Regulation of hepatic LDL metabolism in the guinea pig by dietary fat and cholesterol

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Abstract Studies were carried out to determine the independent and interactive effects of dietary fat and cholesterol on the regulation of hepatic apoB/E receptor expression and its relationship to hepatic cholesterol concentrations and low density lipoprotein (LDL) particle characteristics. Guinea pigs were fed 15% (w/w) fat diets (lard, olive oil, or corn oil) with cholesterol levels corresponding to absorbed intakes of 6 (basal), 50, 100, or 200% endogenous cholesterol synthesis. Guinea pigs maintained stable plasma cholesterol levels until cholesterol intake equaled or exceeded endogenous synthesis ($P < 0.001$). Fat type independently affected plasma total and LDL cholesterol levels such that lard > corn oil, with olive oil being intermediate ($P < 0.05$). Hepatic membrane apoB/E receptor number (B_{max}) decreased as dietary cholesterol increased ($P < 0.001$) without an independent effect of dietary fat saturation. B_{max} values were significantly correlated with plasma LDL cholesterol levels ($r = -0.632$), and with hepatic free ($r = 0.527$) and esterified cholesterol ($r = -0.512$) concentrations, which were both increased with dietary cholesterol ($P < 0.001$). Significant interactions between dietary fat type and cholesterol mediated the extent of hepatic free and esterified cholesterol accumulation. Dietary fat and cholesterol interactions also contributed to changes in LDL particle composition and peak density. The results of these studies do not support the thesis that dietary cholesterol-mediated suppression of apoB/E receptor expression is ameliorated by intake of polyunsaturated fatty acids. Dietary fat type and cholesterol amount interactively affect hepatic cholesterol concentrations and LDL composition and size, which in part determine plasma LDL cholesterol levels.—**Lin, E. C. K., M. L. Fernandez, M. A. Tosca, and D. J. McNamara.** Regulation of hepatic LDL metabolism in the guinea pig by dietary fat and cholesterol. *J. Lipid Res.* 1994. **35:** 446–457.

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Elevated levels of plasma low density lipoprotein (LDL) cholesterol constitute a major risk factor for cardiovascular disease, and extensive clinical data document dietary lipids as a major determinant of plasma LDL levels. Metabolic studies have shown that dietary lipids affect the regulation of LDL synthesis, intravascular processing, and apolipoprotein (apo) B/E receptor-mediated catabolism (1, 2). Clinical studies indicate that the plasma LDL response to changes in the type and amount of dietary fat and in dietary cholesterol exhibit a high degree of inter-individual heterogeneity (3, 4). While most human studies indicate independent effects of dietary fat and cholesterol on plasma cholesterol levels, there are both clinical and animal studies suggesting that these dietary factors interact to determine plasma cholesterol concentrations (reviewed in ref. 1).

Studies in the hamster by Woollett et al. (5) and Spady and Dietschy (6) indicated that the down-regulation of hepatic apoB/E receptors by dietary cholesterol is significantly affected by the type of dietary fat, with unsaturated fatty acids attenuating cholesterol-induced receptor suppression relative to saturated fatty acids. In vivo LDL turnover studies in cebus monkeys support this thesis in that apoB/E receptor expression is higher in animals fed diets containing polyunsaturated corn oil with 0.1% cholesterol as compared to animals fed saturated coconut oil with cholesterol (7). In contrast, LDL kinetic studies in cynomolgus monkeys demonstrated that apoB/E receptor-mediated catabolism of LDL was not consistently related to fat type and cholesterol amount (8).

In a previous study of guinea pigs fed varying levels of dietary cholesterol combined with fats of varying saturation, we did not find significant interactions between dietary fat and cholesterol in determining plasma LDL cholesterol levels or hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (9). To better understand the role of dietary fat saturation and cholesterol in determining plasma LDL cholesterol levels and hepatic apoB/E receptor expression, the present studies were undertaken to determine the effects of three dietary fats (saturated lard, monounsaturated olive oil,

Abbreviations: apo, apolipoprotein; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein. Diet abbreviations: Corn, Lard, and Olive refer to corn oil-, lard-, and olive oil-based diets; Basal, Low, Medium, and High refer to basal (0.01), 0.08, 0.17, and 0.33% dietary cholesterol, respectively.

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and polyunsaturated corn oil) fed with a range of dietary cholesterol levels. These fats have previously been shown to significantly affect hepatic apoB/E receptor expression and in vivo LDL kinetics (10, 11). Four levels of dietary cholesterol (basal, 0.08, 0.17, 0.33%) were chosen to provide absorbed cholesterol masses equivalent to 6, 50, 100, and 200% of the endogenous cholesterol synthesis rate (12). The varying cholesterol levels, from physiological to pharmacological, provide a wide range of responses in plasma cholesterol levels and hepatic cholesterol metabolism (9). Our primary objective was to test the hypothesis that the extent of dietary cholesterol-mediated down-regulation of hepatic apoB/E receptor expression, as measured by hepatic apoB/E receptor number (B_{max}), is modified by the degree of dietary fat saturation. In addition, we wished to determine the relationships and interactions between dietary fat and cholesterol on the physicochemical properties of LDL, which have been shown to significantly affect plasma LDL metabolism (11, 13), and effects on hepatic free and esterified cholesterol levels, which are considered to be important determinants of the regulatory pool of cholesterol in the liver (14).

The present studies were carried out in the guinea pig because in this animal model: *a)* LDL is the major plasma lipoprotein; *b)* both dietary fat and cholesterol modulate plasma LDL levels; *c)* LDL:HDL ratios are greater than 2; *d)* there is an active plasma cholesteryl ester transfer protein (CETP) (15); *e)* dietary fat saturation regulates plasma LDL metabolism in the absence of dietary cholesterol (10, 11, 13, 16); *f)* hepatic free:esterified cholesterol ratios are greater than 1 (17); and *g)* dietary saturated fatty acids plus cholesterol do not cause hypertriglyceridemia (9). These similarities to human lipid and lipoprotein metabolism make the guinea pig an especially interesting animal model for studies of dietary fat and cholesterol effects on LDL metabolism and apoB/E receptor expression.

MATERIALS AND METHODS

Materials

$Na^{125}I$ was purchased from Amersham (Arlington Heights, IL); enzymatic cholesterol assay kits, cholesterol esterase, and cholesterol oxidase were from Boehringer Mannheim (Indianapolis, IN); aprotinin (Trasylol) and enzymatic triglyceride assay kits were from Sigma (St. Louis, MO); halothane was acquired from Halocarbon Lab Inc. (Hackensack, NJ); and rotors and Quick Seal ultracentrifugation tubes were from Beckman Instruments (Palo Alto, CA).

Experimental diets

Semipurified test diets were prepared and pelleted by Research Diets, Inc. (New Brunswick, NJ). The isocaloric (3.8 kcal/g) diet compositions are reported elsewhere (9) and contained identical ingredients except for the 15 g fat/100 diet (lard, olive oil, or corn oil) and amounts of recrystallized cholesterol added (Basal: 0.00, Low: 0.08, Medium: 0.17, and High: 0.33% by weight). Basal diets were all adjusted to 0.01% cholesterol to account for the cholesterol content of the lard diet. Cholesterol levels were calculated to result in absorbed amounts equivalent to 6, 50, 100, and 200% the mass of guinea pig daily endogenous cholesterol synthesis (51 mg/kg-day) (12). Cholesterol intakes for the four levels were 0.03, 0.21, 0.45, and 0.87 mg/kcal, respectively. Test diets were adjusted to the same plant sterol content (0.9 mg sitosterol/g diet) and were formulated to meet NRC-specified nutritional requirements of the guinea pig. Dietary groups are referred to by fat type and cholesterol level; e.g., lard-based diet with 0.33% cholesterol is abbreviated as Lard-High. Commercial nonpurified guinea pig diet was obtained from Teklad (Madison, WI). Composition of the commercial diet (2.9 kcal/g) was reported to be, by weight, 17.9% protein (mainly soybean meal), 1.9% vegetable fat, 49.3% carbohydrate, and 14% fiber (mainly alfalfa).

Animals

Male Hartley guinea pigs (250–350 g) were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) or Sasco, Inc. (Omaha, NE) and randomly assigned to one of 12 dietary groups. Animals were maintained in a light-cycled room (light 07:00 to 19:00) and provided with diet and water ad libitum. After at least 23 d of dietary treatment, nonfasting guinea pigs were exanguinated under halothane anesthesia, and the blood was collected into syringes containing 1 mg/ml EDTA. Plasma was separated and a preservation cocktail (18) consisting of sodium azide (1 μmol/ml), aprotinin (50 kallikrein units/ml), and phenyl methyl sulfonyl fluoride (0.01 μmol/ml) was added. Livers were excised for membrane isolation and cholesterol determinations.

Lipoprotein isolation and characterization

Plasma cholesterol concentrations were determined using a commercial enzymatic kit. Plasma HDL cholesterol was determined after $MgCl_2$-dextran sulfate precipitation of apoE- and B-containing lipoproteins (19). Plasma was adjusted to 1.019 g/ml with KBr/NaCl to obtain the $d < 1.019$ g/ml lipoprotein fraction (chylomicrons, VLDL, and IDL). LDL cholesterol was calculated as the difference between the total cholesterol and the $d < 1.019$ g/ml + HDL cholesterol concentrations.

For determination of LDL peak density, plasma was adjusted to d 1.24 g/ml with KBr and centrifuged for 36 h at 125,000 *g* at 15°C in a Ti50 rotor to isolate lipoproteins. The lipoprotein fraction was adjusted to d 1.3 g/ml in a final 10-ml volume and overlayered with 30 ml of 1.006 g/ml solution in a Quick Seal ultracentrifugation tube.

Lipoprotein fractionation was achieved by centrifugation in a VC-53 vertical rotor for 3 h at 100,000 *g*, 10°C, and collecting fractions for determining lipoprotein cholesterol distribution and density as previous described (10).

Guinea pig plasma LDL composition was determined in the density fractions corresponding to LDL ($1.019 < d < 1.09$ g/ml) by measuring protein, phospholipids, total and free cholesterol, and triglycerides as previously described (11). The diameters of LDL were calculated from the composition data (11). Molecules of phospholipids, free cholesterol, cholesteryl esters, and triglycerides per molecule of LDL apoB were calculated using molecular weights of 734, 386.6, 646, and 885.4, respectively, relative to guinea pig LDL apoB molecular weight of 412,000 (20).

Hepatic membrane LDL binding assay

Guinea pig LDL for binding studies was isolated from animals fed a nonpurified commercial diet and labeled with ^{125}I to specific activities between 220 and 575 cpm/ng (21). Human LDL ($1.019 < d < 1.063$ g/ml), which has previously been shown to be an effective competitor for apoB/E receptor binding of guinea pig LDL at 37°C (11, 22), was added to the incubations at a protein concentration of 1 mg/ml to determine nonspecific ^{125}I-labeled LDL binding. Incubations without excess human LDL represented total binding, and apoB/E receptor-mediated binding was calculated as the difference between total and nonspecific binding. LDL from both guinea pigs and humans were free of contaminating proteins as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Procedures for hepatic membrane isolation and in vitro LDL binding have been described elsewhere (10, 11, 22).

The LDL binding assay used in this study includes minor modifications: 200-μl incubation mixtures containing 200 μg membrane protein, incubation buffer (100 mM NaCl, 0.5 mM $CaCl_2$, 50 mM Tris-HCl, 20 mg/ml bovine serum albumin), 5–60 μg/ml ^{125}I-labeled LDL, with or without 1 mg/ml unlabeled human LDL, were prepared on ice and initiated by incubating at 37°C in a water bath. The mixtures were incubated for 2 h and transferred to an ice bath. Cellulose propionate ultracentrifuge tubes were preincubated with incubation buffer overnight at 37°C to minimize nonspecific binding to the tubes (23). Aliquots (75 μl) of the incubation mixture were overlayered onto 100 μl cold incubation buffer containing 30 mg/ml albumin. The tubes were centrifuged for 1 h at 38,000 rpm, 15°C in a Ti42.2 rotor. Supernatants were aspirated and the membrane pellets were washed by adding 100 μl cold incubation buffer containing 30 mg/ml albumin and centrifuging for an additional 30 min. The supernatant was discarded, and the tube bottoms were clipped for counting radioactivity (LKB Wallac, Clinigamma) in the membrane pellet. ApoB/E receptor number (B_{max}) and affinity (K_d) were calculated using Woolf plots (24).

Hepatic free and esterified cholesterol measurements

Hepatic total and free cholesterol concentrations were determined by enzymatic analysis following lipid extraction as described by Sale et al. (25). Esterified cholesterol concentrations were calculated as the difference between total and free cholesterol values.

Statistical analysis

Two-way ANOVA was used to determine interactions between dietary fat type and cholesterol amount, as well as their independent effects, inclusive of all dietary groups. When significant differences were found, one-way ANOVA was used to determine differences within a fat group or cholesterol level. Differences between mean values were evaluated by the least significant difference test (26). Regression analyses were determined with best fit equations computed using GBSTAT (Dynamic Microsystems, Inc., Silver Spring, MD). Data are presented as mean ± SD, and differences were considered significant at $P < 0.05$.

RESULTS

Final body weights of guinea pigs fed the various diets were decreased ($P < 0.001$) by dietary cholesterol (Basal: 561 ± 54 g, n = 14; Low: 516 ± 39, n = 12; Medium: 495 ± 39, n = 13; High: 478 ± 33, n = 11). However, analysis of covariance using body weight as the covariate indicated that body weight had no significant influence on the observed differences in the measured variables. Dietary fat type had no significant effect on body weight gain.

Dietary effects on plasma cholesterol concentrations

Plasma total and LDL cholesterol concentrations of guinea pigs fed the three test fats with four levels of cholesterol are presented in **Fig. 1.** Inclusive of all dietary groups, plasma total cholesterol was progressively increased ($P < 0.001$) by increasing amounts of dietary cholesterol; however, total cholesterol levels did not increase significantly compared to Basal intakes until absorbed cholesterol equaled the mass of endogenous cholesterol synthesis (Medium 0.17% level). The increments in plasma total cholesterol levels are relatively modest up to the pharmacological High dosage of cholesterol intake.

When normalized for every mg increase in dietary cholesterol over Basal levels per 100 g diet, plasma total cholesterol concentrations increased on average by 0.44 and 0.36 mg/dl with the Low and Medium cholesterol intakes, respectively; however, total cholesterol increased by an average of 0.77 mg/dl with the High cholesterol intake, ranging from an increase of 0.43 mg/dl for the Corn-High

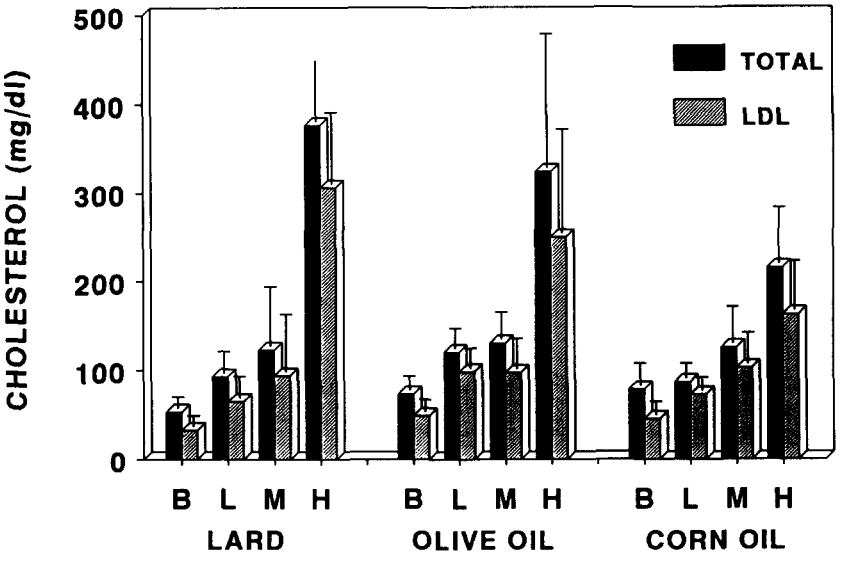

Fig. 1. Plasma total and LDL cholesterol of guinea pigs fed the 12 test diets. Total and LDL cholesterol changes as a result of dietary treatment were parallel and concentrations were correlated (r = 0.991, n= 53, $P < 0.001$). Total and LDL cholesterol concentrations were significantly increased by dietary cholesterol at the Medium (M) cholesterol level as compared to Basal (B) cholesterol intake; High (H) cholesterol feeding further increased plasma cholesterol concentrations significantly ($P < 0.001$). Fat type independently mediated cholesterol levels such that Lard > Corn oil, with Olive oil intermediate and not different from Lard or Corn oil ($P < 0.05$).

group to 1.01 mg/dl for Lard-High. This larger normalized increment in plasma total cholesterol of animals fed Lard-High suggests that saturated fat and high dietary cholesterol interact synergistically to further raise plasma total cholesterol concentrations; however, statistics inclusive of all 12 dietary groups failed to reach significance for dietary interactions (P = 0.10). Inclusive of all groups, plasma LDL cholesterol was significantly increased ($P < 0.001$) with increasing dietary cholesterol parallel to changes in total cholesterol levels.

Plasma LDL cholesterol levels were correlated with total cholesterol levels (r = 0.991, n = 53, $P < 0.001$), consistent with LDL being the lipoprotein primarily affected by diet. However, when analyzed by one-way ANOVA, plasma LDL cholesterol concentrations were significantly increased at only the High cholesterol intake in the Lard and Olive oil groups ($P < 0.003$) whereas the increments in LDL cholesterol concentrations of Corn oil-fed guinea pigs were more gradual with cholesterol intake, such that significant elevations in LDL cholesterol over Basal intake were seen with the Medium cholesterol level ($P < 0.002$). Dietary fat type independently influenced total and LDL cholesterol concentrations significantly such that Lard > Corn oil, with Olive oil being intermediate and not significantly different from either Lard or Corn oil. No interactions between fat and cholesterol were detected relative to effects on plasma total and LDL cholesterol levels.

Dietary effects on LDL characteristics

Table 1 presents the physicochemical characteristics of plasma LDL isolated from guinea pigs fed the 12 test diets. Components of LDL (free cholesterol, cholesteryl esters, phospholipids, and triglycerides) are presented as molecules per molecule of LDL based on one apoB per LDL. Compositions and characteristics of LDL from the Basal cholesterol groups have been reported previously (10) and are presented here for purposes of comparison. The number of free cholesterol molecules in LDL were significantly affected by fat type, cholesterol amount, and there was evidence of an interaction between fat and cholesterol (Table 1). Cholesterol intake increased LDL free cholesterol content in a dose response manner (Basal > Low > Medium > High) and there was more free cholesterol in LDL from guinea pigs fed Lard or Olive oil than from animals fed Corn oil. Interactions of fat type and cholesterol amount were evident: for example, the number of free cholesterol molecules in LDL from animals fed Olive oil-based diets increased 2.4-fold at the Low cholesterol intake over Basal values, and higher cholesterol intakes resulted in little further change (Table 1). In contrast, increases in free cholesterol content of LDL from animals fed Lard or Corn oil with increasing dietary cholesterol were more gradual, although Lard appeared to interact with High cholesterol to augment free cholesterol content further than Corn-High. Unlike free cholesterol, LDL cholesteryl ester content was not affected by dietary fat or cholesterol (Table 1).

Molecules of LDL phospholipid were decreased by dietary cholesterol at the Medium and High intakes overall. The significant dietary interaction observed for effects on the phospholipid content may be due to the 57% decrease in LDL phospholipid from Corn-Low-fed

TABLE 1. Physicochemical characteristics of plasma low density lipoprotein from guinea pigs fed Lard-, Olive Oil-, or Corn Oil-based diets containing 0.00 (Basal), 0.08 (Low), 0.17 (Medium), or 0.33% (High) added cholesterol[1]

Fat Type Cholesterol Amount	LDL Lipid Content				Core:Surface Ratio[2]	Diameter	Peak Density
	Free Cholesterol	Cholesterol Ester	Phospholipid	Triglyceride			
	molecules/particle					*Å*	*g/ml*
Lard							
Basal	453 ± 239	1478 ± 445	571 ± 233	264 ± 153	1.19 ± 0.18	228 ± 29	1.048 ± 0.002
Low	533 ± 110	1281 ± 79	1167 ± 250	907 ± 171	1.11 ± 0.16	215 ± 27	1.050 ± 0.003
Medium	749 ± 92	1393 ± 72	514 ± 150	410 ± 104	1.17 ± 0.06	228 ± 11	1.047 ± 0.004
High	1490 ± 133	1418 ± 81	464 ± 53	121 ± 84	1.00 ± 0.04	200 ± 7	1.047 ± 0.002
Olive oil							
Basal	483 ± 502	1204 ± 583	515 ± 244	210 ± 112	1.15 ± 0.21	224 ± 33	1.045 ± 0.002
Low	760 ± 109	1611 ± 474	515 ± 137	276 ± 56	1.18 ± 0.16	228 ± 24	1.047 ± 0.002
Medium	890 ± 281	1712 ± 156	622 ± 130	270 ± 82	1.12 ± 0.12	217 ± 20	1.045 ± 0.001
High	886 ± 208	1533 ± 408	617 ± 155	260 ± 59	1.02 ± 0.17	201 ± 28	1.045 ± 0.002
Corn oil							
Basal	340 ± 77	1287 ± 159	975 ± 188	264 ± 54	0.85 ± 0.11	174 ± 17	1.055 ± 0.003
Low	494 ± 165	1275 ± 442	417 ± 152	565 ± 239	1.42 ± 0.26	273 ± 38	1.050 ± 0.004
Medium	599 ± 92	1522 ± 239	403 ± 120	382 ± 93	1.41 ± 0.18	269 ± 29	1.047 ± 0.002
High	851 ± 145	1760 ± 632	518 ± 132	453 ± 36	1.36 ± 0.19	255 ± 27	1.044 ± 0.001
Fat type[3]	$P < 0.001$	NS	NS	$P < 0.002$	$P < 0.030$	$P < 0.015$	$P < 0.002$
Cholesterol amount[4]	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.025$	$P < 0.015$	$P < 0.002$
Interaction[5]	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.002$	$P < 0.001$	$P < 0.005$

[2]Values are means ± SD for n = 4 animals per diet group except for Lard-Low (ratios and diameter, n = 3) and Olive-High (peak density, n = 3). Data for basal cholesterol diets were previously reported (10) and are presented for comparison.
[2]Core:Surface ratio = (cholesteryl ester + triglyceride)/(free cholesterol + phospholipid + protein).
[3]Differences due to dietary fat type as determined by two-way ANOVA; NS, not significant.
[4]Differences due to dietary cholesterol amount as determined by two-way ANOVA.
[5]Differences due to interactive effects between dietary fat type and cholesterol amount.

animals contrasted with the increase seen with animals fed Lard-Low diet. LDL triglycerides were also significantly affected by dietary fat saturation and cholesterol amount in an interactive manner (Table 1). Why LDL triglycerides in Lard- and Corn oil-fed animals consuming Low cholesterol increased is unclear considering the return to Basal values with higher cholesterol intake. Animals consuming Olive oil exhibited no change in the number of LDL triglyceride molecules with cholesterol intake, although values were generally lower relative to Lard and Corn oil groups.

The ratios of LDL core (cholesteryl esters + triglycerides) to surface (free cholesterol + phospholipid + protein) components were also affected by fat type and cholesterol quantity interactively. Collectively, LDL from animals fed Corn oil-based diets had higher core:surface ratios than those fed Lard or Olive oil; however, this fat effect was dependent on the dietary cholesterol level, since at the Basal cholesterol intake, LDL from guinea pigs fed Corn oil had the lowest core:surface value (Table 1). Calculated LDL diameters were also affected by fat type and cholesterol amount interactively. Overall, LDL from Corn oil-fed guinea pigs were larger than LDL from animals fed Lard or Olive oil. As with the core:surface ratios, dietary cholesterol was found to affect only the diameters of LDL from animals fed Corn oil. Peak densities of plasma LDL followed a more consistent trend in that cholesterol feeding significantly decreased LDL peak densities from an average of 1.049 g/ml in both Basal and Low cholesterol groups to 1.046 g/ml (Medium) and 1.045 g/ml (High), indicative of a shift to more buoyant particles. Fat type affected LDL peak densities with LDL from Lard- and Corn oil-fed animals being more dense than LDL from Olive oil-fed guinea pigs. Dietary fat type and cholesterol interacted to affect LDL peak density in that while dietary cholesterol altered values of LDL densities from animals fed Lard and Corn oil, values were not affected by dietary cholesterol when the fat was Olive oil.

LDL cholesteryl ester content was found to be significantly related to mean LDL peak densities ($r = 0.709$, n = 12, $P < 0.001$). In contrast, none of the LDL composition components correlated with LDL core:surface ratios or diameters, nor did measured peak density relate to calculated values of core:surface ratios or diameters.

Dietary effects on hepatic LDL receptors

The kinetic parameters of guinea pig ^{125}I-labeled LDL binding to isolated hepatic membranes were determined for all dietary groups (**Table 2**). Dietary cholesterol significantly reduced hepatic membrane LDL receptor number in a dose-dependent manner. Overall, cholesterol

TABLE 2. Kinetics of LDL binding to hepatic membranes from guinea pigs fed Lard-, Olive oil-, or Corn oil-based diets containing 0.00 (Basal), 0.08 (Low), 0.17 (Medium), or 0.33% (High) added cholesterol[1,2]

	Hepatic Membrane LDL Binding Kinetics					
	B_{max}			K_d		
Cholesterol/ Dietary Fat	Lard	Olive	Corn	Lard	Olive	Corn
	$\mu g/mg$			$\mu g/ml$		
Basal	2.04 ± 0.11 (5)[a]	2.07 ± 0.30 (4)[a]	2.59 ± 0.08 (5)[a]	46 ± 14	60 ± 9	60 ± 14[a]
Low	1.92 ± 0.18 (4)[a]	1.58 ± 0.22 (4)[b,c]	1.51 ± 0.36 (4)[b]	34 ± 14	46 ± 28	39 ± 11[b]
Medium	1.47 ± 0.24 (4)[b]	1.80 ± 0.35 (5)[a,b]	1.22 ± 0.30 (4)[b]	30 ± 14	41 ± 24	30 ± 9[b]
High	1.32 ± 0.07 (3)[b]	1.16 ± 0.25 (4)[c]	1.14 ± 0.25 (4)[b]	35 ± 14	39 ± 15	34 ± 11[b]
Fat[3]		NS			NS	
Cholesterol[4]		$P < 0.0001$			$P = 0.006$	
Interaction[5]		$P = 0.0004$			NS	

[1]Values are means ± SD for the indicated (n) number of guinea pigs.
[2]Different superscripts indicate significant differences ($P < 0.05$) within the same dietary fat group.
[3]Differences due to dietary fat type as determined by two-way ANOVA; NS, not significant.
[4]Differences due to dietary cholesterol amount as determined by two-way ANOVA.
[5]Differences due to interactive effects between dietary fat type and cholesterol amount.

intake decreased B_{max} values in the order Basal > Low = Medium > High. Although fat type had no independent effect on estimated B_{max} values, one-way ANOVA indicated significantly higher apoB/E receptor number for Corn oil-fed animals relative to those fed Olive oil or Lard when the diets contained only Basal cholesterol ($P < 0.002$). Dietary cholesterol interacted with fat type to varying extents in suppressing receptor expression. For example, LDL binding to hepatic membranes from Corn oil-fed animals exhibited an immediate and large (42%) decrease in receptor B_{max} values with the Low level of dietary cholesterol, and higher intakes resulted in no significant further suppression. In contrast, membranes from Lard- and Olive oil-fed animals exhibited a gradual reduction in LDL binding capacity with increasing dietary cholesterol (Table 2).

The apparent affinity of the apoB/E receptor for LDL (K_d) was also found to be significantly affected by dietary cholesterol (Table 2). The observed effects were not due to changes in the composition of ligand LDL, which was kept constant by isolating LDL from commercial diet-fed guinea pigs. K_d values were decreased at the Low level of dietary cholesterol suggesting an increased affinity of the hepatic membrane apoB/E receptor for LDL when dietary cholesterol is modestly increased. Higher intakes of dietary cholesterol did not have any additional effect on ligand affinity (Table 2). Although there was no significant dietary interaction in determining K_d values, one-way ANOVA indicated that the major influence of dietary cholesterol on K_d values was associated with changes in hepatic membranes from Corn oil-fed animals.

Dietary effects on hepatic cholesterol concentrations

Both free and esterified hepatic cholesterol levels were significantly increased with intake of cholesterol in the order Basal = Low < Medium < High and hepatic cholesterol accumulation was dependent upon fat type (**Table 3**). Lard intake protected against hepatic cholesterol accumulation which did not increase significantly until the High dietary level. Lard-fed animals also had the lowest free and esterified cholesterol concentrations overall relative to Olive oil and Corn oil groups. In contrast, Olive oil intake promoted hepatocholesterolemia, as hepatic free cholesterol was increased significantly at even the Low level of dietary cholesterol. Overall, Olive oil and Corn oil feeding produced similarly elevated levels of hepatic free cholesterol as compared with Lard. However, hepatic cholesteryl esters were highest with Olive oil intake, intermediate with Corn oil, and lowest with Lard. Dietary interactions were evident in determining hepatic cholesteryl ester levels, such that animals fed Olive oil accumulated 24-times more cholesteryl ester with High dietary cholesterol compared to Basal cholesterol in contrast to 11-fold increases in hepatic cholesteryl ester levels in Lard- and Corn oil-fed guinea pigs.

Correlations

Hepatic apoB/E receptor B_{max} values were significantly related to plasma LDL cholesterol concentrations ($r = -0.632$, n = 50, $P < 0.001$) in a logarithmic relationship (**Fig. 2**). Levels of hepatic free cholesterol ($r = -0.512$, n = 46, $P < 0.001$) (**Fig. 3,** left panel) and cholesteryl esters ($r = -0.527$, n = 46, $P < 0.001$) (Fig. 3, right panel) were negatively correlated with B_{max} values. Hepatic free and esterified cholesterol concentrations were best related to B_{max} values using exponential and logarithmic equations, respectively.

TABLE 3. Hepatic free and esterified cholesterol levels of guinea pigs fed Lard-, Olive oil-, or Corn oil-based diets containing 0.00 (Basal), 0.08 (Low), 0.17 (Medium), or 0.33% (High) added cholesterol[1,2]

	Hepatic Cholesterol					
	Free			Esterified		
Cholesterol/Dietary Fat	Lard	Olive	Corn	Lard	Olive	Corn
			mg/g			
Basal	2.5 ± 0.4^{ax}	2.3 ± 0.5^{ax}	3.5 ± 0.2^{ay}	0.3 ± 0.2^{ax}	0.4 ± 0.1^{axy}	0.7 ± 0.2^{ay}
Low	3.4 ± 0.5^{ax}	4.5 ± 0.7^{by}	2.9 ± 0.3^{ax}	0.7 ± 0.1^{ax}	2.6 ± 0.8^{ay}	1.1 ± 0.2^{ax}
Medium	3.4 ± 0.3^{ax}	6.5 ± 1.7^{bcy}	5.6 ± 1.4^{by}	0.9 ± 0.5^{ax}	7.0 ± 1.9^{by}	2.4 ± 0.5^{ax}
High	5.9 ± 2.2^{b}	7.9 ± 2.1^{c}	9.7 ± 1.5^{c}	3.2 ± 2.2^{bx}	9.6 ± 2.1^{cy}	8.0 ± 3.2^{by}
Fat[3]		$P < 0.001$			$P < 0.001$	
Cholesterol[4]		$P < 0.001$			$P < 0.001$	
Interactions[5]		$P < 0.001$			$P < 0.002$	

[1]Values are means ± SD for three to five guinea pigs per diet.
[2]Different superscripts indicate significant differences ($P < 0.05$) due to fat type (^{xy}, rows) or cholesterol amount (^{abc}, columns).
[3]Differences due to dietary fat type as determined by two-way ANOVA.
[4]Differences due to dietary cholesterol amount as determined by two-way ANOVA.
[5]Differences due to interactive effects between dietary fat type and cholesterol amount.

DISCUSSION

Dietary fat and cholesterol effects on plasma LDL cholesterol levels

Data from cholesterol feeding studies in humans indicate that the plasma cholesterol response to a dietary cholesterol challenge is independent of dietary fat saturation (1). The present study in guinea pigs is consistent with this finding in that, with both physiological and pharmacological intakes of cholesterol, elevations in plasma LDL cholesterol were independent of dietary fat saturation. Generally, saturated Lard was hypercholesterolemic, polyunsaturated Corn oil was hypocholesterolemic, and monounsaturated Olive oil was intermediate. For all parameters measured, dietary cholesterol was a more dominant factor than fat type and plasma cholesterol homeostasis was maintained when dietary cholesterol intakes were within physiological levels. Although plasma cholesterol levels were significantly increased when absorbed cholesterol equaled endogenous synthesis, the increments were modest until the pharmacological input of twice endogenous synthesis. By extrapolation, assuming appropriate homeostatic regulatory capacity (i.e., excluding noncompensators), a 70-kg man with an endogenous cholesterol synthesis of 13 mg/kg-d, 60% cholesterol absorption, and 2500 kcal intake would have to consume 1500 mg of cholesterol/day (0.61 mg/kcal) to have an absorbed dietary cholesterol input equal to the mass of endogenous cholesterol synthesis (1); an intake three times greater than the cholesterol intake on an average Western diet. Our findings emphasize the importance of considering cholesterol intake relative to endogenous cholesterol synthesis: input below the mass of endogenous synthesis is within regulatory capacity and results in small changes in plasma cholesterol levels, whereas input above synthetic capacity overwhelms regulatory mechanisms and results in a substantial increase in plasma LDL cholesterol. Based on these considerations, it would seem reasonable to relate dietary cholesterol intakes to a physiological equivalent incorporating absorption rates and endogenous synthesis when comparing studies in different species with the objective of relating the data to the physiological state of humans. The present findings demonstrate that there is no dietary fat–cholesterol interaction in determining guinea pig

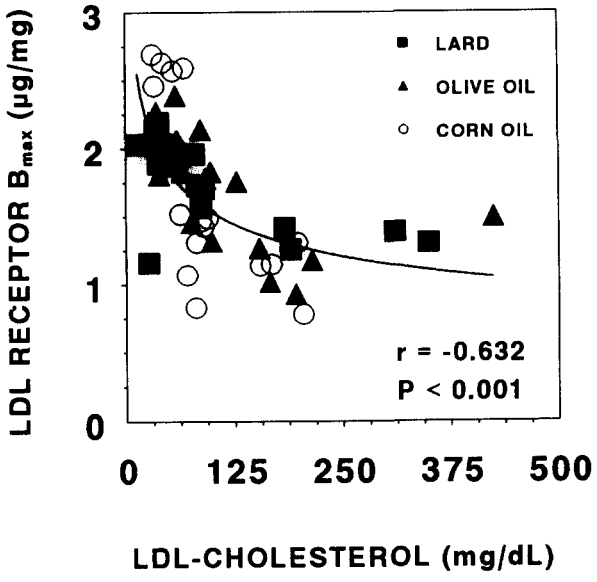

Fig. 2. Plasma LDL cholesterol levels as related to hepatic LDL (apoB/E) receptor number (B_{max}) in guinea pigs fed the 12 test diets. A significant logarithmic relationship was found ($r = -0.632$, n = 50, $P < 0.001$), indicative of the role of hepatic LDL receptors in determining plasma LDL cholesterol concentrations.

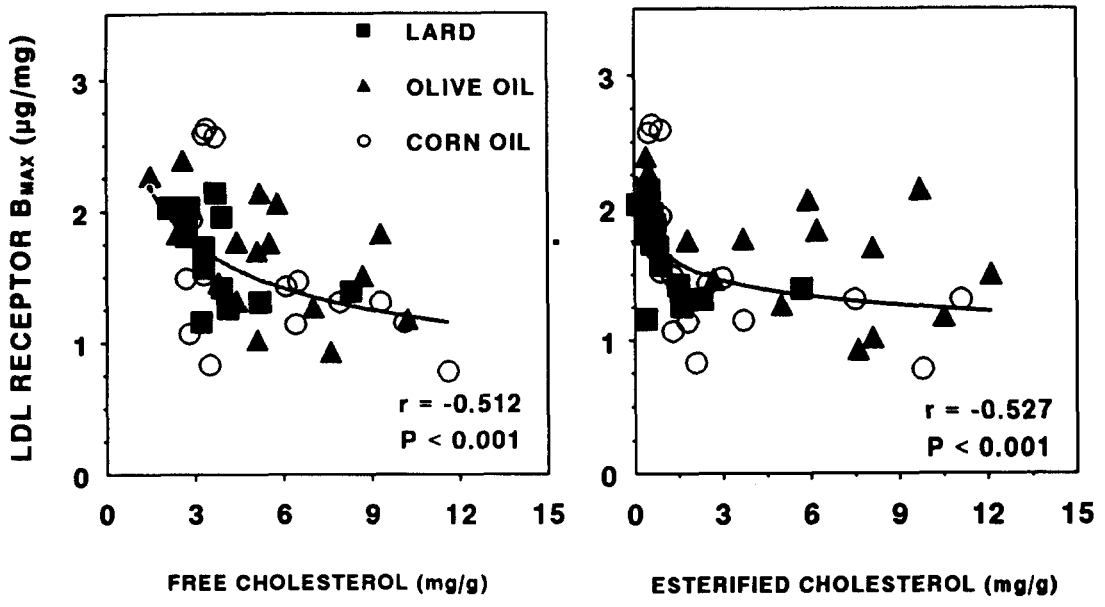

Fig. 3. Left panel: Hepatic free cholesterol concentrations as related to hepatic LDL (apoB/E) receptor number (B_{max}) in guinea pigs fed the 12 test diets. An exponential equation was found to best fit the data ($r = -0.512$, n = 46, $P < 0.001$). Hepatic free cholesterol appeared to regulate hepatic LDL receptor expression across the entire range of hepatic free cholesterol concentrations. Right panel: Hepatic cholesteryl ester concentrations as related to hepatic apoB/E receptor number (B_{max}) in guinea pigs fed the 12 test diets. The data were best fit using a logarithmic curve ($r = -0.527$, n = 46, $P < 0.001$). Hepatic cholesteryl ester levels did not appear to influence hepatic LDL receptor expression when concentrations exceeded approximately 4 mg/g, indicative of the more inert role of esterified cholesterol in receptor regulation.

plasma LDL cholesterol concentrations; however, possible interactions operating at levels of metabolic regulation have been suggested by other studies (27–29).

Dietary interactions on LDL characteristics

All components of LDL, except cholesteryl ester, were altered by dietary treatments. Not surprisingly, LDL free cholesterol, rather than cholesteryl ester, was progressively increased with dietary cholesterol intake, as has been reported in guinea pigs fed 1% cholesterol (30). The 52% increase in total cholesterol molecules per LDL particle between the Basal and High cholesterol intakes indicates that the increase in plasma LDL cholesterol with High cholesterol intake is due to both an increase in LDL particles and an increase in the amount of cholesterol per particle. Guinea pig LDL cholesterol content is similar to that reported for cebus monkeys fed corn oil or coconut oil with or without 0.1% cholesterol (7). Dietary treatment also did not alter monkey LDL cholesteryl ester:protein ratios, and free cholesterol:protein values were higher in LDL from animals fed saturated fat. We have previously demonstrated that guinea pigs fed the Corn-Basal diet produce smaller, more rapidly catabolized LDL particles and have more rapid rates of in vivo LDL catabolism by receptor-specific processes compared to animals fed either Lard-Basal or Olive-Basal diets (11). These additive effects of diet-increased receptor expression and increased particle catabolism demonstrate a significant relationship between LDL composition and size and in vivo LDL turnover (11), leading us to speculate that the physicochemical changes in LDL observed in the present study would also influence in vivo LDL turnover rates.

Dietary effects on apoB/E receptor expression

ApoB/E receptor B_{max} was inversely associated with increases in plasma LDL cholesterol levels, consistent with receptor-mediated catabolism being a major regulator of LDL concentrations (31). Inclusive of all 12 dietary groups, hepatic apoB/E receptor number was not independently affected by dietary fat type; however, previous studies using hepatic membranes from guinea pigs fed Corn-Basal, Olive-Basal, or Lard-Basal diets, and tested with homologous LDL ligands, found significantly higher apoB/E receptor B_{max} values for animals fed Corn-Basal (11). One-way ANOVA also indicated significantly higher apoB/E receptor numbers for animals fed Corn-Basal relative to Olive-Basal or Lard-Basal. These data suggest that dietary fat saturation influences hepatic LDL receptor expression only when dietary cholesterol is minimal (0.01%) and that higher intakes of cholesterol result in a dominant effect of cholesterol in suppressing apoB/E receptor expression. The pattern of guinea pig hepatic apoB/E receptor down-regulation was similar to that found in cynomolgus monkeys (8). When monkeys were fed diets with minimal cholesterol (0.01 mg/kJ), hepatic apoB/E receptor activity, as measured by ^{125}I-labeled tyramine-cellobiose LDL uptake, was 64% higher in animals fed a more unsaturated fat diet (P:S = 0.9 vs.

0.5); however, with higher cholesterol intakes (0.06 mg/kJ), LDL receptor activity decreased 61% with the unsaturated fat diet in contrast to only an 11% decrease with the saturated fat diet. The end result was a higher absolute hepatic LDL protein transport with intake of the saturated fat/high cholesterol diet. These authors (8) reported a significant interaction between dietary cholesterol and P:S ratio on hepatic LDL transport, consistent with our findings of interactive regulation of apoB/E receptor number.

Our data are in agreement with the theory of Dietschy, Woollett, and Spady (32) that plasma LDL increases markedly only after 50% of hepatic apoB/E receptor expression is lost. High cholesterol feeding in the guinea pig depressed hepatic apoB/E receptor B_{max} in the Lard and Olive oil groups to 59% and 52% of Basal apoB/E receptor expression (average Basal B_{max} = 2.24 μg/mg) (**Fig. 4**). Accordingly, plasma LDL cholesterol levels in these two groups did not increase significantly until the High cholesterol intake. In contrast, apoB/E receptor B_{max} of animals fed Corn-Medium were depressed to 54% of average Basal B_{max}, and plasma LDL cholesterol was significantly elevated at the Medium cholesterol intake.

It should be noted that while hepatic apoB/E receptor expression was similar at the High cholesterol intakes, plasma LDL cholesterol levels varied almost 2-fold with differing fat saturation. This is not unexpected considering that dietary fat saturation affects rates of apoLDL production which also determine plasma LDL cholesterol concentrations (2, 11, 13). We have previously reported that guinea pigs fed the Lard-based diet with 0.25% cholesterol have increased LDL flux rates, as well as decreased receptor-mediated uptake relative to animals fed Lard-Basal (11, 33). [Hepatic apoB/E receptor B_{max} of animals fed the Lard-based diet with 0.25% cholesterol (33) is included in Fig. 4 to show the dose-dependent down-regulation of LDL receptor activity by cholesterol.] These data suggest that rates of LDL production, either due to increased secretion of VLDL or more rapid conversion of VLDL to LDL, are also affected by dietary fat and cholesterol.

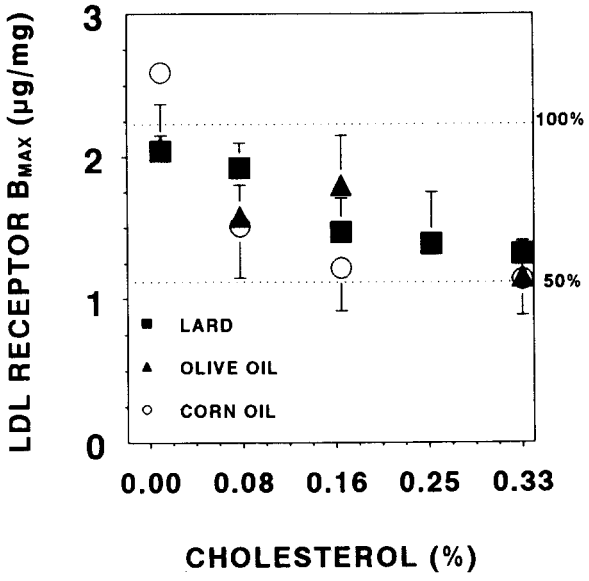

Fig. 4. LDL (apoB/E) receptor B_{max} as related to dietary cholesterol intake. LDL receptor B_{max} values were averaged from all Basal groups to represent 100% activity. Receptor activity was most sensitive to cholesterol regulation in the Corn oil groups, where even 0.17% (Medium) cholesterol intake resulted in near 50% receptor suppression. B_{max} values from guinea pigs fed Lard with 0.25% cholesterol (33) were included to demonstrate the dose-dependent suppression of B_{max} by increasing cholesterol intake.

Mechanisms of the cholesterol-lowering effect of polyunsaturated fatty acids: species differences

There is a major difference between our data and studies that suggest polyunsaturated fat decreases LDL cholesterol by attenuating cholesterol-mediated down-regulation of apoB/E receptor expression (5–7, 27, 34). Apparent differences in metabolic characteristics of the animal models studied could account for these discrepancies. For example, cebus monkeys fed corn oil with or without 0.1% cholesterol had higher receptor-mediated apoB fractional catabolic rates than animals fed coconut oil (7). Interestingly, however, the usually constant nonreceptor-mediated catabolism was also affected by dietary fat type and dietary fat saturation had no effect on LDL production rates (7). In the hamster, dietary safflower oil results in higher specific uptake of LDL relative to hydrogenated coconut oil despite increases in dietary cholesterol from 0.06 to 0.24% (6). However, saturated fat and cholesterol produce severe hypertriglyceridemia in the hamster (5, 35, 36) and intake of 0.12% cholesterol plus hydrogenated coconut oil produces 5-fold higher VLDL-cholesterol concentrations than feeding polyunsaturated safflower oil with cholesterol (5). The increased levels of plasma VLDL apparently result from increased VLDL production with cholesterol feeding (37), which is altered by dietary fat type in the hamster (27) and exacerbated by the relatively low expression of hepatic LDL receptors in this model (28). It is possible that the observed effects of dietary fat saturation and cholesterol on in vivo rates of ^{125}I-labeled LDL clearance in hamsters are due to both a decrease in hepatic apoB/E receptor expression as well as competition between plasma VLDL remnants and IDL with the ^{125}I-labeled LDL tracer for receptor-mediated catabolism. This would not occur in hamsters fed polyunsaturated fats with cholesterol as the plasma levels of the competing lipoproteins are minimal. To what extent increased plasma levels of apoE-rich IDL particles confound the in vivo measurement of receptor-mediated LDL catabolism in hamsters fed saturated fatty acids plus cholesterol has not been determined; however, studies have recently

demonstrated significant relationships between receptor-mediated LDL uptake and hepatic apoB/E receptor protein and mRNA levels in hamsters fed fat and cholesterol (38).

In the guinea pig, addition of up to 0.17% cholesterol to 15% Corn oil, Olive oil, or Lard-based diet did not alter plasma VLDL or triglyceride levels above Basal levels; 0.33% cholesterol intake resulted in an approximately 3-fold increase in VLDL-cholesterol levels and a less than 2-fold increase in plasma triglyceride concentrations (9; E. C. K. Lin, unpublished observations). There were no differences in VLDL-cholesterol or plasma triglyceride concentration due to feeding polyunsaturated Corn oil versus saturated Lard at any level of dietary cholesterol (9; E. C. K. Lin, unpublished observations). We have previously demonstrated a significant correlation between hepatic apoB/E receptor B_{max} determined in vitro and receptor-mediated LDL catabolism determined in vivo (11), as would be predicted in the absence of lipoprotein particles competing with tracer LDL for receptor-mediated catabolism.

Increased LDL degradation in isolated monocytes or hepatocytes from animals fed polyunsaturated fat and cholesterol versus saturated fat plus cholesterol have been demonstrated (27, 34), consistent with the thesis that dietary polyunsaturated fat increases apoB/E receptor activity. Hepatic apoB/E receptor mRNA levels have been measured in the baboon (39, 40), cebus monkey (41), African green monkey (42), hamsters (38, 43), and mice (44) under various dietary conditions. Unfortunately, species differences probably account for the conflicting results regarding significant fat type effects on apoB/E receptor mRNA levels. Interestingly, in the baboon (40) and cebus monkey (41), the trend is for cholesterol intake to result in a greater decrease in apoB/E receptor mRNA levels when animals are fed polyunsaturated fat than when they were fed saturated fat, similar to the pattern we observed with B_{max}. Just the opposite pattern is found in the hamster in that apoB/E receptor mRNA levels decrease to a greater degree with saturated fat feeding (38).

Dietary interactions on hepatic cholesterol accumulation

Both hepatic free and esterified cholesterol concentrations were increased by dietary cholesterol; however, the extent of increase was dependent on dietary fat type. Saturated fat intake resulted in the least hepatic free and esterified cholesterol accumulation, whereas intake of monounsaturated olive oil increased accumulation of cholesteryl esters as previously reported in studies of rats, hamsters and rabbits (45, 46). Polyunsaturated fat was generally more hepatocholesterolemic than saturated fat even though plasma cholesterol levels were lower. We have recently observed lower VLDL triglyceride secretion rates in Corn-Basal-fed guinea pigs relative to animals fed a Lard-Basal diet (47), suggesting that decreased hepatic VLDL production, in part, explains the elevated hepatic cholesterol and lower plasma LDL cholesterol in animals fed polyunsaturated fat. Decreased LDL production rates in Corn-Basal-fed guinea pigs relative to animals fed Lard-Basal or Olive-Basal are consistent with lower VLDL production (11).

Hepatic free cholesterol levels in the guinea pig correlated with hepatic apoB/E receptor B_{max} in contrast to other animal species such as hamsters (5) and cebus monkeys (7). The diet-mediated differences in hepatic free and esterified cholesterol distribution could partially explain why the guinea pig is sensitive to hepatic cholesterol-mediated regulation of LDL receptors. The relationship of guinea pig hepatic apoB/E receptor number with hepatic free cholesterol along the entire range of free cholesterol concentrations is consistent with the concept that the free cholesterol pool serves as the regulator of LDL receptor expression. In contrast, cholesteryl ester concentrations were logarithmically related to hepatic LDL receptor B_{max}, such that cholesteryl ester levels above approximately 4 mg/g resulted in no further decrease in receptor expression. The linear relationship of cholesteryl ester concentration and LDL receptor B_{max} only at low cholesteryl ester concentrations are suggestive of a carry-over effect by free cholesterol due to the correlation of free with esterified cholesterol. The elevated hepatic cholesterol levels in animals fed polyunsaturated fat appear in contradiction to the greater apoB/E receptor expression at the Basal cholesterol intake; however, identical results have been observed in hamsters fed saturated and polyunsaturated fats with varying amounts of cholesterol (38). The possibility of specific fatty acids affecting a regulatory pool of cholesterol separate from the assayable free and esterified cholesterol pools remains to be established.

Our results indicate that dietary fat and cholesterol interactions act at levels of metabolic regulation (LDL physicochemical characteristics, hepatic cholesterol, and apoB/E receptor B_{max}) that contribute to plasma LDL cholesterol levels. The responses of plasma LDL cholesterol, hepatic free and esterified cholesterol, and apoB/E receptor expression to dietary fat saturation and cholesterol intake are highly variable among different species, and mechanistic responses to dietary fat and cholesterol in humans are largely undefined. The guinea pig exhibits independent effects of dietary fat and cholesterol on plasma LDL cholesterol concentrations and has alterable pools of hepatic free and esterified cholesterol related to hepatic apoB/E receptor expression which contribute to plasma LDL levels. Lower plasma LDL cholesterol concentrations in this model with polyunsaturated fat intake were not due to attenuation of cholesterol-mediated down-regulation of the apoB/E receptor, suggesting that other hypocholesterolemic mechanisms attributable to polyunsaturated fat intake ex-

ist. Whether these mechanisms parallel human responses are as yet unknown.

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