

Regulation of guinea pig very low density lipoprotein secretion rates by dietary fat saturation

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Abstract We investigated the effects of dietary fat saturation on very low density lipoprotein (VLDL) production in guinea pigs fed semipurified diets containing 15% (w/w) fat, either corn oil (CO, 58% linoleic acid), lard (LA, 42% oleic and 24% palmitic acids) or palm kernel oil (PK, 52% lauric and 18% myristic acids) for 4 weeks. Animals were given an intravenous injection of Triton WR 1339 to block VLDL catabolism and rates of VLDL triacylglycerol (TAG) and apolipoprotein (apo) B secretion were measured over time. Plasma TAG concentrations increased linearly for 8 h ($r = 0.99$) and VLDL-TAG secretion rates were significantly higher ($P < 0.01$) in guinea pigs fed LA (72.7 ± 14.7 mg/kg-h, $n = 12$) compared to animals fed PK (55.4 ± 13.4 mg/kg-h, $n = 12$) or CO (48.6 ± 17.5 mg/kg-h, $n = 15$). VLDL apoB secretion rates were highest in PK-fed animals (3.1 ± 1.8 mg/kg-h) compared to guinea pigs fed LA (1.5 ± 0.8 mg/kg-h) or CO (1.1 ± 0.6 mg/kg-h) diets ($P < 0.005$). Concurrent with analysis of VLDL secretion, turnover of ¹²⁵I-labeled LDL was measured. Low density lipoprotein (LDL) fractional catabolic rates were not altered by Triton treatment and LDL apoB specific radioactivity (cpm/ μ g) did not change over time indicating that: *a*) the Triton blockage of VLDL catabolism was complete, and *b*) there was no direct secretion of LDL by the liver. **Key words:** These data demonstrate that intake of lard increases the rate of VLDL-triacylglycerol secretion and that nascent VLDL particles from the lard and corn oil diet groups have the same relative triacylglycerol content, whereas palm kernel oil intake increases secretion of VLDL particles which have a reduced triacylglycerol content. These results demonstrate that dietary fat chain length and saturation have specific effects on VLDL secretion rates affecting both particle number and composition.—**Abdel-Fattah, G., M. L. Fernandez, and D. J. McNamara.** Regulation of guinea pig very low density lipoprotein secretion rates by dietary fat saturation. *J. Lipid Res.* 1995. **36**: 1188–1198.

Supplementary key words low density lipoproteins • apolipoprotein B • triacylglycerol secretion

Studies have shown that the type and amount of dietary fat significantly affect plasma low density lipoprotein (LDL) levels and metabolism (1). When the amount of dietary cholesterol remains constant, plasma LDL cholesterol levels are regulated by dietary fatty acid chain length and saturation. The association between intake of saturated fat and elevated plasma LDL cholesterol levels

is due to both an increase in LDL flux rates and a decrease in apoB/E (LDL) receptor-mediated LDL catabolism (1). This saturated fat-mediated increase in LDL flux could be due to either an increase in very low density lipoprotein (VLDL) production rates, increased conversion of VLDL to LDL, increased direct secretion of LDL by the liver, or various combinations of these factors (2).

Studies by Hegsted et al. (3) demonstrated that dietary fatty acids of different chain length and saturation have differential effects on plasma cholesterol levels. The most hypercholesterolemic fatty acid was myristic acid (C14:0) followed by palmitic acid (C16:0) with little effect of stearic acid (C18:0). Similarly, Bonanome and Grundy (4) reported that stearic acid has a hypocholesterolemic effect in normolipidemic subjects when compared to palmitic acid, and Denke and Grundy (5) reported that intake of lauric acid (C12:0) increases plasma LDL cholesterol levels in humans compared to stearic acid. In general, studies in animal models have resulted in similar findings (2, 6–10).

While effects of dietary fatty acids on plasma cholesterol levels are fairly consistent, there is considerable uncertainty regarding effects of dietary fat type on VLDL triacylglycerol (TAG) and apoB secretion. Studies in humans suggest that dietary saturated fat increases VLDL apoB production rates (11); however, the data are from only four subjects. Animal model studies have not provided a definitive answer in that the results have been highly variable. Studies in gerbils (6) indicated that intake of safflower oil increased VLDL-TAG secretion compared to coconut oil intake suggesting that polyunsaturated dietary fat increased hepatic secretion of VLDL-TAG.

Abbreviations: VLDL, very low density lipoprotein; CO, corn oil; LA, lard; PK, palm kernel oil; TAG, triacylglycerol; apo, apolipoprotein; LDL, low density lipoprotein; CETP, cholesteryl ester transfer protein; FCR fractional catabolic rate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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Ohtani et al. (12) reported increased rates of VLDL cholesterol and TAG secretion by hepatocytes from hamsters fed 5% (w/w) linoleic as compared to palmitic acid in the presence of 0.1% (w/w) cholesterol. Similar results have been reported for non-human primates in that intake of safflower oil increases VLDL-TAG secretion compared to coconut oil in both squirrel and cebus monkeys (13). However, not all non-human primate studies support the thesis that intake of polyunsaturated fat increases VLDL-TAG secretion (14). Studies in rhesus monkeys suggest that intake of palm oil as compared to coconut oil increased VLDL apoB flux rates while decreasing the independent production of LDL apoB (15). Groot et al. (16) reported no significant differences in hepatic VLDL-TAG secretion in rats fed either sunflower seed oil- or palm oil-containing diets. Consistent with these data is the report of Lai et al. (17) of similar VLDL-TAG secretion rates in rats fed corn oil versus coconut oil; however, intake of beef tallow increased VLDL-TAG secretion.

Because of these conflicting reports, and due to the variance of the data from the limited studies in human subjects, the present studies were undertaken to determine the effects of dietary fat saturation and chain length on hepatic VLDL secretion and composition in guinea pigs. Previous studies in guinea pigs have shown significant effects of dietary fat type and amount on plasma LDL levels and metabolism (8-10). Therefore, these studies compared effects of a polyunsaturated fat (corn oil) with two saturated fats of varying chain length (palm kernel oil and lard) on VLDL-TAG and apoB secretion rates in the guinea pig. In addition, studies tested whether dietary fat saturation and chain length affected direct hepatic production and secretion of LDL apoB.

Guinea pigs were selected as the animal model because of numerous similarities to humans (8-10): guinea pigs have an LDL:HDL ratio greater than 2 and LDL is the major plasma carrier of cholesterol; the plasma cholesterol responses to dietary fatty acid type and

amount are similar to humans in that primary effects are on LDL and these effects occur even in the absence of cholesterol feeding; they have an active plasma cholesteryl ester transfer protein (CETP) that transfers cholesteryl ester between high density lipoprotein and apoB-containing lipoproteins resulting in intravascular lipoprotein processing similar to humans (18); they have a distribution of tissue cholesterol synthesis and a relative ratio of hepatic free:esterified cholesterol similar to humans (19); and finally, guinea pigs have been shown to have significant changes in hepatic apoB/E receptor number and in LDL flux rates and catabolic rates in response to changes in dietary fatty acid type and amount (8-10).

MATERIALS AND METHODS

Materials

Tyloxapol (Triton WR 1339), triacylglycerol reagent, triacylglycerol calibrator, and aprotinin were purchased from Sigma Chemicals (St. Louis, MO); Na ¹²⁵I from Amersham (Arlington Heights, IL); enzymatic cholesterol assay kits from Boehringer Mannheim (Indianapolis, IN); halothane from Halocarbon (Hackensack, NJ); Quickseal ultracentrifuge tubes from Beckman Instruments (Palo Alto, CA).

Diets

Diets were prepared and pelleted by Research Diets, Inc. (New Brunswick, NJ). All diets were isocaloric (15.9 kJ/gm) as reported previously (9, 10) and contained identical ingredients except for fat type. Fat content was 15% (wt/wt) as either corn oil (CO), lard (LA), or palm kernel oil (PK). The fatty acid compositions of the experimental diets (Table 1) were measured by gas chromatography as previously reported (8). The diets were formulated to meet NRC-specified nutritional requirements of guinea pigs. Plant sterol (0.9 mg sitosterol/g diet) and cholesterol (0.1 mg cholesterol/g diet) were normalized for all diets as previously described (8).

Animals

Male Hartley guinea pigs from Sassco Sprague-Dawley (Omaha, NE) weighed between 250 and 300 g. Animals were randomly assigned to one of three diet groups and after 4 weeks on the test diets were used for *in vivo* kinetic studies and for isolation of plasma VLDL and LDL. Previous studies have shown that this time is sufficient to attain constant plasma cholesterol levels and a metabolic steady state (8). Animals were housed in a light-cycle room (light from 07:00 to 19:00), provided water and diet *ad libitum*, and the drinking water was supplemented with vitamin C. All animals consumed equal amounts of diet and there were no differences in the rates of growth or the final body weights (see Results).

TABLE 1. Fatty acid composition of semipurified diets

Fatty Acid	Experimental Diets		
	Corn Oil	Lard	Palm Kernel
	%		
12:0	0	0	52.4
14:0	0.2	1.6	18.0
16:0	11.6	24.0	8.5
18:0	2.2	13.6	14.0
18:1	25.0	42.3	4.1
18:2	58.4	10.7	1.4
18:3	1.4	1.2	0
20:0	0.4	2.4	0
P/S ^a	4.11	0.29	0.02

^aP/S, ratio of polyunsaturated to saturated fatty acids.

All animal experiments were conducted in accordance with U.S. Public Health Service and U.S. 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 and TAG were measured by enzymatic analysis (20, 21), and the composition of nascent and mature plasma VLDL was analyzed by measuring protein, TAG, phospholipids, and total cholesterol as previously described (8). Plasma was isolated from blood samples from guinea pigs anesthetized with halothane vapors and collected by cardiac puncture into syringes containing EDTA (1 mg/ml) as anticoagulant. Plasma was isolated by centrifugation and a preservative mixture was added [aprotinin (50 kallikrein units/ml), sodium azide (1 μ mol/ml), and phenyl methyl sulfonyl fluoride (0.01 μ mol/ml)] to minimize changes in lipoprotein composition during isolation (22). Plasma lipoproteins were isolated by sequential ultracentrifugation at 125,000 *g* using a Ti 50 rotor as previously described (8-10).

In vivo VLDL-TAG and apoB secretion rates

For analysis of the effects of dietary fat on VLDL-TAG and apoB secretion rates, VLDL catabolism was blocked by Triton injection (23). Triton WR 1339 was diluted to a final concentration of 20% with 0.9% NaCl (pH = 7.4) (24) and injected at a dose of 100 mg/kg body weight through an indwelling silastic catheter inserted in the internal carotid artery. After injection, 0.5- to 1.0-ml blood samples were collected from nonanesthetized animals via an indwelling catheter into EDTA-containing tubes (1 mg/ml) at 0, 10, 15, 20, 35, 50, 75, 120, 180, 300, and 480 min post-injection and used for analysis of plasma triacylglycerol levels and for isolation of plasma VLDL and LDL (see below). Animals were fasted 12 h prior to the experiment and all through the 8-h study. At the end of 480 min animals were anesthetized with halothane vapors and exsanguinated by cardiac puncture and plasma VLDL was isolated by ultracentrifugation at density 1.006 g/ml, dialyzed against 0.09% NaCl, and used to measure nascent VLDL composition.

For analysis of VLDL apoB secretion, VLDL apoB was isolated by precipitation with isopropanol (25). Total protein and protein in the supernatant were determined by a modified Lowry procedure (26). ApoB concentrations were determined by subtracting protein concentration in the supernatant from the total protein concentration. ApoB of VLDL particles accounted for 31-33% of the total VLDL proteins. As each VLDL particle contains a single apoB molecule (27), VLDL-TAG per apoB protein ratios were used to determine apoB secretion rates at the 480 min post-Triton injection time point.

LDL kinetic studies

LDL was isolated by ultracentrifugation between density 1.019-1.09 g/ml, dialyzed against 0.9% NaCl and 0.01% EDTA for 24 h, and concentrated to 1.5-2 mg/ml protein (9, 10). The purity of LDL was assessed by SDS-PAGE electrophoresis. Iodination of LDL was carried out according to the method of Goldstein, Basu, and Brown (28). Radiolabeled LDL was used within 2-3 days after iodination to minimize possible effects due to radiation oxidation (29). 125 I-labeled LDL specific activity ranged between 200 and 400 cpm/ng apoB.

Animals injected with Triton WR 1339 for analysis of VLDL secretion rates were also injected with autologous 125 I-labeled LDL (80 μ g) for analysis of LDL turnover rates and to test for direct secretion of LDL. Blood samples collected for analysis of plasma VLDL-TAG and apoB secretion rates were used to measure LDL kinetics. Twenty- μ l plasma samples were used to measure plasma LDL radioactivity in a gamma counter (LKB Wallac Clinigamma, Finland). The data were fitted to a two-pool model (30) and fractional catabolic rate (FCR) values were calculated as previously described (9, 10).

Nascent and mature VLDL isolation

Plasma from Triton-injected animals was used for isolation of nascent VLDL and non-Triton-injected fasting animals were used to isolate mature VLDL. VLDL was collected at a density of 1.006 g/ml and further purified by washing with a buffer solution at 1.006 g/ml. The composition of nascent and mature VLDL was analyzed by measuring protein, TAG, phospholipids, and total cholesterol as previously described (8). SDS-PAGE showed that nascent VLDL contained apoB-100, apoC, and apoE, with no detectable apoB-48, consistent with the reported absence of apoB mRNA editing by the guinea pig liver (31).

To determine whether the presence of Triton WR 1339 affects the determination of VLDL composition, *in vitro* tests were conducted. Plasma VLDL was isolated in the absence or presence of Triton, at a concentration equivalent to that of plasma (100 mg/kg body weight \times 4.0% body weight plasma volume = 2.5 mg Triton WR 1339/ml) and VLDL composition was analyzed. There were no significant differences in the concentrations of VLDL-TAG (control 130 \pm 6 mg/dl vs. Triton 132 \pm 3 mg/dl, *n* = 3), phospholipid (control 39 \pm 2 mg/dl vs. Triton 35 \pm 2 mg/dl, *n* = 3), or cholesterol (control 8 \pm 1 mg/dl vs. Triton 7 \pm 1 mg/dl, *n* = 3) between control and Triton-treated VLDL. Measurements of bovine serum albumin by the procedure of Markwell et al. (26) were not affected by a 15-fold range of Triton concentrations. To determine effects of Triton WR 1339 on the isopropanol precipitation of apoB, isolated guinea pig plasma LDL was precipitated in the absence or presence of 2.5 mg/ml Triton and analyzed for total and supernatant protein.

There were no significant differences between either total or percent apoB protein values (control $79 \pm 4\%$ vs. Triton $79 \pm 3\%$ apoB, $n = 3$).

LDL isolation and analysis of apoB radioactivity

Blood samples collected at the 50, 120, 300 min time points were used to isolate LDL by ultracentrifugation between densities 1.019 and 1.09 g/ml. LDL radioactivity was measured in a gamma counter and used to quantitate apoB specific radioactivity activity after precipitation with isopropanol (25, 32) to determine apoB mass (μg) and radioactivity (cpm). Briefly, LDL (800–1000 μl) was precipitated with an equal volume of 100% isopropanol in 12×75 mm conical glass centrifuge tubes. After vigorous mixing for 1 min, samples were incubated overnight at 4°C . The samples were centrifuged for 30 min at 1000 g. The precipitated pellet containing apoB was re-dissolved with 1 M NaCl. Total protein and apoB protein in the precipitate were measured by a modified Lowry method (26). Total LDL and apoB radioactivity were determined and LDL apoB specific activity was calculated as cpm/ μg protein.

Statistics

Data are expressed as mean \pm SD. One-way analysis of variance (ANOVA) was used to assess differences in plasma cholesterol, TAG, and lipoprotein levels, nascent VLDL composition among diet groups, and LDL FCR values. Differences between mean values were evaluated by the Student-Newman-Keuls Multiple Comparisons Test and were considered significant at $P < 0.05$. Statistical analyses of the kinetic data were best fitted using a two-pool model (JANA, SCI Software, Lexington, KY). Student's *t*-test was used to assess differences between the compositions of nascent and mature VLDL of animals on the same diet. Linear regression analysis was used to determine correlation coefficients between variables.

RESULTS

Growth rates and plasma lipids

Final body weights were not significantly different among guinea pigs fed the three diets (Table 2) and all animals had similar growth rates indicative of comparable dietary intakes. There were significant differences in plasma total cholesterol levels with animals fed the PK diet having the highest plasma cholesterol levels followed by animals fed the LA diet and the lowest concentrations occurring in guinea pigs fed the CO diet (Table 2). Fasting plasma TAG levels were not different for the three dietary fat groups.

VLDL-TAG secretion rates

Plasma TAG concentrations increased linearly over the 8-h period following Triton WR 1339 injection ($r = 0.99$) in all three dietary fat groups (Fig. 1). The highest rate of VLDL-TAG accumulation (mg/kg-h) occurred in guinea pigs fed the LA diet relative to CO- or PK-fed animals (Fig. 1). The rate of TAG accumulation in the plasma compartment was significantly higher (+40%) in animals fed LA compared to CO- and PK-fed animals (Table 3). There was a weak correlation ($r = 0.38$, $P < 0.02$) between VLDL-TAG secretion rates and fasting plasma TAG levels of guinea pigs fed the three test diets (Fig. 2) indicating that differences in VLDL-TAG secretion rates account for only a small percentage of the variance in the low levels of plasma TAG in the guinea pig.

VLDL-apoB secretion rates

VLDL-apoB secretion rates were significantly higher (+133%) in PK-fed guinea pigs compared to either CO- or LA-fed animals (Table 3). As there is only one apoB-100 molecule per VLDL particle, increased rates of VLDL apoB flux with PK feeding, while TAG secretion is low, indicates production and secretion of a greater

TABLE 2. Body weights and plasma lipids of guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil

Dietary Fat (n)	Weight Gain	Final Weight	Plasma Lipids	
			Cholesterol	Triacylglycerol
	<i>g/day</i>	<i>g</i>		<i>mg/dl</i>
Corn oil (25)	8.2 ± 2.2	613 ± 78	54 ± 14^a	74 ± 34
Lard (23)	8.7 ± 2.9	579 ± 79	68 ± 17^b	79 ± 51
Palm kernel (23)	8.3 ± 2.5	592 ± 90	124 ± 33^c	87 ± 49

Data are presented as mean \pm SD for (n) animals. Values in the same column with different superscripts are significantly different ($P < 0.002$) as determined by ANOVA and the Student-Newman-Keuls Multiple Comparisons Test.

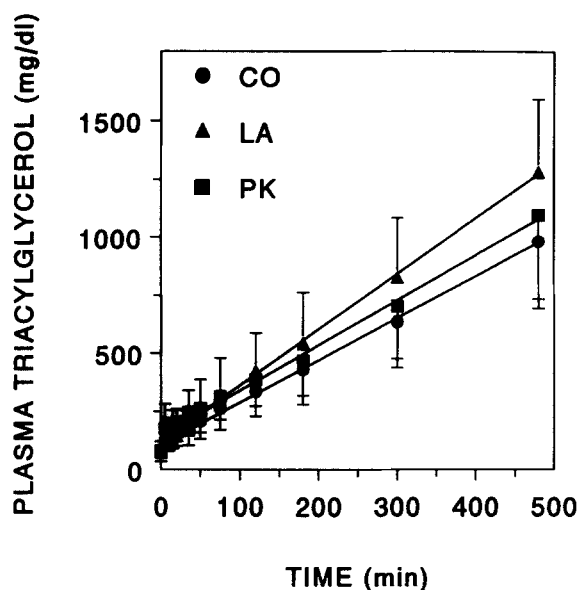


Fig. 1. Plasma triacylglycerol (mg/dl) accumulation over time in guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil. Values represent the mean \pm SD of $n = 15$ determinations for animals fed the corn oil diet and $n = 12$ determinations for animals fed the lard or the palm kernel oil diets.

number of VLDL particles with a decreased TAG content per particle compared to CO or LA feeding (see composition data below). The ratio of TAG to apoB secretion for CO-fed animals was 52 ± 27 ($n = 15$), for LA-fed animals 54 ± 27 ($n = 12$), and for PK-fed guinea pigs 21 ± 10 ($n = 12$) ($P < 0.001$) indicating that, compared to animals fed the CO diet, intake of LA increased secretion of a comparable TAG-rich VLDL particle (since total TAG secretion was increased) whereas intake of PK increased secretion of TAG-poor VLDL particles.

Compositions of nascent and mature VLDL

Analysis of the composition of nascent VLDL obtained from Triton WR 1339-treated guinea pigs at the 8-h time point indicated that nascent VLDL from PK-fed animals had a significantly different composition than nascent

TABLE 3. VLDL triacylglycerol and apoB secretion rates in guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil

Dietary Fat (n)	VLDL Secretion	
	Triacylglycerol	ApoB
	mg/kg-hr	
Corn oil (15)	48.6 ± 17.5^a	1.14 ± 0.63^a
Lard (12)	72.7 ± 14.7^b	1.53 ± 0.83^a
Palm kernel (12)	55.4 ± 13.4^a	3.11 ± 1.84^b

Data presented as mean \pm SD for (n) animals. Values in the same column with different superscripts are significantly different ($P < 0.002$) as determined by ANOVA and the Student-Newman-Keuls Multiple Comparisons Test.

VLDL isolated from CO- or LA-fed animals (Table 4). Intake of the PK diet resulted in newly secreted VLDL particles with a reduced lipid content including a lower number of TAG molecules per particle (Table 4) compared to VLDL from animals fed the LA- or CO-based diets.

Based on the observed differences in nascent VLDL composition in response to differences in dietary fat, and as a measure of potential dietary fatty acid effects on VLDL intravascular processing, circulating (mature) VLDL was isolated from each dietary group and the composition was analyzed. For the CO and LA fat groups the conversion of nascent to circulating VLDL involved a decrease in the number of TAG molecules per particle and for all three dietary fat groups a decrease in the cholesterol content (Table 4). The overall VLDL lipid content was decreased in animals fed the CO and LA diets and increased in those fed the PK diet. The data are consistent with the theory that during intravascular processing there is decrease in the core components of the particles with a increase in the surface components. Mature VLDL from guinea pigs fed the LA diet had an increased TAG and decreased phospholipid content compared to CO or PK. It is clear from the comparisons of nascent and mature VLDL that there are significant dietary fat effects on both the composition of the newly secreted particle and the intravascular processing of the particles to form the mature VLDL.

In vivo metabolism of LDL

Plasma ^{125}I -labeled LDL exhibited a significantly faster turnover with CO feeding followed by LA then PK (Fig. 3) as determined from kinetic studies measured over 8 h

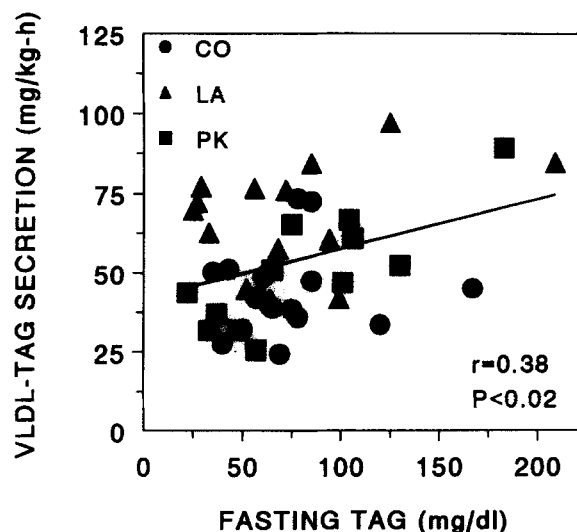


Fig. 2. Correlation between fasting plasma triacylglycerol (TAG) concentrations (mg/dl) and VLDL-TAG secretion rates (mg/kg-h) for guinea pigs fed semipurified diets containing 15% (w/w) corn oil (●), lard (▲), or palm kernel oil (■) ($r = 0.38$, $P < 0.02$).

TABLE 4. Composition of nascent and mature VLDL from guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil

Dietary Fat (n)	Molecules/Nascent VLDL Particle		
	Triacylglycerol	Phospholipid	Cholesterol
Corn oil (15)	24418 ± 14197 ^a	5098 ± 1373	4687 ± 1975
Lard (12)	26570 ± 15204 ^a	5373 ± 1748	4421 ± 2143
Palm kernel (12)	9468 ± 4429 ^b	4208 ± 1650	4098 ± 1428
Dietary Fat (n)	Molecules/Mature VLDL Particle		
	Triacylglycerol	Phospholipid	Cholesterol
Corn oil (4)	6385 ± 1213 ^b	5732 ± 1719 ^b	680 ± 163
Lard (4)	7561 ± 257 ^{a,b}	2753 ± 193 ^c	452 ± 277
Palm kernel (4)	9306 ± 1488 ^c	10647 ± 2342 ^a	1143 ± 912

Data presented as mean ± SD for (n) animals. Calculations based on VLDL containing a single apoB molecule of molecular weight 412,000 (9). Values in the same column with different superscripts are significantly different ($P < 0.002$) as determined by ANOVA and the Student-Newman-Keuls Multiple Comparisons Test.

in the presence of Triton (Table 5). The data demonstrate that CO intake increases LDL FCR compared to the two saturated dietary fats and, interestingly, the data are virtually indistinguishable from previously reported values of LDL FCR in guinea pigs fed the identical diets and the turnover kinetics were measured over 33 h in ad libitum fed animals in the absence of Triton (Table 5). The results indicate that Triton injection does not interfere with LDL catabolism in vivo and that its primary effect is limited to blocking VLDL. A negative correlation was found between plasma total cholesterol levels and LDL-FCR values for the three dietary fat groups ($P < 0.01$) (Fig. 4) consistent with previous reports that LDL-FCR is a major determinant of plasma LDL cholesterol levels (9, 10).

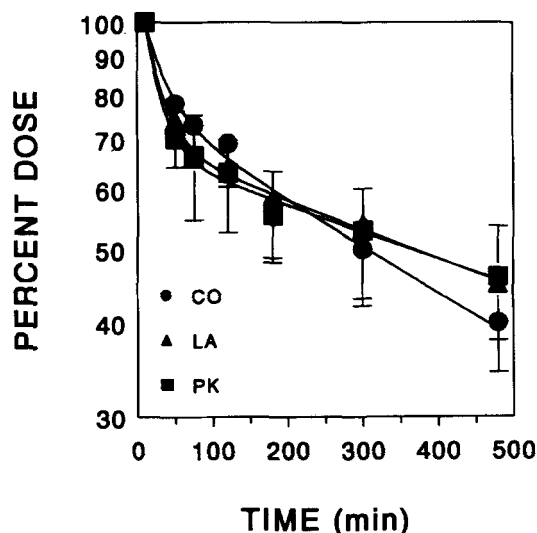


Fig. 3. Plasma disappearance curves of homogenous LDL isolated from guinea pigs fed semipurified diets containing 15% (w/w) corn oil (CO), lard (LA), or palm kernel oil (PK) injected into animals fed the same diets. Values represents the means ± SD of n = 10 determinations for animals fed corn oil and n = 9 determinations for animals fed the lard or palm kernel oil diet.

ApoB-LDL specific activity

LDL was isolated at three time points during the 8-h VLDL secretion/LDL turnover analysis for determination of LDL apoB specific activity (cpm/μg) to document the completeness of the Triton blockage of VLDL catabolism to LDL. In addition, analysis of plasma LDL apoB specific activity would determine whether guinea pigs exhibit direct secretion of LDL apoB and, if there was direct secretion, whether dietary fatty acid chain length and saturation had a significant effect on rates of secretion. Shown in Fig. 5 are the values for LDL apoB specific activity presented as a percentage of the 50-min time point. The data clearly demonstrate that LDL apoB specific radioactivity was unchanged over time for all dietary groups consistent with the complete blockage of the conversion of VLDL to LDL and the absence of any direct hepatic secretion of LDL apoB in the guinea pig.

TABLE 5. Plasma LDL fractional catabolic rates (FCR) in guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil

Dietary Fat	LDL FCR	
	+ Triton ^a	- Triton ^b
	<i>h</i> ⁻¹	
Corn oil	0.122 ± 0.021 (10) ^c	0.112 ± 0.009 (5) ^c
Lard	0.086 ± 0.022 (9) ^d	0.087 ± 0.008 (6) ^d
Palm kernel oil	0.076 ± 0.031 (9) ^d	0.073 ± 0.016 (5) ^d

Data presented as mean ± SD for (n) animals. Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by ANOVA and the Student-Newman-Keuls Multiple Comparisons Test.

^aData from the present study of LDL kinetics measured over 8 h after injection of Triton WR 1339 (100 mg/kg body weight).

^bData from previously reported studies (9, 10) measuring LDL kinetics over 33 h without Triton WR 1339 injection in guinea pigs fed the identical diets.

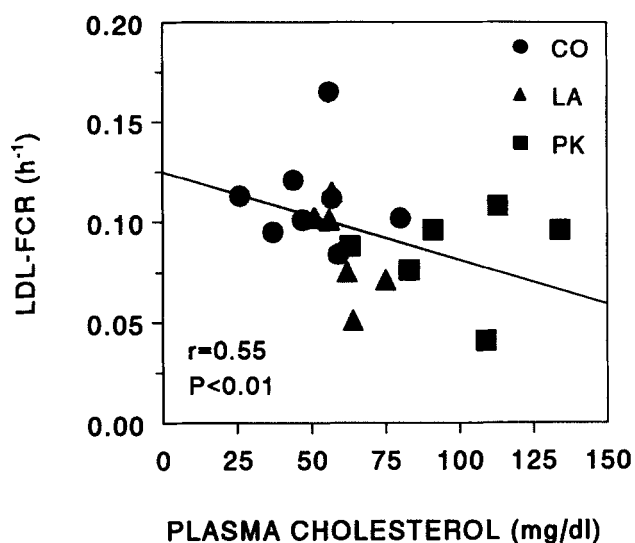


Fig. 4. Correlation between plasma cholesterol concentrations (mg/dl) and LDL-FCR (h^{-1}) values for guinea pigs fed semipurified diets containing 15% (w/w) corn oil (CO), lard (LA), or palm kernel oil (PK) ($r = 0.55$, $P < 0.01$).

The data also provide evidence that the rates of turnover of the radiolabeled LDL and endogenous unlabeled LDL were identical.

DISCUSSION

The present study used two saturated fats of different chain length: lard, rich in C16:0 palmitic and C18:0 stearic acids, and palm kernel oil, high in medium chain C12:0 lauric and C14:0 myristic acids, compared to polyunsaturated corn oil which is high in C18:2 linoleic acid, to study effects of dietary fat chain length and saturation on plasma VLDL and LDL metabolism in the guinea pig. The objective was to determine whether the observed increases in plasma LDL cholesterol levels and flux rates (9, 10) with intake of saturated fatty acids were due to increased production of VLDL, and the associated increase in conversion to LDL, or to similar VLDL production rates and an increase in the fraction of the VLDL converted to LDL. A secondary objective was to determine whether there was direct secretion of LDL by the liver in the guinea pig and, if so, whether dietary fat saturation and chain length had any effect on hepatic LDL secretion rates.

Dietary fat saturation effects on plasma lipids

Our results show that intake of the PK diet significantly increased plasma cholesterol levels compared to intake of the LA or CO diets, as shown in previous studies in the guinea pig (9, 10, 33) and consistent with studies in humans (1). The data also demonstrate that even when fast-

ing plasma TAG levels are not significantly different with changes in dietary fat chain length and saturation, there are significant effects on VLDL-TAG and apoB secretion rates. This is in contrast to the report of Nicolosi et al. (34) that plasma TAG concentrations were significantly higher in rhesus monkeys fed 30% fat as coconut oil compared to corn oil. Similarly, Lai et al. (17) found significant differences in plasma TAG concentrations of rats fed coconut oil rich in lauric and myristic acids and beef tallow rich in palmitic and stearic acids relative to corn oil. Human studies have also been variable and apparently dependent upon the baseline TAG level. Chait et al. (35) reported that serum TAG were reduced by feeding polyunsaturated fat as compared to saturated fat for normal and hyperlipidemic subjects, whereas Demacker et al. (36) reported that serum TAG levels were not different when normolipidemic volunteers were fed either polyunsaturated or saturated fats. It has been proposed that fasting plasma TAG levels are correlated with VLDL-apoB fractional synthetic rates and that VLDL removal is a saturable process (37) yet there are apparently substantial species and individual variations in fasting plasma TAG concentrations that are not necessarily reflective of the amount and composition of dietary fatty acids.

Dietary fat and TAG secretion rates

Since the first report that intravenous administration of Triton WR 1339 leads to hyperlipidemia (38), this detergent has been widely used to study lipid metabolism (13, 14, 23). Triton is a nonionic detergent that coats the

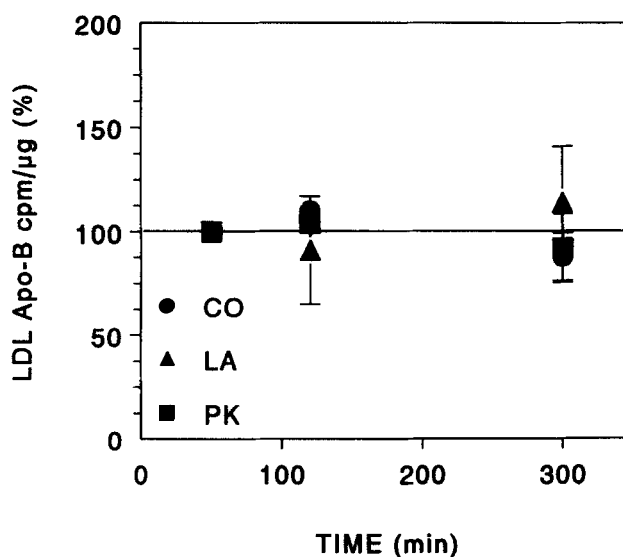


Fig. 5. LDL apoB specific activity time course. Values represents mean \pm SD of LDL apoB specific activity (%) of the 50-min time point for $n = 3$ determinations in guinea pigs fed semipurified diet containing 15% (w/w) corn oil (CO), lard (LA), or palm kernel oil (PK) and injected with Triton WR 1339 and ^{125}I -labeled LDL.

VLDL particle preventing its catabolism by lipoprotein lipase (LPL), and lipoproteins accumulating in the plasma of a Triton-treated animal represent newly secreted lipoproteins. In this study Triton was used to block VLDL catabolism and verification of the extent of blockage was determined by simultaneously injecting ^{125}I -labeled LDL and analysis of plasma apoB specific activity. Consistent with other findings of blocked clearance of VLDL-TAG from the plasma (23, 39), we found that intravenous injection of Triton completely blocked VLDL conversion to LDL.

Our results demonstrated that LA feeding significantly increased VLDL-TAG secretion rates compared to CO- and PK-containing diets. Comparable results were reported by Lai et al. (17) of significantly higher TAG secretion rates in rats fed beef tallow compared to corn oil- or coconut oil-fed animals, suggesting differential effects of saturated fatty acid chain length and saturation on TAG secretion. In contrast, Groot et al. (16) reported that postprandial TAG concentrations were significantly higher in rats fed palm oil than animals fed sunflower oil even though TAG synthesis rates were not different between the diet groups. Nicolosi et al. (6, 13) reported that dietary saturated fatty acids reduced the rate of TAG secretion compared to polyunsaturated fatty acids in both the gerbil and non-human primates and suggested that the hypercholesterolemia induced by dietary saturated fat is not due to overproduction of VLDL-TAG. It should be noted, however, that other studies in non-human primates suggest just the opposite effect with polyunsaturated fat intake resulting in decreased VLDL-TAG secretion compared to saturated fat (14). It could be hypothesized that one reason for the contrasting results is the evidence that saturated fatty acids of different chain lengths have different effects on VLDL-TAG secretion, as seen here where intake of the PK and CO diets had similar effects on VLDL-TAG secretion whereas intake of the LA diet significantly increased VLDL-TAG secretion.

A further complication in the interpretation of VLDL secretion studies is the finding that, as seen in this study, dietary fatty acids have differential effects on VLDL-TAG and VLDL apoB secretion rates. While intake of the PK diet did not alter VLDL-TAG secretion as compared to intake of the CO diet, there was a significant increase with intake of the saturated PK diet on VLDL apoB secretion resulting in secretion of VLDL particles of altered composition. These data are consistent with results from the only VLDL apoB turnover study in humans fed saturated and polyunsaturated fatty acid diets (11) and indicate that certain dietary saturated fatty acids can increase VLDL apoB production.

The VLDL-TAG:apoB ratio of nascent VLDL was significantly affected by the composition of the dietary fat. In a similar manner, studies in Zucker rats treated with lovastatin demonstrated decreased secretion of VLDL-TAG but not VLDL apoB, resulting in production of

smaller, TAG-depleted VLDL particles that had the TAG:apoB ratio decreased from 184 to 91 (40). These data demonstrate that analysis of the secretion of a single component of VLDL does not necessarily represent the effects of interventions on VLDL production. Obviously, the type of saturated fat in the diet can have significant differential effects on both VLDL-TAG and VLDL apoB secretion rates.

Dietary fat saturation effects on nascent VLDL composition

Our data indicate that nascent VLDL are rich in TAG and cholesterol compared to their circulating counterparts. VLDL cholesterol was predominately free cholesterol with undetectable cholesteryl ester as noted in studies using perfused guinea pig liver where cholesteryl ester constituted only 0.3% of nascent VLDL (41). The data also indicate that the guinea pig, like humans, secretes VLDL particles containing only apoB-100 and there is no apoB-48 secretion by the liver (31). Ingestion of different types of fats results in unique effects on nascent VLDL composition. These data indicate that intake of the PK diet resulted in secretion of a greater number of VLDL particles with low TAG content even though the TAG secretion rate was the same as in the CO diet group. There is evidence that large and TAG-rich VLDL particles are removed by direct hepatic uptake, whereas smaller TAG-poor particles are directed into the LDL pathway (42). This would suggest that the nascent TAG-rich VLDL of LA- and CO-fed guinea pigs are primarily removed by the liver whereas the TAG-poor VLDL secreted by PK-fed animals are converted to LDL and this is consistent with the increased LDL flux rate in PK-fed guinea pigs (9, 10). Interestingly, Nicolosi et al. (13) found an inverse relationship between VLDL-TAG secretion rates and plasma LDL cholesterol levels in squirrel and cebus monkeys, whereas in the present study we found a significant positive correlation (**Fig. 6**) between apoLDL and VLDL apoB flux rates consistent with the increased LDL cholesterol levels being due to increased production of the VLDL precursor.

Dietary fat saturation effects on LDL kinetics in vivo

Our results are consistent with previous studies using analysis of LDL turnover over a 33-h period and demonstrate that CO-fed animals have the fastest LDL FCR followed by LA and the slowest FCR occurs with PK feeding (9, 10). Surprisingly, this relationship could be demonstrated using an 8-h analysis and carried out in the presence of Triton. This demonstrates that the major effect of Triton was to block LPL activity by coating the VLDL particles preventing catabolism and causing accumulation of VLDL-TAG in the plasma compartment that did not affect LDL turnover. The data can also be interpreted to suggest that in the guinea pig, which does not

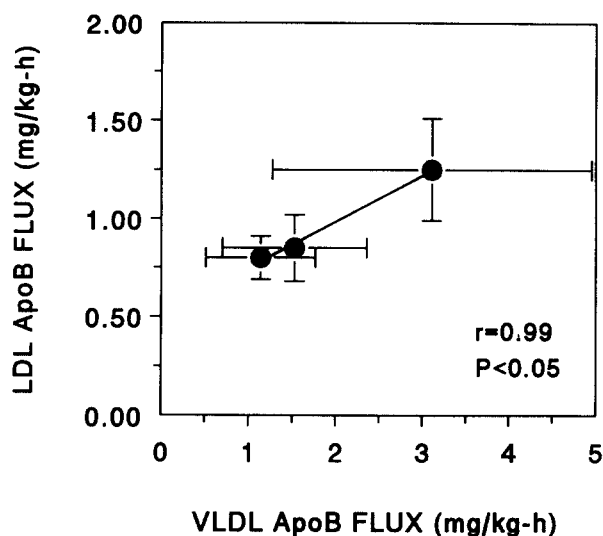


Fig. 6. Correlation between VLDL apoB flux rate (mg/kg-h) and LDL apoB flux rate (mg/kg-h) for guinea pigs fed semipurified diets containing 15% (w/w) corn oil, lard, or palm kernel oil. Values represents the mean \pm SD of $n = 15$ determinations for animals fed corn oil and $n = 12$ determinations for animals fed the lard or palm kernel diets used for VLDL apoB flux calculations and LDL apoB flux rate data from previous studies (9, 10).

develop hypertriglyceridemia, nascent VLDL does not compete with LDL for the apoB/E receptor (43).

Dietary saturated fat effects on apoB secretion

^{125}I -labeled LDL apoB specific radioactivity (cpm/ μg) was measured at various time points during the VLDL secretion/ ^{125}I -labeled LDL kinetic studies and the results indicate that LDL apoB specific radioactivity remained constant over a 5-h period. These data are consistent with two conclusions: *a*) the Triton blockage of VLDL catabolism and conversion to LDL was complete, and *b*) there was no direct secretion of LDL from the liver. Studies in rhesus monkeys have suggested that dietary fat saturation affects plasma LDL levels by effects on the independent production of LDL apoB (15). While there is considerable debate regarding interpretation of the kinetic analysis of VLDL turnover to determine direct production of LDL apoB (44-48), the present study does not rely on turnover kinetics and modeling to determine direct LDL production but rather measures changes in LDL apoB specific radioactivity. If LDL apoB specific radioactivity had decreased over time it could have been due to either direct production of LDL apoB, to leakage of VLDL past the Triton blockage, or both. In this study the fact that LDL apoB specific radioactivity did not change, and that LDL FCR values were comparable to a non-Triton-treated data set, is consistent with the absence

of any independent hepatic production of LDL apoB. Similar findings have been reported from studies using hamster hepatocytes which also secrete only apoB-100 and only VLDL apoB (49). These data are also similar to the findings of Goldberg et al. (50) which demonstrated that in the LPL-inhibited cynomolgus monkey there was minimal independent secretion of LDL by the liver.

In conclusion, our data indicate that saturated fats with different chain lengths induce elevated plasma cholesterol levels by different mechanisms and that intake of the PK diet increases the number of VLDL particles secreted by the liver while intake of the LA diet increases VLDL-TAG secretion rates compared to the CO diet. One hypothesis for the decrease in plasma LDL with intake of polyunsaturated fatty acids is that the increased expression of hepatic apoB/E receptors increases the catabolism of VLDL and IDL, reducing the available pool of the LDL precursor, and that there is no specific effect on VLDL production, even though changes occur in LDL flux rates (2). In contrast, the data from these studies indicate that dietary fatty acids have unique and specific effects on VLDL secretion rates and that the mechanisms responsible for the decrease in plasma LDL levels with intake of polyunsaturated fatty acids in part depend on the comparative dietary fat. Comparing intakes of polyunsaturated CO to PK, high in lauric and myristic acids, it is clear that the CO diet reduces secretion of VLDL apoB and consequently the number of VLDL particles, particles which apparently have a high rate of conversion to LDL. Exchanging CO for LA, with its palmitic and stearic acid content, lowers the rate of VLDL-TAG secretion with an associated minor effect on VLDL apoB secretion, an effect consistent with the modest changes in LDL apoB flux rates. In either case it is clear that the effects of dietary fat saturation and chain length on plasma LDL cholesterol levels in the guinea pig are, in part, due to effects on VLDL production and that associated changes in the flux of VLDL to LDL, as well as changes in LDL receptor-mediated FCR, determine plasma LDL cholesterol levels in the guinea pig. The observed dietary fat-mediated changes in guinea pig VLDL metabolism are consistent with the reported changes in humans (1, 11) and further define the unique effects of specific fatty acids on lipoprotein metabolism. ■

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