pigs (Table 7). The source of LDL, as noted above, affected the FCR when injected into animals fed the commercial diet (Table 7 and Fig. 4). These data suggest that differences in LDL kinetics observed in guinea pigs fed 15% CO, OL, or lard diets are due to multiple effects of dietary fat saturation on LDL physicochemical properties, flux rate, and receptor-mediated turnover that interact to determine plasma LDL levels in vivo.

A significant relationship ($r = 0.987$) was found between maximal LDL binding capacity (B_{max}), estimated from in vitro LDL binding to guinea pig hepatic membranes, and in vivo receptor-mediated LDL FCR for guinea pigs fed the different dietary fats both when the labeled LDL was from the homologous and from the nonpurified diet (Fig. 6). The y-intercept (LDL FCR for 0 receptor number) equaled 0.063 pools/h for both sets of data, a value indistinguishable from the experimental value of 0.057 ± 0.009 pool/h obtained for LDL receptor-independent FCR.

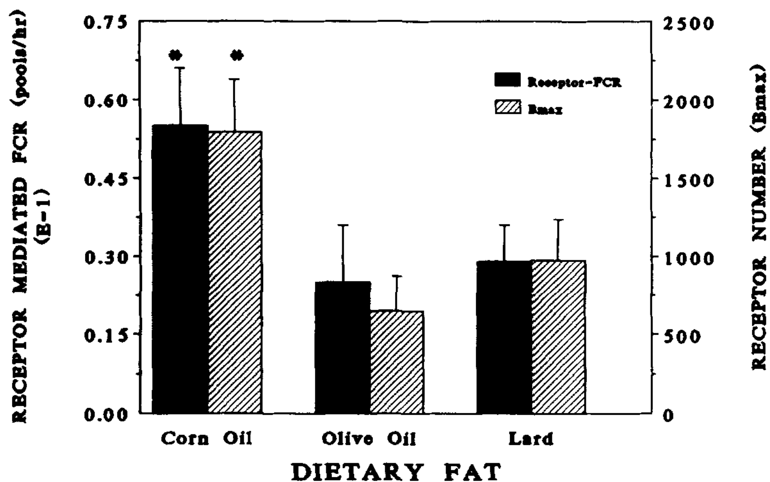

Fig. 3. ApoB/E receptor expression comparing hepatic membranes B_{max} values determined by in vitro LDL binding to guinea pig hepatic membranes at 37°C and receptor-mediated FCR of LDL determined by in vivo LDL kinetics in animals fed corn oil, olive oil, and lard diets. B_{max} values were 1796 ± 335, 647 ± 225, and 976 ± 261 ng bound/mg membrane protein for animals fed corn oil, olive oil, and lard diets, respectively. Values represent mean ± SD of three determinations. Receptor-mediated turnover values were 0.055 ± 0.009, 0.025 ± 0.009, and 0.029 ± 0.006 pools/h for animals fed corn oil, olive oil, and lard diets, respectively. Values represent mean ± SD of four determinations.

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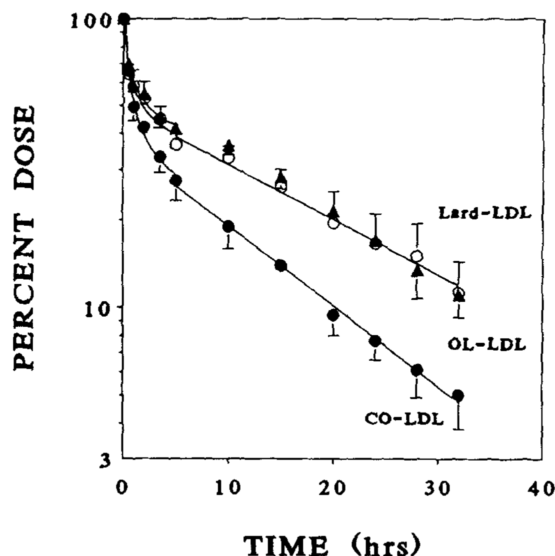

Fig. 4. Plasma decay curves of LDL isolated from guinea pigs fed 15% corn oil, olive oil, and lard diets, and injected into animals fed a commercial nonpurified diet. CO-LDL (●) and OL-LDL (▲) represent the mean ± SD of three and lard-LDL (○) of four determinations. Identical amounts of LDL protein were injected into the guinea pigs. Plasma decay curves were analyzed as described in Experimental Procedures.

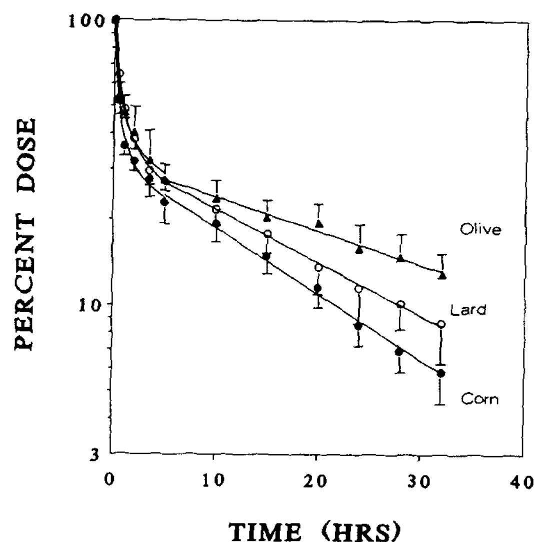

Fig. 5. Plasma decay curves of LDL isolated from guinea pigs fed the commercial nonpurified diet and injected into guinea pigs fed corn oil-(●), olive oil-(▲), and lard (○)-based diets. Corn oil represents the mean ± SD of six and olive oil and lard of four determinations. Identical amounts of LDL protein were injected in the guinea pigs. Plasma decay curves were analyzed as described in Experimental Procedures.

DISCUSSION

Dietary fat saturation and plasma LDL levels and metabolism

Both clinical and animal studies have indicated that changes in LDL flux rates and catabolism occur when dietary fat saturation is changed from SFA to PUFA (4–6, 8–11) and the composite data indicate that both processes are involved. In addition, many of these studies have presented evidence for dietary fat-mediated changes in LDL composition which in itself can have an effect on in vivo and in vitro LDL metabolism (6, 7, 10, 12, 13, 17). Human and animal studies indicate that intake of PUFA increases LDL turnover rates (6, 8, 9) and decreases LDL flux rates (4, 5, 11) compared to SFA intake.

TABLE 6. Metabolic parameters of LDL isolated from guinea pigs fed corn oil, olive oil, and lard diets

LDL Source (n)	FCR	Half Life	
		Pool A	Pool B
	pools/h	*h*	
Corn oil (3)	0.166 ± 0.018^a	0.58 ± 0.34^a	11.7 ± 1.7^a
Olive oil (3)	0.087 ± 0.008^b	0.27 ± 0.18^a	16.0 ± 1.1^b
Lard (4)	0.090 ± 0.018^b	0.47 ± 0.43^a	16.9 ± 3.0^b

LDL isolated from animals fed the three dietary fats were radiolabeled and injected into guinea pigs fed commercial nonpurified diets. Values are presented as mean ± SD for (n) determinations. Values in the same column with different superscripts are significantly different as determined by ANOVA and least significant difference test ($P < 0.01$).

There are still a number of unanswered questions regarding effects of dietary fat saturation on in vivo LDL metabolism, including the possibility of interactions between fat-mediated changes in LDL composition and in vivo turnover, effects of dietary fat saturation on receptor-mediated and nonspecific catabolism of LDL, and the relationship between in vitro analysis of hepatic LDL receptor number to in vivo receptor-mediated LDL turnover.

In the present study we have demonstrated that, when compared to animals fed MONO or SFA diets, guinea pigs fed PUFA diets have two major determinants that account for the reduced plasma LDL cholesterol level, a decrease in LDL apoB flux rates and an increase in receptor-mediated FCR. These results agree with studies indicating that both mechanisms contribute to the hypocholesterolemic effects of PUFA diets in humans (4–6).

As suggested by Grundy and Denke (37), the decrease in LDL apoB flux rates associated with intake of PUFA diets could be due to either a decreased conversion of VLDL to LDL, mediated by increased clearance of VLDL remnants as a result of an increased number of apoB/E receptors, or to a reduced synthesis of VLDL apoB resulting in a decreased substrate pool for the conversion of VLDL to LDL. The latter view is supported by a single clinical study which found that intake of PUFA diets lowered VLDL apoB flux rates with no effect on VLDL apoB FCR (4). Determination of plasma VLDL synthesis and catabolism in guinea pigs fed CO, OL, and lard diets

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TABLE 7. Fractional catabolic rates (pools/hr) of LDL isolated from guinea pigs fed 15% fat diets (corn oil, olive oil, lard) and commercial diet and injected into animals fed the indicated diets

Diet of LDL Donor	Diet of LDL recipient			
	Commercial	Corn Oil	Olive Oil	Lard
Commercial	0.120[a]	0.153 ± 0.020 (6)	0.088 ± 0.016 (4)	0.127 ± 0.019 (4)
Corn oil	0.166 ± 0.018 (3)	0.112 ± 0.009 (4)		
Olive Oil	0.087 ± 0.008 (3)		0.082 ± 0.009 (4)	
Lard	0.090 ± 0.018 (4)			0.086 ± 0.006 (4)

Values are indicated as mean ± SD for the number of determinations in parentheses.
[a]Average of two determinations.

would be required to determine whether dietary fat saturation lowers LDL apoB flux rates directly or decreases the conversion of VLDL to LDL in animals fed PUFA diets.

In order to have a better understanding of the regulatory mechanisms that increase LDL apoB FCR in guinea pigs fed CO diets, experiments were carried out to separate the effects of differences in LDL composition and size from effects on LDL receptor expression mediated by dietary fat saturation.

Dietary fat saturation and apoB/E receptor-mediated LDL turnover

Previous experiments have shown that dietary fat saturation affects LDL composition and peak density in the guinea pigs and that animals fed CO diets have increased expression of hepatic membrane apoB/E receptors compared to animals fed OL or lard diets

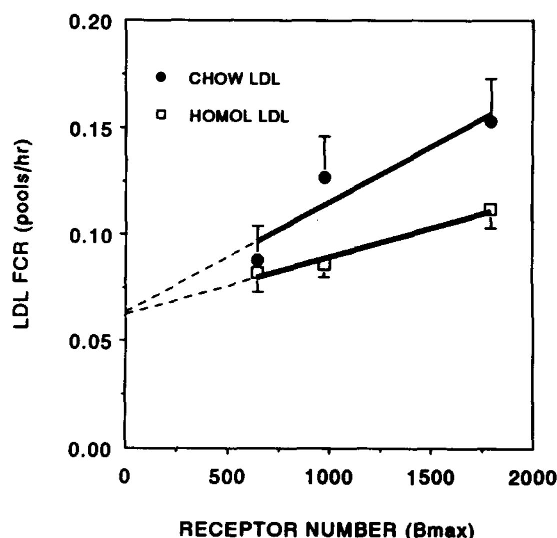

Fig. 6. Correlation between number of hepatic apoB/E receptors (B_{max}) determined by in vitro LDL binding and in vivo LDL turnover in guinea pigs fed corn oil, olive oil, and lard diets. LDL was isolated from guinea pigs fed the homologous diets (□) and from guinea pigs fed the commercial nonpurified diet (●). FCR values represent the mean ± SD of at least four and B_{max} of three determinations ($r = 0.987$ for homologous LDL; $r = 0.953$ for commercial diet LDL, $P < 0.05$).

(12, 13). From these observations one can hypothesize two potential mechanisms by which dietary fat saturation affects plasma LDL turnover; *a*) smaller LDL particles of animals fed PUFA diets have a preferential uptake compared to larger LDL from guinea pigs fed MONO and SFA diets; and *b*) LDL receptor-mediated uptake is increased in animals fed PUFA diets.

In agreement with reported findings in the hamster (8), Cebus monkey (9), and squirrel monkey (11), an increase in receptor-mediated LDL turnover was observed in guinea pigs fed PUFA-containing diets as compared to animals fed MONO and SFA diets when animals were injected with LDL from the homologous diet. In addition, a significant relationship was found between receptor expression of the recipient animals and catabolism of LDL from animals fed the commercial diet. Animals fed CO diets expressed the highest number of apoB/E receptors and had the most rapid catabolism of LDL isolated from animals fed the nonpurified diet (Fig. 5 and Table 7). Similarly, OL-fed guinea pigs had the lowest expression of apoB/E receptors and the slowest turnover rate for this small LDL particle. Receptor expression was slightly higher in lard-fed, relative to OL-fed guinea pigs, and turnover rates of LDL isolated from guinea pigs fed nonpurified diets had an intermediate value in these animals. These results indicate that hepatic apoB/E receptor expression makes a significant contribution to the rate of LDL catabolism whether LDL was isolated from the homologous or from the nonpurified diet.

The significance of apoB/E receptor number of the recipient animal to LDL FCR is apparent as shown in Fig. 6 in which a significant relationship is demonstrated between the two variables. Furthermore, the y-intercept provides a theoretical estimate of the FCR in the absence of LDL receptors and corresponds surprisingly well to the experimental value of LDL receptor-independent FCR in animals fed the three dietary fats irrespective of which tracer LDL is used.

Receptor-independent LDL turnover values in animals fed a commercial nonpurified diet were

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found to be similar to reported values using glycosylated guinea pig LDL (31) or methylated guinea pig LDL (34) which support use of methylated human LDL as a tracer for analysis of receptor-independent catabolism. Contrary to what has been observed in studies of LDL turnover in Cebus monkeys (9), dietary fat saturation had no effect on receptor-independent LDL turnover in that methylated human LDL turnover rates were identical in all animals fed the 15% fat diets.

Dietary fat saturation and LDL composition

Data obtained in the present study demonstrate that dietary fat saturation affected LDL size, composition, and peak density as previously reported (12, 13, 17). The results of LDL kinetic studies indicate that these physicochemical modifications of LDL have a significant effect on in vivo LDL turnover. The comparatively smaller LDL isolated from animals fed the CO diet had a more rapid FCR than the larger LDL isolated from OL- and lard-fed guinea pigs (Table 6 and Fig. 4). In a similar manner, the smaller LDL isolated from animals fed the commercial nonpurified diet had an even more rapid FCR when injected into animals fed the 15% fat diets (Fig. 5, Table 7) which was directly related to the number of apoB/E receptors expressed by the recipient animals (Fig. 6).

Witztum et al. (31) and Berglund et al. (36) reported that administration of either cholestryramine or lovastatin modified the size and composition of guinea pig LDL when compared to LDL isolated from animals fed a control nonpurified commercial diet. However, contrary to what we observed in the present study, these investigators found that the smaller LDL particles induced by cholesterol-lowering drugs had a slower FCR than the larger LDL obtained from control animals (31, 36). One possible explanation for the different metabolic patterns of smaller, more dense LDL found in these two studies could be that the LDL from both the cholestyramine- and lovastatin-treated guinea pigs were isolated in a density range of 1.02–1.063 g/ml which corresponds to a larger, less dense LDL subfraction. LDL from guinea pigs fed commercial diets extends to a density range of 1.1 g/ml and the smaller particles (d 1.063 – 1.1 g/ml) could have made a contribution to the total FCR values of the drug-treated animals.

In vitro studies have also shown that compositional modifications of LDL affect its metabolic characteristics (38, 39). When LDL is incubated in the presence of VLDL and lipid transfer proteins, the resulting cholesteryl ester-poor, triglyceride-rich particle exhibits reduced competition for binding and decreased degradation by human fibroblasts compared to control unmodified LDL. It should be noted, however, that previous studies in guinea pigs fed the same test diets used in these studies failed to document any differences in the in vitro binding affinity of LDL particles to guinea pig hepatic membranes (12, 13).

Swinkels et al. (40) isolated two LDL subfractions from human plasma that were tested in guinea pigs to determine in vivo LDL turnover rates. The denser LDL fraction was catabolized more rapidly than the more buoyant particle suggesting a direct relationship between LDL size and catabolism. Luc and Chapman (35) reported that smaller LDL particles isolated in a density range of 1.05–1.1 g/ml from guinea pigs fed a commercial diet had a faster in vivo turnover rate than larger LDL particles isolated over a density range of 1.024 – 1.05 g/ml. However, FCR values were not different between particles due to intravascular conversion of the smaller LDL to larger LDL particles (35).

Similar to the findings of the present study, Kuo et al. (10) reported an increased rate of LDL degradation by mononuclear cells isolated from Cebus monkeys when LDL was isolated from animals fed a diet high in corn oil versus LDL from animals on a coconut oil-based diet. Checovich, Aiello, and Attie (41) have reported that pigs prone to develop atherosclerosis have an increased number of large buoyant LDL that are catabolized at a slower rate than dense LDL present in normal animals. In agreement with the data obtained in these pigs, Rudel, Haines, and Sawyer (42) found smaller plasma LDL particles in African green monkeys fed PUFA diets compared to LDL particles isolated from animals fed MONO or SFA diets and, in this animal model, smaller particles have been related to decreased coronary atherosclerosis (42). In the present investigation, guinea pigs fed PUFA diets had LDL particles that contained less cholesteryl ester, were smaller in size, and were catabolized at a significantly faster rate than the large, cholesteryl ester-rich LDL from animals fed MONO or SFA diets. These data are consistent with the hypothesis that PUFA diets lead to formation of less atherogenic LDL particles (42). Further studies need to be carried out examining the extent of atherosclerotic lesions in guinea pigs fed PUFA, MONO, and SFA diets for longer periods of time to test this possibility.

It is possible that some of the observed differences in turnover rates of LDL could be due to variations in intravascular processing of the LDL tracer mediated by the saturation of dietary fat. Guinea pigs have significant levels of cholesteryl ester transfer protein (CETP) which is involved in the regulation of plasma cholesteryl ester transport (43). Guinea pigs fed 15% dietary fat have moderate levels of plasma HDL

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cholesterol, 12 to 15 mg/dl (44), with a cholesteryl ester pool that has a rapid turnover rate (45), and would be preferentially transferred to LDL, the major source of plasma cholesteryl ester in these animals. Since all three sets of turnover data suggest that LDL particles of smaller size are removed more rapidly from the plasma, one could predict that an increase of cholesteryl ester transfer from HDL to LDL in animals fed OL- and lard-based diets would lead to the formation of larger particles accounting for the observed decrease in FCR of LDL from the homologous diet and LDL isolated from guinea pigs fed the commercial diet. In agreement with these observations, Stein et al. (46) reported that hamsters fed SFA diets had higher levels of plasma cholesterol which correlated with increased CETP activity when compared to animals fed PUFA diets. Further studies are needed to determine the effects of dietary fat saturation on CETP-mediated transfer of cholesteryl ester from HDL to LDL and how differences in transfer rates affect LDL composition and turnover rates. Since the in vivo and in vitro data indicate that animals fed CO-based diets have higher expression of apoB/E receptors, a decreased LDL residence time in the plasma of PUFA-fed guinea pigs would, in theory, result in decreased CETP-mediated cholesteryl ester transfer and could also account for the more rapid disappearance of the denser, cholesteryl ester-depleted LDL particles.

Limitations of study and new questions

Similar to humans, guinea pigs carry plasma cholesterol mainly in LDL, making the guinea pig a unique animal model for studies of plasma LDL turnover. When compared to an SFA diet, intake of a PUFA diet resulted in increased receptor-mediated FCR values and decreased LDL flux rates analogous to reported data from clinical studies (4–6). One unusual aspect of this animal model is its response to dietary olive oil which results in hypercholesterolemia. Unique responses of cholesterol metabolism in OL-fed guinea pigs have been previously reported (12, 14). MONO- and SFA-fed guinea pigs exhibited virtually identical LDL metabolic responses. They had similar LDL composition, LDL FCR, expression of hepatic apoB/E receptors (B_{max}), and plasma LDL turnover when injected with homologous LDL. Animals fed lard- and OL-based diets differed only in terms of the slower FCR values in MONO-fed animals when injected with the smaller LDL isolated from guinea pigs fed the commercial nonpurified diet. These results suggest that intravascular processing might be different between lard- and OL-fed guinea pigs in that intake of MONO diets might accelerate formation of larger LDL particles.

A possible limitation in this study is the use of the two-pool or Mathews model to analyze LDL turnover rates. Effects of dietary fat saturation on LDL turnover were determined with the assumption that LDL populations from a given diet are kinetically homogeneous. Since LDL subfractions, which differ in composition, have been observed in animals fed the different dietary fats, the possibility of kinetically heterogeneous LDL exists and, if that is the case, the two-pool model might not be the most appropriate to analyze LDL turnover.

These studies demonstrated that differences in both LDL receptor expression and LDL composition mediated by dietary fat saturation independently affect plasma LDL turnover. However, LDL receptor number of the recipient animal was found to be the major determinant of plasma LDL turnover. In addition, the use of a different source of LDL (isolated from animals fed a commercial nonpurified diet) suggest that LDL intravascular processing in animals fed the different dietary fats might also make a contribution to measured rates of plasma LDL turnover (Table 7).

Since dietary fat saturation affected LDL apoB flux and FCR rates and possibly LDL intravascular processing, several new questions can be raised based on these data. PUFA-fed animals had lower LDL apoB flux rates than animals fed MONO or SFA diets. Whether the decrease in apoLDL flux observed in animals fed PUFA diets is related to increased catabolism of VLDL apoB, mediated by the increased number of apoB/E receptors, or to decreased synthesis of apoB VLDL remains to be determined. Since direct LDL synthesis can make a contribution to LDL input rates, as shown in several animal models (41) and in familiar hypercholesterolemia patients (47), it is possible that variations in direct LDL production could occur in response to differences in dietary fat saturation. Based on data in the literature (48), differences in direct LDL production mediated by dietary fat saturation could kinetically contribute to the observed differences in LDL apoB flux rates.

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