Carbohydrate-Fat Exchange and Regulation of Hepatic Cholesterol and Plasma Lipoprotein Metabolism in the Guinea Pig

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Adult female guinea pigs were fed semipurified diets containing increasing concentrations of saturated fat (2.5%, 7.5%, 15%, and 25% wt/wt) to determine effects of exchanging fat-carbohydrate calories on lipoprotein metabolism. Plasma very–low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) did not vary but plasma low-density lipoprotein (LDL) concentrations increased with increasing fat calories. LDL cholesterol values were 42 ± 25, 61 ± 17, 92 ± 25, and 98 ± 21 mg/dL (mean ± SD, n = 5), respectively. The relative proportion of cholesteryl ester increased and triacylglycerol (TAG) decreased for VLDL, LDL, and HDL as dietary fat increased. Plasma lecithin cholesterol acyltransferase (LCAT) activity was positively correlated with HDL cholesteryl ester content. Hepatic cholesterol and TAG concentrations were highest in animals fed 25% fat ($P < .01$). Hepatic apolipoprotein (apo) B/E receptor maximal binding capacity (Bmax) was 30% higher in animals fed 2.5% and 7.5% fat as compared with those fed 15% and 25% fat ($P < .01$) and inversely correlated with plasma LDL ($r = -.85$, $P < .01$). In contrast, HDL binding to guinea pig hepatic membranes exhibited a significant positive correlation with dietary fat quantity ($r = .98$, $P < .001$), consistent with a dose-response with increasing fat calories. The activity of hepatic 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase was not affected by the amount of dietary fat, whereas the activity of acyl CoA:cholesterol acyltransferase (ACAT) was significantly increased in animals fed 25% fat ($P < .05$). Hepatic free-cholesterol and ACAT activity exhibited a positive correlation for all dietary groups ($r = .75$, $P < .001$). These results demonstrate that exchange of saturated dietary fat for carbohydrate calories results in significant modifications in the regulation of metabolic pathways that determine plasma LDL concentrations and hepatic cholesterol homeostasis.

HIGH-CARBOHYDRATE, low-fat diets have been recommended to decrease elevated levels of plasma low-density lipoprotein (LDL) cholesterol, which are correlated with increased risk of coronary heart disease.[1] However, there is evidence that consumption of diets high in carbohydrates (low-fat) is related to increased plasma triacylglycerol (TAG) levels and decreased plasma high-density lipoprotein (HDL) concentrations, which are also associated with increased risk of coronary heart disease.[2]

Clinical studies have reported that compared with a high-fat diet (35% to 43% calories from carbohydrate), intake of a low-fat diet (60% calories from carbohydrate) results in a decrease of plasma HDL cholesterol concentrations independently of the type of dietary fatty acids.[3-6] In these studies, an increase in plasma TAG concentrations was only observed in subjects fed simple sugars in a liquid formula[3] or in non–insulin-dependent diabetic patients,[4] suggesting that the hypertriglyceridemia effects are due more to the type (simple *v* complex) than to the amount of dietary carbohydrate. The low levels of plasma HDL resulting from intake of a high-carbohydrate diet have been attributed to a decrease in apolipoprotein (apo) A-I synthesis rates,[7,8] although an increase in apo A-I fractional catabolic rates has also been reported.[8]

Low-fat diets (60% energy from carbohydrate) have been shown to decrease the conversion of very–low-density lipoprotein (VLDL) to intermediate-density lipoprotein (IDL) and LDL[9,10] and to increase the VLDL apo B pool,[11] which could partly explain the decrease of plasma LDL concentrations. An increased direct uptake of VLDL and a decreased uptake of VLDL TAG were observed in one study,[9] suggesting a dissociation between VLDL apo B and VLDL TAG catabolism, possibly related to changes in VLDL composition and/or intravascular processing. Changes in LDL composition associated with increased degradation of these modified particles by human skin fibroblasts have been proposed as a mechanism by which high-carbohydrate diets might decrease plasma LDL concentrations.[12] However, the evidence is from a study that used an extreme situation in which carbohydrate contributed 85% of calories and fat accounted for less than 1%.[12]

The present studies were undertaken to investigate the effects of exchanging fat-carbohydrate calories on the regulation of hepatic cholesterol and lipoprotein metabolism. Four levels of dietary fat ranging from 7% to 53% of total calories were tested to monitor effects on lipoprotein metabolism with gradual increases in dietary fat. Guinea pigs were used as the experimental animal model based on a number of similarities with humans in cholesterol and lipoprotein metabolism, including the lipoprotein profile (high LDL/low HDL), presence of plasma lipid transfer proteins,[13] distribution of hepatic cholesterol pools,[14] and responses to dietary fat saturation,[15] dietary cholesterol,[16] and dietary fiber.[17]

MATERIALS AND METHODS

Materials

DL-Hydroxy-[3-^{14}C]methyl glutaryl coenzyme A (1.81 GBq/mmol), DL-[5-^{3}H]mevalonic acid (370 GBq/mmol), cholesteryl [1,2,6,7-^{3}H]oleate (370 GBq/mmol), Aquasol, and Liquifluor were purchased from New England Nuclear (Boston, MA). Oleoyl-[1-^{14}C] coenzyme A (1.8 GBq/mmol) and DL-3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) were from Amersham (Clearbrook, IL). Cholesteryl oleate was from Sigma (St Louis, MO).

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Enzymatic cholesterol assay kits, cholesterol oxidase, cholesterol esterase, and hydroperoxide were purchased from Boehringer Mannheim (Indianapolis, IN). Plasma free-cholesterol and phospholipid enzymatic assay kits were from Wako Pure Chemical Industries (Osaka, Japan). Quickseal tubes were from Beckman (Palo Alto, CA), and halothane was from Halocarbon (Hackensack, NJ).

Diets

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ). The semipurified diets contained 2.5%, 7.5%, 15%, and 25% (wt/wt) fat, which corresponds to 59%, 51%, 42%, or 26% (wt/wt) dietary carbohydrate (Table 1). The fat was a 50:50 mixture of butter fat and palm oil (C14:0 6.8%, C16:0 33.6%, C18:1 32%, and C18:2 7.8%), and the amounts of protein, vitamins, and minerals were adjusted such that all diets had the same amount of nutrients per caloric content. Cholesterol and sitosterol content were adjusted for all diets by combining the amount present in butter fat and palm oil with the added cholesterol and sitosterol to a final concentration of 0.12 g/1,000 kcal and 0.23 mg/1,000 kcal, respectively (Table 1).

Animals

Female Hartley guinea pigs at stable weight (800 to 1,200 g) were purchased from Sasco Sprague-Dawley (Omaha, NE) and randomly assigned to one of four dietary groups. After 4 weeks, animals were killed by anesthesia with halothane vapor and exsanguination by cardiac puncture to obtain liver and plasma for isolation of hepatic microsomes and lipoproteins. All animals consumed equal calories as estimated by stable body weights throughout the experimental feeding period. All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines, and experimental protocols were approved by the University of Arizona Institutional Animal Care and Use Committee.

Plasma and Liver Lipids

Plasma total and lipoprotein cholesterol concentrations and plasma TAG levels were determined by enzymatic analysis[18,19] using commercial kits. VLDL plus IDL, LDL, and HDL were separated by sequential density-gradient ultracentrifugation in an L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 125,000 × *g* at 15°C for 19 hours in a Ti-50 rotor for guinea pigs from all dietary groups. Separation was based on the following density fractionations as previously determined for guinea pigs[19]: 1.006 to 1.019 g/mL for VLDL plus IDL, 1.019 to 1.09 for LDL, and 1.09 to 1.24 for HDL. Purity of lipoprotein fractions was ensured by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (data not shown). Hepatic concentrations of TAG and total and free cholesterol were measured according to the method reported by Carr et al,[20] and hepatic esterified cholesterol concentrations were estimated by subtracting free from esterified cholesterol.

Lipoprotein Characterization

In addition to lipoprotein isolation, an aliquot of plasma (1.2 mL) was adjusted to a density of 1.3 g/mL with KBr and overlayered with 4 mL of a NaCl-KBr solution of density 1.006 g/mL in a 5.2-mL Quickseal ultracentrifugation tube. A density gradient was generated by centrifugation in a VTi 65.2 vertical rotor for 45 minutes at 200,000 × *g* at 10°C, and the lipoprotein profile and density distribution were determined by measuring cholesterol level and refractive index in all collected fractions as previously described.[21] Peak densities were determined for LDL from each dietary group to evaluate effects of fat calories on LDL size distribution as previously reported.[15,21] VLDL plus IDL, LDL, and HDL composition were calculated by determining protein,[22] free and esterified cholesterol,[23] TAG, and phospholipids as previously described.[21] The number of component molecules of LDL was calculated assuming 1 apo B molecule per LDL with a molecular weight of 412,000 as reported for the guinea pig.[23]

Table 1. Composition of Test Diets

	Percent Fat							
	2.5		7.5		15		25	
Component	g/100 g	kcal (%)	g/100 g	kcal (%)	g/100 g	kcal (%)	g/100 g	kcal (%)
Nutrient (%)								
Protein (soybean/casein)*	18.8	22.6	20.0	22.6	21.8	22.6	24.1	22.6
Fat (butter/palm oil)†	2.5	6.7	7.5	19.0	15.0	35.1	25.0	52.8
Starch‡	23.8	28.9	15.0	24.8	16.2	16.9	10.3	10.1
Sucrose	34.3	41.7	35.8	35.7	23.4	24.2	14.7	14.5
Vitamins	0.9	—	1.0	—	1.0	—	1.0	—
Minerals	7.1	—	7.5	—	8.2	—	9.0	—
Fiber (cellulose/guar)§	11.8	—	12.5	—	13.6	—	15.0	—
Cholesterol	0.037	—	0.033	—	0.027	—	0.019	
Sitosterol	0.077	—	0.081	—	0.086	—	0.093	
kcal/g	3.34		3.54		3.85		4.26	
Nutrient caloric density (g/1,000 kcal)								
Fiber	35.3		35.2		35.3		35.2	
Minerals	21.3		21.2		21.3		21.1	
Vitamins	2.69		2.82		2.86		2.82	
Protein	56.5		56.4		56.6		56.6	
Cholesterol	0.12		0.12		0.12		0.12	

*60% to 40% ratio.

†50% to 50% ratio.

‡Concentration adjusted to 41% of total carbohydrates.

§Cellulose to guar gum ratio, 2.8.

Previous studies involving selective precipitation of LDL protein with isopropyl alcohol indicated that apo B constitutes 96% to 98% of total LDL protein.[15] Molecular weights of TAG, free and esterified cholesterol, and phospholipids used for the calculations were 885.4, 386.6, 646, and 734, respectively.

LDL and HDL Binding Assays

Hepatic membranes from animals fed the four test diets were isolated as previously described.[21] For LDL binding assays, pooled LDL samples from the 2.5%, 7.5%, 15%, and 25% fat were used for LDL binding to guinea pig hepatic membranes from the four dietary fat groups, and human apo E free HDL was used for HDL binding assays. Previous studies in guinea pigs have demonstrated that human HDL is an appropriate ligand for measurement of HDL binding protein content.[24] Both pooled guinea pig LDL and human apo E free HDL were radioiodinated using the method reported by Goldstein et al[25] to produce a final specific activity between 150 and 400 cpm/ng. Hepatic membranes were incubated with ^{125}I-LDL over a range of 10 to 80 μg LDL protein/mL or 5 to 60 μg human ^{125}I-apo E free-HDL protein/mL for 2 hours at 37°C in the presence or absence of 1 mg/mL of the corresponding unlabeled lipoprotein. Values for maximal binding capacity (Bmax) and the ligand dissociation constant (K_d) were calculated from Woolf plots by plotting free LDL or HDL (μg/mL) versus free/bound lipoprotein (micrograms per milliliter divided by micrograms per milligram membrane protein).[26]

Microsome Isolation

Guinea pigs were killed at the nadir of the diurnal rhythm (9 AM), livers were removed, and hepatic microsomes for HMG CoA reductase and acyl CoA:cholesterol acyltransferase (ACAT) assays were isolated by pressing liver tissue through a tissue grinder into 1:3 homogenization buffer (50 mmol/L KH_2PO_4, 0.1 mol/L sucrose, 50 mmol/L KCl, 50 mmol/L NaCl, 30 mmol/L EDTA, and 2 mmol/L dithiothreitol, pH 7.2). This preparation was further homogenized with a Potter-Elvenjhem homogenizer. A microsomal fraction was isolated by two 15-minute centrifugations at 10,000 × *g* (JA-20 rotor in a J2-21 centrifuge, Beckman Instruments) followed by a 1-hour centrifugation at 100,000 × *g* in a Ti-50 rotor at 4°C. Microsomes were resuspended in the homogenization buffer and centrifuged an additional hour at 100,000 × *g*. After centrifugations, microsomal pellets were homogenized and stored at −70°C. Microsomal protein was determined according to the method reported by Markwell et al.[22]

Plasma Lecithin Cholesterol Acyltransferase Assay

Lecithin cholesterol acyltransferase ([LCAT] EC 2.3.1.43) activity was determined by measuring the decrease in the mass of unesterified cholesterol in incubated plasma samples. Freshly isolated plasma samples from each guinea pig were incubated at 37°C for 6 hours and compared with a control sample containing dithiobis-nitrobenzoic acid (DTNB) (1.5 mmol/L) and incubated at 0°C. Free cholesterol concentrations were determined by enzymatic analysis for both control and incubated plasma samples using a Titertrek Multiscan Plus microtiter plate reader (Flow Lab, McLean, VA) at 492 nm.[20] The rate of cholesterol esterification is expressed as micrograms per milliliter per hour.

Hepatic HMG CoA Reductase Assay

Microsomal HMG CoA reductase (EC 1.1.1.34) activity was measured as previously described.[27] Briefly, 200 μg microsomal protein was incubated with 7.5 nmol (0.33 GBq/nmol) [3-^{14}C]HMG CoA, 4.5 μmol glucose-6-phosphate, 3.6 μmol EDTA, 0.45 μmol NADP, and 0.3 IU glucose-6-phosphate dehydrogenase for 15 minutes at 37°C to a final volume of 0.05 mL. [^{3}H]mevalonic acid was used as an internal recovery standard (0.024 GBq per assay). HCl 10 mol/L (0.025 mL per assay) was added to stop the reaction, and samples were further incubated at 37°C for 30 minutes. After incubation, microsomal protein was precipitated by microfuging for 1 minute, and an aliquot of the supernatant was applied to silica gel thin-layer chromatography (TLC) plates (Alltech, Deerfield, IL). Plates were developed in acetone:benzene (1:1), the area containing mevalonate (Rf 0.6 to 0.9) was scraped and mixed with 5 mL Aquasol, and radioactivity was measured using a scintillation counter. HMG CoA reductase activity is expressed as picomoles of [^{14}C]mevalonate produced per minute per milligram microsomal protein. Recoveries of [^{3}H]mevalonate were between 60% and 80%.

Hepatic ACAT Assay

ACAT (EC 2.3.2.26) activity was determined by preincubating microsomal protein 0.8 to 1.0 mg per assay with 84 g/L albumin, an amount of albumin equivalent to the molar ratio of the substrate (1:1 albumin:^{14}C-oleoyl CoA)[28] and buffer for ACAT isolation (50 mmol/L KH_2PO_4, 1 mol/L sucrose, 50 mmol/L KCl, 30 mmol/L EDTA, and 50 mmol/L NaF) to a final volume of 0.18 mL. After 5 minutes at 37°C, 20 μL (500 μmol/L) oleoyl-[1-^{14}C]coenzyme A (0.15 GBq/pmol) was added, and the reaction proceeded for 15 minutes at the same temperature. The reaction was stopped by addition of 2.5 mL chloroform:methanol (2:1). A [^{3}H]cholesteryl oleate recovery standard (0.045 GBq per assay) was added, and the sample was vortexed and allowed to stand overnight at room temperature. The aqueous phase was removed, and after evaporation of the organic phase to dryness, samples were resuspended in 150 μL chloroform containing 30 μg unlabeled cholesteryl oleate. Samples were applied to 20 × 20-cm silica gel TLC plates (Alltech) and developed in hexane:diethyl ether (9:1 vol/vol). Cholesteryl oleate was visualized with iodine vapors and scraped from the TLC plates, and 5 mL Liquifluor was added and counted in a scintillation counter. Recoveries of [^{3}H]cholesteryl oleate were between 75% and 90%.

Statistical Analysis

One-way ANOVA and the Student-Newman-Keuls multiple comparison test (INSTAT, San Diego, CA) were used to determine differences in plasma cholesterol, triglycerides, lipoprotein composition, hepatic lipid concentrations, LDL receptor Bmax, HDL binding protein Bmax, plasma LCAT activity, and activities of the hepatic enzymes HMG CoA reductase and ACAT. Linear regressions were performed to assess significant correlations, and values were considered significantly different at P less than .05.

RESULTS

Plasma Lipids

Body weights were not affected by increasing the amount of fat in the diets. Animals maintained initial body weights throughout the duration of the feeding experiment. Final body weights are listed in Table 2. Plasma cholesterol concentrations increased as dietary fat calories increased (r = .954, P < .05), and for every 1% (wt/wt) increase in saturated dietary fat, plasma cholesterol increased 2.4 mg/dL. Animals fed the 25% fat diet had plasma cholesterol levels almost twofold the values for guinea pigs fed the 2.5% fat diet (P < .01; Table 2). In contrast, plasma TAG concentrations were not different among the four dietary fat groups (Table 2); however, since the animals were not

Table 2. Body Weight and Plasma Lipids of Guinea Pigs Fed Varying Levels of Dietary Fat

Fat (%)	Body Weight (g)	Plasma Lipids (mg/dL)	
		Cholesterol	TAG
2.5	1,032 ± 83[a]	62 ± 27[a]	111 ± 32[a]
7.5	1,082 ± 105[a]	80 ± 22[ab]	119 ± 66[a]
15.0	867 ± 218[a]	107 ± 23[bc]	111 ± 11[a]
25.0	1,053 ± 138[a]	115 ± 25[c]	108 ± 19[a]
r	—	.95	—
P	—	<.05	—

NOTE. Values are the mean ± SD for n = 5 guinea pigs. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .01$).

fasted at the time of death, slight differences might not be detectable.

Plasma VLDL and HDL cholesterol concentrations were not affected by dietary fat quantity whereas plasma LDL cholesterol levels followed the pattern of total cholesterol (Table 3). Animals fed the 15% and 25% fat diets had twofold higher plasma LDL cholesterol than guinea pigs fed 2.5% fat. Animals fed 7.5% fat had an intermediate value that was not significantly different from the value of the 2.5% fat dietary group (Table 3). There was a significant exponential correlation between plasma LDL cholesterol concentrations and fat calories ($r = .96, P < .02$).

The compositions of the three lipoprotein fractions were significantly modified by dietary fat quantity (Table 4). VLDL from the 25% fat group contained more protein and less TAG than VLDL from the 2.5% and 7.5% fat groups, resulting in a particle with a greater surface to core ratio. In addition, VLDL from the 25% fat group had a higher percentage of cholesteryl ester. Phospholipids and free cholesterol in VLDL were not different among dietary groups. The increases in VLDL protein and cholesteryl ester concentrations and decreases in TAG were significantly correlated with increases in dietary fat ($r = .997$ and $P < .005$, r = .953 and $P < .05$, and $r = -.978$ and $P < .05$, respectively).

LDL from the 15% and 25% fat groups had significantly higher proportions of cholesteryl ester than LDL from guinea pigs fed the 2.5% fat diet, whereas LDL from the 7.5% fat diet had an intermediate value (Table 4). The

Table 3. Lipoprotein Cholesterol of Guinea Pigs Fed Varying Levels of Dietary Fat

Fat (%)	Lipoprotein Cholesterol (mg/dL)		
	VLDL + IDL	LDL	HDL
2.5	7 ± 3[a]	45 ± 25[a]	11 ± 2[a]
7.5	7 ± 6[a]	61 ± 17[a]	11 ± 2[a]
15.0	6 ± 2[a]	92 ± 25[b]	11 ± 3[a]
25.0	8 ± 3[a]	98 ± 21[b]	11 ± 2[a]
r	—	.96	—
P	—	<.02	—

NOTE. Values are the mean ± SD for n = 5 determinations. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .01$).

percentage of LDL TAG decreased as the amount of dietary fat increased, whereas LDL phospholipids were highest in animals fed the 25% fat diet (Table 4). Again, significant correlations were found between increases in LDL percent phospholipids ($r = .983, P < .025$), decreases in LDL percent TAG ($r = -.997$, $P < .005$), and the amount of calories from dietary fat, indicating a dose-response to dietary fat. No significant differences in LDL free cholesterol were found among diets. LDL particles contained less cholesteryl ester molecules and a higher number of TAG molecules when animals were fed the 2.5% fat diet versus the 15% and 25% fat diets. Animals fed 7.5% fat had an intermediate number of cholesteryl ester and TAG molecules per LDL particle, which was not significantly different from the other dietary fat groups (Fig 1). Other differences include a higher number of phospholipid molecules per LDL particle from animals fed the 25% and 15% fat diets (Fig 1).

The composition of HDL was not modified by dietary fat quantity, except for percent cholesteryl ester, which was significantly higher in animals fed 15% and 25% fat versus 2.5% fat (Table 4). Significant correlations were found between increases in dietary fat and increases in the relative percentage of HDL free cholesterol ($r = .983$, $P < .05$) and decreases in percent TAG ($r = -.989$, $P < .05$).

Higher fat intakes resulted in lower LDL peak densities, higher cholesteryl ester to protein ratios, and higher molecular weights of LDLs, indicating that increasing dietary fat calories results in the formation of larger, more buoyant LDL particles (Table 5).

Hepatic Lipids

Hepatic free cholesterol and TAG concentrations were significantly higher in animals fed 25% fat versus the other three dietary fat groups ($P < .01$; Table 6). As observed for the dose-response to dietary fat and changes in plasma lipoprotein composition, significant correlations existed between hepatic lipids and the amount of dietary fat for free cholesterol ($r = .969$, $P < .05$), cholesteryl ester ($r = .942, P = .058$), and hepatic TAG ($r = .955, P < .05$).

Activities of Plasma and Hepatic Enzymes

Plasma LCAT activity was not significantly changed by dietary fat quantity (Table 7); however, there was a trend suggesting increased plasma LCAT activity with increasing fat intake, which plateaued at 15% fat (Table 7). HDL cholesteryl ester content and LCAT activity exhibited a significant correlation, indicating that the higher HDL cholesteryl ester content in animals fed high-fat diets is associated with increased LCAT activity (Fig 2).

Hepatic HMG CoA reductase activity was not affected by dietary fat amount, but ACAT activity was found to be significantly different by ANOVA, suggesting an effect of 25% fat intake; the post hoc test demonstrated no significant differences among means, probably due to the high standard deviation in animals fed 25% fat (Table 7). Hepatic free cholesterol concentrations and ACAT activi-

Table 4. Percent Composition of VLDL + IDL, LDL, and HDL From Guinea Pigs Fed Varying Levels of Dietary Fat

Fat (%)	Protein	Cholesteryl Ester	Free Cholesterol	Phospholipids	TAG
VLDL					
2.5	7.8 ± 0.3[a]	1.5 ± 0.5[a]	10.3 ± 2.4[a]	7.0 ± 0.7[a]	73.6 ± 2.9[a]
7.5	8.6 ± 0.7[ab]	2.4 ± 0.9[a]	10.2 ± 0.4[a]	7.7 ± 3.5[a]	71.7 ± 2.1[a]
15.0	10.7 ± 1.9[bc]	2.7 ± 0.5[a]	9.3 ± 0.7[a]	7.4 ± 0.8[a]	70.0 ± 2.6[a]
25.0	12.7 ± 1.5[c]	5.7 ± 0.6[b]	9.7 ± 2.3[a]	8.3 ± 0.6[a]	63.7 ± 3.1[b]
LDL					
2.5	25.9 ± 1.5[a]	30.6 ± 4.3[a]	7.2 ± 0.7[a]	15.7 ± 2.2[a]	16.9 ± 1.6[a]
7.5	25.7 ± 1.7[a]	36.1 ± 1.6[ab]	6.8 ± 1.1[a]	15.9 ± 1.1[a]	15.2 ± 2.2[ab]
15.0	22.8 ± 3.0[a]	40.3 ± 3.8[b]	7.0 ± 1.5[a]	18.2 ± 2.0[ab]	11.7 ± 3.5[bc]
25.0	22.7 ± 0.2[a]	40.2 ± 0.8[b]	8.5 ± 0.5[a]	20.0 ± 0.8[b]	8.5 ± 0.4[c]
HDL					
2.5	35.6 ± 2.6[a]	6.8 ± 0.8[a]	10.1 ± 0.5[a]	23.4 ± 0.6[a]	24.0 ± 1.2[a]
7.5	31.1 ± 0.5[a]	9.5 ± 0.9[ab]	10.6 ± 1.2[a]	25.9 ± 1.5[a]	23.0 ± 2.0[a]
15.0	32.5 ± 3.1[a]	12.1 ± 3.3[b]	11.1 ± 0.8[a]	22.0 ± 3.0[a]	22.0 ± 3.5[a]
25.0	33.6 ± 3.5[a]	11.4 ± 0.6[b]	12.7 ± 0.9[a]	22.7 ± 1.6[a]	19.3 ± 1.7[a]

NOTE. Values are the mean ± SD for n = 3 determinations. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .05$).

ties exhibited a significant positive correlation for all dietary fat groups ($r = .77, P < .01$; Fig 3).

LDL and HDL Binding

Equilibrium binding parameters K_d and Bmax were measured for LDL and HDL binding to hepatic membranes of guinea pigs from all fat groups. Expression of apo B/E (LDL) receptors (Bmax) was increased in animals fed 2.5% and 7.5% fat as compared with the higher-fat groups (Table 8). A significant negative correlation was found between LDL Bmax and plasma LDL cholesterol concentrations for guinea pigs from all diets ($r = -.82, P < .01$; Fig 4). K_d was not different among dietary groups, indicating no effect of dietary fat quantity on the affinity of hepatic apo B/E receptors for LDL.

Expression of hepatic membrane HDL binding protein was not significantly changed among dietary fat groups (Table 8); however, there was a significant correlation between dietary fat calories and hepatic HDL binding protein expression ($r = .98$, $P < .005$; Fig 5). K_d values were unaffected by increasing the amount of dietary fat (Table 8).

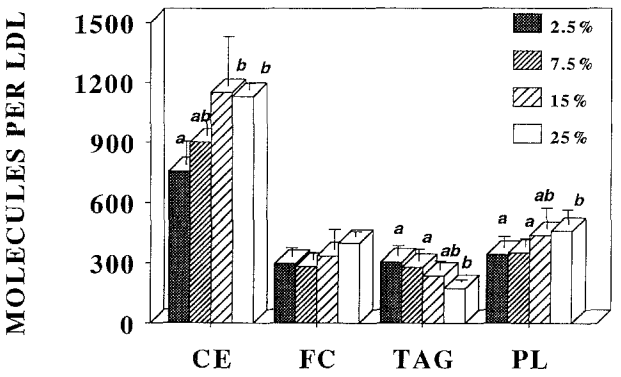

Fig 1. Plasma LDL composition (cholesteryl ester [CE], free cholesterol [FC], TAG, and phospholipid [PL]) in guinea pigs fed varying concentrations of dietary fat. The number of CE molecules was lowest in animals fed 2.5% fat and highest in animals fed 15% and 25% fat; animals fed 7.5% fat had an intermediate value. The number of TAG molecules was lowest in animals fed 25% fat and highest in the group fed 2.5% fat. The number of PL molecules was higher in animals fed 15% and 25% fat diets. Values with different superscripts for each LDL component are significantly different ($P < .01$).

DISCUSSION

Although a number of clinical studies have investigated effects of dietary fat quantity on plasma lipid and lipoprotein levels, there is a surprising scarcity of animal studies investigating the mechanisms involved in these significant changes in plasma lipoprotein concentrations (reviewed in McNamara[2]). Many of the animal studies that have been reported are difficult to interpret due to problems with the approaches used for diet formulation. Preparation of high-fat diets by simply adding fat or oil to commercial natural-ingredient diets results in two significant variables, the variable under study, which is the fat, plus variables resulting from modification of the caloric density and variations in the intake of all other nutrients. The added fat dilutes the concentration of other nutrients in the diet, and decreased consumption due to the higher caloric density of the diet results in a reduction in the intakes of protein, fiber, vitamins, and minerals. It can be estimated that under conditions of stable caloric intake, addition of 20% (wt/wt) fat to a commercial diet results in a 40% to 50% (wt/wt)

Table 5. Characteristics of Plasma LDL From Guinea Pigs Fed Varying Levels of Dietary Fat

	LDL Characteristics		
Fat (%)	Cholesteryl Ester to Protein Ratio	Molecular Weight (× 10^6)	Peak Density (g/mL)
2.5	1.19 ± 0.18[a]	1.53 ± 0.15[a]	1.064 ± 0.003[a]
7.5	1.41 ± 0.12[ab]	1.60 ± 0.11[a]	1.060 ± 0.005[ab]
15.0	1.80 ± 0.38[b]	1.83 ± 0.23[a]	1.056 ± 0.003[b]
25.0	1.77 ± 0.05[b]	1.81 ± 0.02[a]	1.049 ± 0.002[c]

NOTE. Values are the mean ± SD for n = 3 determinations. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .01$).

Table 6. Hepatic Lipids of Guinea Pigs Fed Varying Concentrations of Dietary Fat

Fat (%)	Hepatic Lipids (mg/g liver)		
	Free Cholesterol	Cholesteryl Ester	TAG
2.5	2.43 ± 0.26[a]	0.20 ± 0.11[a]	5.8 ± 3.1[a]
7.5	2.58 ± 0.30[a]	0.26 ± 0.28[a]	6.5 ± 2.3[a]
15.0	2.73 ± 0.21[a]	0.27 ± 0.15[a]	10.0 ± 5.4[a]
25.0	3.35 ± 0.37[b]	0.47 ± 0.38[a]	21.0 ± 5.3[b]
r	.97	.94	.96
P	<.05	.058	<.05

NOTE. Values are the mean ± SD for n = 5 guinea pigs. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .01$).

reduction in the intakes of protein, fiber, vitamins, and minerals, all factors that on their own can alter plasma lipid levels and lipoprotein metabolism. In the present study, we formulated the test diets by exchanging fat for carbohydrate mass and increasing the concentrations of the other nutrients, thereby maintaining the nutrient caloric density (grams per kilocalorie) constant for all dietary components except the variables of interest, carbohydrate and fat. In an effort to mimic an American diet, the protein mixture was 60% animal (casein) and 40% vegetable (soybean) protein, and the changes in carbohydrate calories maintained the ratio of sucrose to starch calories constant. In addition, the diets contained 0.12 g/1,000 kcal cholesterol, which for the guinea pig is the physiologic equivalent (ie, absorbed cholesterol relative to endogenous cholesterol synthesis) of a human intake of 300 mg/d.[16]

Since the fat mix used for the diets of this experiment is highly saturated, the possibility of essential fatty acid (EFA) deficiency in animals fed the 2.5% fat diet could be a concern in the interpretation of data. The EFA requirement of guinea pigs is 1.8% of total calories provided by linoleic acid,[29] and animals fed the 2.5% fat diet had only 0.5% of calories provided by this EFA. However, studies by Reid[30] have shown that the symptoms of EFA deficiency are present only after 7 weeks in growing guinea pigs fed a fat-free diet, and that these symptoms are reversed by inclusion of 10, 30, or 60 mg/d linoleic acid in the diet. Reid also observed that the first symptom of EFA deficiency was greatly reduced weight gain as compared with control

Table 7. Activities of Plasma LCAT and Hepatic HMG CoA Reductase and ACAT of Guinea Pigs Fed Varying Concentrations of Dietary Fat

Fat (%)	LCAT (μg/mL · h)	HMG CoA Reductase (pmol/min · mg)	ACAT (pmol/min · mg)*
2.5	3.6 ± 1.6[a]	1.8 ± 0.8[a]	17.0 ± 5.0[a]
7.5	7.8 ± 3.8[a]	3.5 ± 2.4[a]	14.2 ± 6.8[a]
15.0	10.9 ± 4.5[a]	1.5 ± 0.6[a]	16.4 ± 8.6[a]
25.0	9.5 ± 4.7[a]	2.4 ± 1.0[a]	38.0 ± 24.3[a]

NOTE. Values are the mean ± SD for n = 5 guinea pigs. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Neuman-Keuls multiple comparison test ($P < .01$).

*Values are significantly different as determined by ANOVA. The post hoc test did not show any significant differences among dietary groups.

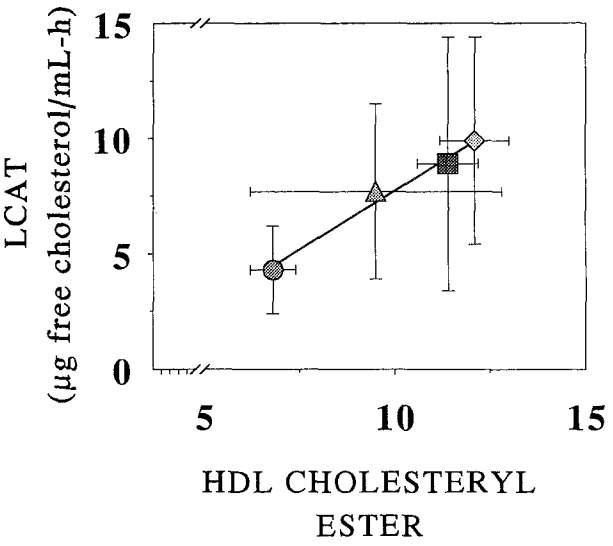

Fig 2. Correlation between plasma HDL cholesteryl ester content (% weight) and plasma LCAT activity (μg cholesterol esterified/mL · h) in guinea pigs fed 2.5% (●), 7.5% (▲), 15% (◆), and 25% (■) dietary fat ($r = .99$, $P < .01$). Each data point represents the mean ± SD of 4 determinations.

animals, and that after feeding 60 mg/d linoleic acid the symptoms of EFA deficiency did not return.[30] In our experimental design, the use of adult guinea pigs, the duration of the experiment (4 weeks), the lack of changes in body weights among dietary groups, and the fact that animals consumed on average 50 g/d diet, which corresponds to 98 mg linoleic acid/d, suggest that EFA deficiency was not a concern. Further, previous studies in guinea pigs fed palm kernel oil, which contains no linoleic acid, showed that these animals have 32% of this fatty acid in hepatic membranes, an amount similar to that found in

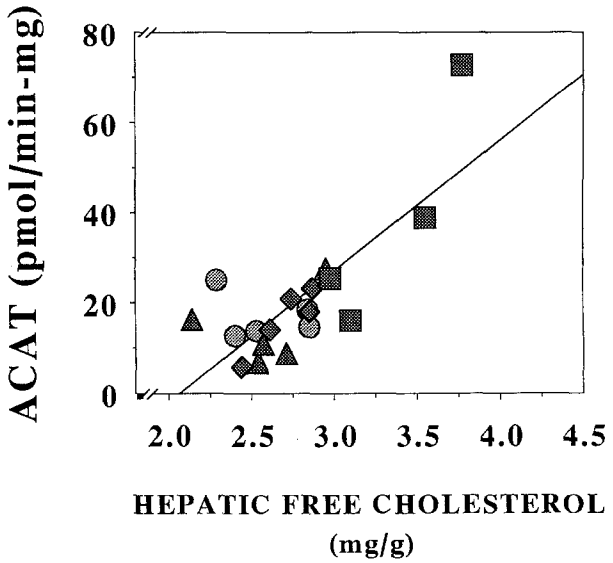

Fig 3. Correlation between hepatic free cholesterol concentrations (mg/g) and hepatic ACAT activity (pmol cholesterol esterified/min · mg) in guinea pigs fed 2.5% (●), 7.5% (▲), 15% (◆), and 25% (■) dietary fat ($r = .77$, $P < .01$).

Table 8. LDL and HDL Binding Parameters of Hepatic Membranes From Guinea Pigs Fed Varying Concentrations of Dietary Fat

	LDL		HDL	
Fat (%)	Bmax (μg/mg protein)	K_d (μg/mL)	Bmax (μg/mg protein)	K_d (μg/mL)
2.5	2.57 ± 0.18[a]	28 ± 9[a]	1.24 ± 0.18[a]	33 ± 12[a]
7.5	2.60 ± 0.43[a]	30 ± 12[a]	1.35 ± 0.20[a]	25 ± 8[a]
15.0	2.10 ± 0.25[b]	42 ± 13[a]	1.40 ± 0.07[a]	34 ± 6[a]
25.0	1.94 ± 0.22[b]	28 ± 1[a]	1.42 ± 0.04[a]	37 ± 15[a]
r	−.82	—	.98	—
P	<.01	—	<.005	—

NOTE. Values are the mean ± SD for n = 4 animals. Values in a column with different superscripts are significantly different as determined by ANOVA and Student-Newman-Keuls multiple comparison test ($P < .01$).

guinea pigs fed corn oil diets (high in linoleic acid),[15,31] which demonstrates that EFA deficiency was not present under those experimental conditions.

Saturated Fat Quantity and Plasma Lipids and Lipoproteins

To study mechanisms by which dietary saturated fat quantity modifies plasma LDL cholesterol concentrations, we have used the guinea pig, an animal model that carries most of its cholesterol in LDL, to determine effects on some of the primary regulatory sites of cholesterol and lipoprotein metabolism. In these studies, we have demonstrated that although no changes in plasma TAG, VLDL plus IDL, or HDL concentrations occurred with increasing saturated fat, there were significant increases in plasma LDL concentrations. Using density-gradient ultracentrifugation, we isolated VLDL plus IDL between density 1.006 and 1.019 g/mL; however, since the IDL fraction contributes 2% to 3% of total lipoprotein cholesterol in guinea pigs,[32] this fraction can be considered representative of VLDL. Although no changes in plasma VLDL or HDL cholesterol concentrations were evident with increasing dietary fat, significant modifications in the composition of all plasma

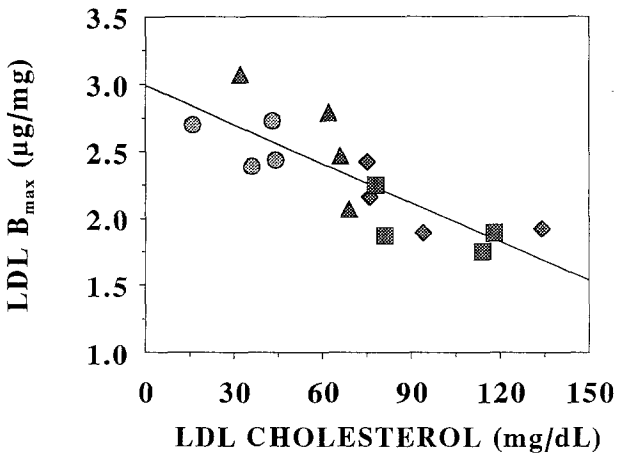

Fig 4. Correlation between plasma LDL cholesterol concentrations (mg/dL) and hepatic LDL receptor Bmax (μg LDL bound/mg membrane protein) in guinea pigs fed 2.5% (●), 7.5% (▲), 15% (◆), and 25% (■) dietary fat ($r = -.82$, $P < .01$).

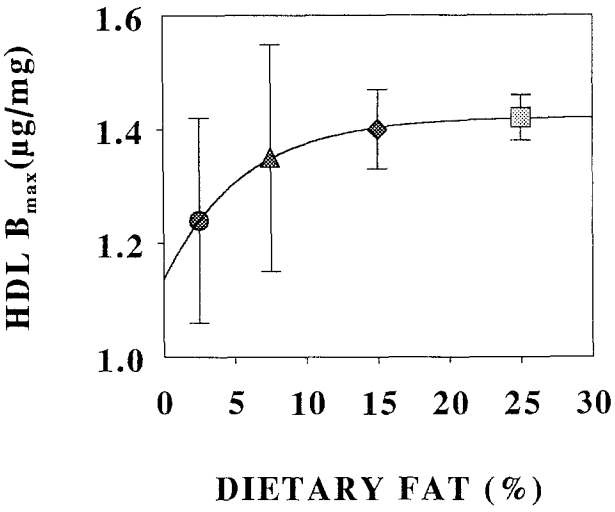

Fig 5. Correlation between hepatic membrane HDL binding protein Bmax (μg HDL bound/mg membrane protein) and dietary fat quantity in guinea pigs fed 2.5% (●), 7.5% (▲), 15% (◆), and 25% (■) fat ($r = .989$, $P < .01$). Each point represents the mean ± SD of 4 determinations.

lipoproteins were observed, suggesting significant fat-mediated alterations in the synthesis, intravascular processing, and catabolism of lipoproteins.

Since guinea pigs have low hepatic ACAT activity, similar to humans,[28] cholesteryl esters in mature VLDL are primarily derived from the actions of plasma LCAT and cholesteryl ester transfer protein. We have shown in previous studies of the effects of dietary fat saturation on VLDL metabolism[33] that nascent VLDL contains negligible amounts of cholesteryl ester and that mature VLDL particles acquire cholesteryl ester during intravascular processing through the actions of plasma LCAT and cholesteryl ester transfer protein. The higher plasma LCAT activities of animals fed the 15% or 25% fat diets correlate with the higher VLDL cholesteryl ester content, consistent with a contribution of LCAT to VLDL cholesteryl ester levels in guinea pigs fed increasing dietary fat. However, animals fed 25% fat also had higher hepatic ACAT activity, as well as the highest concentration of cholesteryl ester into VLDL, suggesting that with very high fat intake (25%) ACAT could contribute to the incorporation of cholesteryl esters to VLDL, as has been observed in the African green monkey.[34] It would be predicted that nascent VLDL from guinea pigs fed 25% fat diets would have a higher cholesteryl ester content as compared with lower fat intakes.

LCAT activity was highly correlated with increased cholesteryl ester content of plasma HDL, suggesting that high-fat diets favor the formation of the less dense HDL_2, which is associated with increased reverse cholesterol transport.[35] Although no changes in plasma total HDL cholesterol concentrations were observed with varying amounts of dietary fat, subclasses of HDL probably were altered, as suggested by the higher percentage of cholesteryl ester in HDLs from high-fat groups. We found a

significant dose-response relationship between hepatic membrane HDL binding protein Bmax[36] and dietary fat quantity, suggesting that high-fat diets (15% and 25% fat) increased expression of hepatic HDL binding protein. We have previously found that dietary fat saturation and chain length[37,38] significantly modify HDL binding to guinea pig hepatic membranes, and that under some circumstances this appears to be related to plasma HDL cholesterol concentrations.[38] The data in this study demonstrate that dietary fat quantity also affects expression of the hepatic HDL binding protein.

Several mechanisms could be responsible for the lower plasma LDL concentrations and lower ratio of LDL cholesteryl ester to protein observed in animals fed low-fat diets. Similar to our findings, Abbot et al[10] reported that Pima Indians consuming 60% of energy from carbohydrates had reduced plasma LDL levels and a decrease of the LDL cholesteryl ester to apo B ratio. This and other studies have shown that the decrease in plasma LDL induced by a high-carbohydrate diet can result in part from a decreased conversion of VLDL to IDL and LDL.[9,10] In our studies, although no differences in plasma VLDL concentrations were observed, VLDLs from high-fat diet groups had a reduced percentage of TAG, suggesting an increased catabolism by lipoprotein lipase as has been observed in the rat,[39] resulting in a particle containing more surface (higher amounts of phospholipid) and less core (less TAG); this smaller VLDL could be more readily converted to LDL. More dense VLDL containing less TAG have been shown to be more efficiently converted to LDL.[40]

Abbott et al[10] also found increases in apo B clearance rates in subjects fed low-fat diets, which is consistent with our finding of higher expression of hepatic LDL receptors in guinea pigs fed 2.5% and 7.5% fat diets. We have previously shown that hepatic LDL receptor expression is significantly modified by dietary fatty acid saturation and composition, and that these modifications are highly correlated with plasma LDL concentrations and with receptor-mediated LDL catabolism in vivo.[15,32] In the present studies, similar results were obtained in that the higher plasma LDL concentrations of animals fed high-fat diets were significantly correlated with low expression of hepatic apo B/E receptors. Compositional changes in LDL that resulted in smaller, denser particles with low-fat diets would also be predicted to make a contribution to the lower plasma LDL cholesterol levels of guinea pigs fed these diets. Studies in humans have shown that high-carbohydrate diets modify LDL composition, and these modifications increase receptor-mediated LDL degradation by human fibroblasts.[12] In the present studies, although we found an increased number of receptors in animals fed low-fat diets of 2.5% and 7.5%, no differences in receptor affinity related to the composition of LDL particles were noted. However, we have previously demonstrated that small LDL particles induced by other dietary interventions have an increased fractional catabolic rate,[15,32] and if true for the low-fat diet effects on LDL composition, the combined effects of smaller LDL particles and increased LDL receptor numbers would significantly decrease plasma LDL concentrations in animals fed 2.5% and 7.5% fat diets.

Saturated Fat Quantity and Hepatic Cholesterol Metabolism

A significant dose-response correlation was observed between intake of dietary fat from 2.5% to 25% and hepatic free cholesterol and TAG concentrations. Intake of 25% fat induced the highest hepatic free cholesterol and TAG concentrations, suggesting that some compensatory mechanism becomes saturated with 25% fat intake. This response could, in part, explain the decrease in hepatic apo B/E receptor number associated with high-fat intake in that the 15% and 25% fat diets theoretically increase the putative regulatory pool of hepatic free cholesterol, which would suppress apo B/E receptor expression.[41] ACAT activity was also related to the concentration of hepatic free cholesterol, whereas HMG CoA reductase activity was not modified by dietary fat. Values for hepatic HMG CoA reductase were lower than previously reported for guinea pigs using diets varying in saturation of fatty acids.[18] One possible explanation is that 60% of the dietary protein used in the present study was casein, whereas in our previous experiments soy was used as the only protein source,[18] and it has been shown in animal studies that casein suppresses HMG CoA reductase as compared with diets containing vegetable proteins.[42] In addition, the diets used in this study contained a small amount of added cholesterol (0.12 g/1,000 kcal), and we have previously shown that the guinea pig suppresses hepatic HMG CoA reductase activity as the first compensatory mechanism when challenged with dietary cholesterol.[16] Dietary fat quantity did not regulate the activity of hepatic HMG CoA reductase, which was an unexpected finding because the correlation between hepatic cholesterol concentrations and the amount of dietary fat was significant. The mechanisms that induce an increase in hepatic cholesterol pools in response to increasing dietary fat are unknown at present, but could be due to a dose-response increase in absorption of dietary cholesterol with increasing dietary fat or to an increased reabsorption of bile acids with high-fat diets.

Higher hepatic ACAT activity was observed with increasing dietary fat from 2.5% to 25%, and the increase correlated with increases in hepatic free cholesterol. We have previously demonstrated that ACAT activity is regulated by the amount of absorbed dietary cholesterol in guinea pigs.[17] In the present study, we also had increases in hepatic cholesterol in guinea pigs fed 25% fat diets that were apparently related to increased ACAT activity, although the mechanisms of the increase in hepatic cholesterol are unknown.

From these studies, we conclude that changes in saturated fat–carbohydrate caloric intake result in significant modifications in hepatic cholesterol/lipoprotein metabolism and lipoprotein composition that partly explain the observed changes in plasma LDL concentrations. If we analyze the metabolic alterations induced by the 25% fat diet, we can account for the higher plasma LDL by (1)

modifications in VLDL composition that could be due to secretion of smaller nascent VLDL particles by the liver or related to changes in tissue LPL, resulting in a remnant particle that is readily converted into LDL; (2) the increased hepatic ACAT activity, which increases the rate of incorporation of cholesteryl esters into VLDL, leading to formation of larger LDL containing increased amounts of cholesteryl ester and the associated decrease in LDL turnover[15,29]; and (3) decreased hepatic apo B/E receptor Bmax values, possibly due to increases in the regulatory pool of hepatic free cholesterol. In addition to these metabolic changes that directly affected plasma LDL levels, high-fat diets also altered HDL metabolism by increasing LCAT activity, altering the composition of HDL, and increasing expression of hepatic HDL binding protein.

However, it should be noted that these metabolic responses to the exchange of carbohydrate for fat calories could be limited to dietary fats that have a high saturated fatty acid content as in the present study, and in addition, the responses could vary in male guinea pigs. The effects of exchanging carbohydrate with polyunsaturated fat calories require analysis to determine potential differences and interactions between dietary fat type and amount on cholesterol and lipoprotein metabolism.

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