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Regulation of guinea pig hepatic acyl-CoA:cholesterol acyltransferase activity by dietary fat saturation and cholesterol

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We measured the interactive effects of dietary cholesterol and fat on the regulation of hepatic acyl-CoA: cholesterol acyltransferase (ACAT) activity and its relationship to hepatic microsomal lipid composition in guinea pigs fed 15 g/100 g (w/w) fat diets (corn oil, olive oil, or lard) with 0.01, 0.08, 0.17, or 0.33 g/100 g (w/w) added cholesterol. Guinea pigs exhibited a dose dependent increase in hepatic microsomal ACAT activity, with increasing levels of cholesterol intake ($P < 0.001$*) in all dietary fat groups. Animals fed monounsaturated olive oil had the highest hepatic ACAT activity with the exception of the 0.33 g/100 g cholesterol diet (*$P < 0.001$*). There were no differences in ACAT activity with intake of polyunsaturated corn oil or saturated lard. Dietary cholesterol resulted in increased microsomal free cholesterol (FC) concentrations in a dose dependent manner but had no effects on microsomal phosphatidylcholine (PC) concentrations. Guinea pigs fed olive oil generally had the highest microsomal FC/PC molar ratios, and hepatic ACAT activities correlated significantly with this parameter. After modification of the lipid compositions of the microsomes from guinea pigs fed the 12 test diets with FC/PC liposome treatment, microsomal ACAT activities remained significantly related to the microsomal FC/PC molar ratios, and dietary fat type did not affect this correlation. Our findings do not support the hypothesis that the stimulation of hepatic ACAT activity with cholesterol intake is enhanced by polyunsaturated fat intake. The data demonstrate that although dietary fat type and cholesterol amount have differential effects on hepatic ACAT activity, substrate availability, expressed as microsomal FC/PC molar ratio, is a major regulator of hepatic microsomal ACAT activity.* (J. Nutr. Biochem. 10:172–180, 1999)

Keywords: acyl-coA:cholesterol acyltransferase; liver; low density lipoprotein; dietary cholesterol; dietary fat; guinea pig

Introduction

Elevated plasma cholesterol levels are strongly associated with increased incidence of atherosclerosis[1] and, because plasma low density lipoprotein (LDL) carries the majority of plasma cholesterol in humans, LDL cholesterol levels are positively correlated with risk of cardiovascular disease. Dietary intervention studies have shown that dietary lipids are a significant determinant of plasma LDL cholesterol concentrations,[2,3] and evidence from animal and human studies have documented that dietary saturated fatty acids (SFA) have a hypercholesterolemic effect whereas intake of polyunsaturated fatty acids (PUFA) have a hypocholesterolemic effect. Dietary monounsaturated fats (MUFA), when fed as olive oil, have been shown to have contradictory effects on plasma cholesterol levels in human versus animal studies.[4–6] Feeding very high levels of cholesterol increases plasma LDL cholesterol levels in most animal model studies, whereas the intake of low to moderate dietary cholesterol has not consistently demonstrated a hypercholesterolemic effect.[3,6,7]

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The liver plays the central role in regulating whole body cholesterol and plasma LDL metabolism through biliary secretion of cholesterol and bile acids regulating sterol excretion from the body. Hepatic LDL receptors account for 80% of plasma LDL clearance[8] and the synthesis and secretion of plasma very low density lipoprotein (VLDL). Therefore, alterations in hepatic cholesterol homeostasis by dietary or drug interventions significantly influence whole body cholesterol balance and plasma LDL cholesterol levels.

Acyl-CoA:cholesterol acyltransferase (ACAT; EC 2.3.2.26), an integrated membrane enzyme, is involved in regulating intracellular cholesterol homeostasis by catalyzing formation of cholesteryl ester from free cholesterol (FC) and long-chain acyl-CoA. A high cholesterol diet is associated with increased hepatic microsomal ACAT activity,[9,10] which is consistent with the observation that in vitro addition of cholesterol to the microsomal fractions significantly increases ACAT activity.[11,12] These findings support the concept that availability of microsomal FC is a major determinant of ACAT activity.[13] However, studies in rats fed polyunsaturated safflower oil demonstrated that the animals had more PUFA in the fatty acyl chains of the microsomal phospholipids, which was associated with higher hepatic ACAT activity compared with rats fed saturated tristearin.[14] These data suggest that membrane fluidity, which is determined by fatty acyl groups of phospholipids, also may play a role in regulating ACAT activity.

Although studies suggest that both dietary fat and cholesterol alter hepatic ACAT activity, most previous studies used pharmacologic levels of dietary cholesterol (i.e., an amount of absorbed cholesterol in substantial excess of endogenous synthesis). In addition, there are limited data regarding hepatic ACAT activity with intake of physiologic levels of dietary cholesterol or possible interactions of dietary cholesterol with dietary fat saturation. In the present study, guinea pigs were fed 15 g/100 g (w/w) fat diets containing either polyunsaturated corn oil, monounsaturated olive oil, or saturated lard with 0.01, 0.08, 0.17, or 0.33 g/100 g cholesterol to investigate the regulation of hepatic ACAT activity by the interactions of dietary fat saturation with physiologic and pharmacologic levels of dietary cholesterol.

These studies were designed to test the hypothesis that the effects of fat type on ACAT activity are evident only with physiologic levels of dietary cholesterol whereas pharmacologic cholesterol intake would overwhelm the fat-mediated changes in hepatic ACAT activity. Regulation of ACAT activity by changes in the amount versus the activity of the enzyme in response to dietary interventions is difficult to distinguish. Experiments using cholesterol/phosphatidylcholine (C/PC) liposome to modify microsomal cholesterol and PC content were performed to determine whether dietary fat and cholesterol altered the amount of ACAT protein or, through changes in substrate availability, the activity of the enzyme.

The guinea pig was chosen as the animal model for these studies due to a number of lipid and lipoprotein metabolic similarities to humans: (1) LDL is the major carrier of plasma cholesterol,[5] (2) guinea pigs have an active plasma cholesterol ester transfer protein,[15] (3) dietary fat saturation with or without added cholesterol modulates plasma LDL cholesterol concentrations,[7,16] and (4) the distribution of tissue cholesterol synthesis and the hepatic free:esterified cholesterol ratio in the guinea pig are similar to that of humans.[17,18]

Table 1 Composition of experimental diets

Component	Weight (g/100g)	Energy (%)
Carbohydrate*	39.6	41.9
Fat†	15.1	35.1
Protein‡	22.3	23.2
Fiber§	13.6	–
Mineral Mix‖	8.2	–
Vitamin Mix‖	1.1	–
Cholesterol	0.01, 0.08, 0.17, or 0.33	–

*Sucrose/starch ratio of 1.43:1.
†Three test fats were corn oil, lard, and olive oil.
‡Soy bean protein.
§Cellulose:guar gum ratio of 4:1.
‖Mineral and vitamin mixture were formulated to match NRC requirements for the guinea pig.[19]

Materials and methods

Materials

Cholesterol enzymatic assay kits, cholesterol standards, cholesterol oxidase, and cholesterol esterase were purchased from Boehringer Mannheim (Indianapolis, IN USA); FC and phospholipids enzymatic assay kits were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); purified cholesterol and PC, dl-dithiotheritol (DTT), bovine serum albumin (BSA), 96% purity, and cholesteryl oleate were obtained from Sigma (St. Louis, MO USA); cholesteryl-[1,2,6,7-^{3}H]oleate (370 GBq/mmol) and liquifluor (toluene concentrate) were obtained from Du Pont NEN (Boston, MA USA); oleoyl-[1-^{14}C]CoA (1.8 GBq/mmol) was obtained from Amersham (Clearbrook, IL USA); quick seal ultracentrifugation tubes were obtained from Beckman Instruments (Palo Alto, CA USA); and Triton X-100 was obtained from Fisher Scientific (Fair Lawn, NJ USA).

Diets

Experimental diets were prepared and pelleted by Research Diets, Inc. (New Brunswick, NJ USA). The composition of test diets is shown in *Table 1*. The 12 test diets had identical caloric densities (3.8 kcal/g) and compositions except for the fat source and the cholesterol content. Diets were formulated to meet National Research Council nutrient requirements for guinea pigs.[19] The 15 g/100 g fat diets contained either lard [polyunsaturated:saturated fat ratio (P:S) = 0.29], olive oil high in MUFA with 72% 18:1, or corn oil (P:S = 4.11), and four levels of recrystallized added cholesterol (basal, 0.01%; low, 0.08%; medium, 0.17%; and high, 0.33% by weight). The basal diets were normalized for the cholesterol content of lard (0.01%) and amounts of absorbed cholesterol equal to 6%, 50%, 100%, and 200% of guinea pig daily endogenous cholesterol synthesis (51 mg/kg/day[5,20]) as previously described.[6,7] Each test diet is referred to by fat type and cholesterol level (i.e., corn-high refers to the corn oil-based diet with 0.33% cholesterol).

Animals

Forty-eight male Hartley guinea pigs from Harlan Sprague-Dawley, Inc. (Indianapolis, IN USA) weighing between 250 and 350 g were randomly assigned to one of 12 dietary groups. Animals were housed under a controlled light cycle (light from 7:00 AM to 7:00 PM) with constant humidity and temperature. After 4 weeks on the test diets to establish steady-state plasma cholesterol levels, nonfasting animals were anesthetized with halothane vapors and blood was obtained by cardiac puncture in syringes containing EDTA (1 mg/mL). Plasma was separated from blood cells by centrifugation for measurement of plasma lipids and lipoprotein, and the livers were excised for analysis of free and esterified cholesterol concentrations and for isolation of hepatic microsomes for analysis of ACAT activity and lipid content. All experiments were conducted in accordance with U.S. Public Health Service and U.S. Department of Agriculture guidelines and were approved by the University of Arizona Institutional Animal Care and Use Committee.

Plasma and hepatic cholesterol assay

After separation from cellular component, plasma samples were mixed with a preservation solution containing sodium azide (1 nmol/mL plasma), aprotonin (50 kallikrein units/mL plasma), and phenyl methyl sulfonyl fluoride (10 pmol/mL plasma),[21] and an aliquot of plasma was used to measure plasma total cholesterol concentrations. Plasma lipoproteins of $d < 1.019$ g/mL including chylomicrons, VLDL, and intermediate density lipoprotein were obtained by sequential ultracentrifugation. Plasma total cholesterol, $d < 1.019$ g/mL lipoprotein cholesterol, and high density lipoprotein (HDL) cholesterol, after precipitating apo-B,E containing lipoprotein with $MgCl_2$,[22] were analyzed using a commercial enzymatic assay kit (Boehringer Mannheim). LDL cholesterol was calculated by subtracting $d < 1.019$ g/mL lipoprotein cholesterol and HDL cholesterol from total cholesterol. Hepatic lipids were extracted according to the method of Folch et al.,[23] and hepatic free and total cholesterol concentrations were measured enzymatically as described by Sale et al.[24] Cholesteryl ester concentrations were determined by subtracting free from total cholesterol values.

Hepatic microsomal cholesterol and PC assay

Hepatic microsomes were isolated as described previously.[25] Microsomal protein content was measured by a modified Lowry assay[26] and microsomal lipids were extracted according to the method of Folch et al.[23] Lipids were extracted from 2 to 3 mg of microsomal protein with 20 vol of chloroform:methanol (2:1), dried under nitrogen, and solubilized in 1 mL H_2O containing 1% Triton X-100. Lipid extracts were transferred to microtiter plates. Microsomal FC and PC were determined using a microtiter assay[27] with cholesterol and choline as standards. The assays were done in duplicates.

Preparation of C/PC liposome

C/PC liposomes were prepared using the method of Garcia-Gonzalez et al.[10] A mixture of cholesterol and egg PC (C/PC, 1:1 mol/mol) was dissolved in sufficient volume of chloroform: methanol (2:1) and dried under nitrogen. The dried lipids were resuspended by incubating at 40°C for 45 minutes in a shaking bath to a final concentration of 8 mmol of lipid in 1 mL microsomal isolation buffer. The lipid-buffer mixture was sonicated on ice for 10 minutes under nitrogen at 50 mHz with a Sonic Dismembrator (Fisher Scientific), followed by centrifugation for 1 hour at 100,000 × g at 4°C in a Ti-50 rotor. The supernatant solution was used as the source of C/PC liposome.

Hepatic ACAT assay

Guinea pig hepatic ACAT activity was assayed as previously described.[24] Microsomal protein (0.8–1.0 mg) was preincubated with BSA (84 mg/mL) and buffer (50 mM KH_2PO_4, 1 M sucrose, 50 mM KCl, 30 mM EDTA, and 50 mM NaF) to a final volume of 0.18 mL at 37°C for 5 minutes. The reaction was initiated by the addition of 20 mL (10 nmoles) of oleoyl-[1-^{14}C]CoA (0.15 GBq/pmol) with incubation at 37°C for 15 minutes. Reactions were terminated by the addition of 5 mL of chloroform:methanol (2:1) and 1 mL of 0.05% H_2SO_4 in H_2O. [^{3}H]Cholesteryl oleate (0.045 GBq per assay) was added as a recovery standard. Samples were mixed and left overnight at room temperature, and the organic phase was collected and dried under nitrogen. Samples were resuspended in chloroform, loaded on glass silica gel TLC plates (Alltech) and developed in hexane:diethyl ether (9:1, v:v). Cholesteryl oleate visualized with iodine vapors was scraped from the TLC plates, solubilized in Liquifluor, and counted in a scintillation counter. Recoveries of the [^{3}H]cholesteryl oleate standard were greater than 75%.

To enrich hepatic microsomes with exogenous cholesterol, 4 mg of microsomal protein were incubated with either C/PC liposomes (3.5 mmol of lipid) or isolation buffer as control (total volume 8 mL in quick seal tubes) at 37°C for 1 hour in a shaking bath. Following incubation, the mixtures were cooled on ice, layered over 3 mL of a 20% sucrose solution, and centrifuged at 100,000 × g for 1 hour. Lipid was removed following tube slicing and the microsomal pellet was resuspended in isolation buffer. Aliquots were taken for analysis of microsomal lipid, protein content, and ACAT activities as described above.

Statistics

Data are presented as mean ± SD. Differences due to the dietary cholesterol amount, fat type, or interactions were determined by two-way analysis of variance (ANOVA).[28] One-way ANOVA was used to compare plasma total and LDL cholesterol of guinea pigs fed the three types of fat. Linear regression was used to assess significant correlations between variables. A *P*-value of less than 0.05 was considered significant.

Results

Plasma total and LDL cholesterol levels

Plasma total and LDL cholesterol concentrations of guinea pigs fed the 12 test diets are presented in *Table 2*. Plasma total cholesterol levels of guinea pigs fed the three types of fat exhibited a dose response to the four levels of dietary cholesterol ($P < 0.001$). Plasma cholesterol concentrations were higher as the level of dietary cholesterol increased (*Table 2*), as indicated by two-way ANOVA. Dietary fat saturation influenced plasma cholesterol levels only at basal and low levels of cholesterol intake as determined by one-way ANOVA (calculations not shown), with the olive oil and lard groups having higher plasma cholesterol levels than the corn oil group at the basal level of cholesterol intake ($P < 0.05$). At medium and high cholesterol intakes dietary cholesterol apparently dictates changes in plasma cholesterol concentration, and dietary fat effects are not significant. There was no significant interaction between dietary cholesterol amount and fat saturation in determining plasma total cholesterol concentrations.

Plasma LDL cholesterol accounts for 70 to 80% of plasma total cholesterol concentrations in all dietary groups

Table 2 Plasma total and LDL cholesterol concentrations of guinea pigs fed semi-purified diets containing 15 g/100 g corn oil, olive oil or lard with 0.01% (basal), 0.08% (low), 0.17% (medium), or 0.33% (high) dietary cholesterol*

	Total (mmol/L)			LDL (mmol/L)		
	Corn oil	Olive oil	Lard	Corn oil	Olive oil	Lard
Cholesterol level						
Basal	1.35 ± 0.10	1.78 ± 0.12	1.62 ± 0.11	0.84 ± 0.06	1.40 ± 0.13	1.10 ± 0.08
Low	2.22 ± 0.39	3.34 ± 0.51	1.76 ± 0.48	1.89 ± 0.32	2.66 ± 0.46	1.25 ± 0.47
Medium	2.73 ± 0.17	3.34 ± 0.61	2.88 ± 0.92	2.26 ± 0.15	2.28 ± 0.54	2.24 ± 1.02
High	5.77 ± 1.75	7.34 ± 2.72	8.52 ± 1.89	4.56 ± 1.23	5.53 ± 2.07	6.89 ± 1.69
Two-way ANOVA†						
Fat		NS			NS	
Cholesterol		$P < 0.001$			$P < 0.001$	
Interaction		NS			NS	

*Values are means ± SD for $n = 4$ guinea pigs per dietary group.
†Differences due to dietary cholesterol, fat type, and interactions were determined by two-way analysis of variance (ANOVA).
LDL–low density lipoprotein.

and changes in plasma LDL cholesterol with dietary intervention paralleled those of total cholesterol.

Hepatic cholesterol concentrations

Both hepatic free and esterified cholesterol concentrations increased as a result of increasing intake of dietary cholesterol ($P < 0.001$; *Table 3*). Significant increases in hepatic FC were found in guinea pigs fed lard and olive oil at low levels of dietary cholesterol and in those fed corn oil at the medium level of cholesterol. Intake of the olive oil diet resulted in the highest hepatic FC content up to the medium cholesterol intake. High cholesterol intakes resulted in 5.5-, 3.3-, and 3.0-fold increases in hepatic FC concentrations of guinea pigs fed corn oil, olive oil, and lard, respectively, compared with the basal level of dietary cholesterol.

Hepatic cholesteryl ester accumulations were evident in all fat groups with cholesterol intake in a dose response manner ($P < 0.001$). Guinea pigs fed the olive oil diet generally had the highest cholesteryl ester concentrations, whereas those fed lard had the lowest values ($P < 0.001$). There were interactions between dietary cholesterol and fat in determining both hepatic free and esterified cholesterol concentrations ($P < 0.001$).

Hepatic microsomal lipid

Hepatic microsomal lipid concentrations of guinea pigs fed the 12 test diets are presented in *Figure 1*. Hepatic microsomal FC concentrations were gradually increased with increasing dietary cholesterol for all fat groups (*Figure 1A*), with significant difference found at the medium level of dietary cholesterol ($P < 0.001$). Intake of the high cholesterol diet resulted in a twofold increase in microsomal FC concentrations, compared with the basal cholesterol diet. Dietary fat type had no significant effects on microsomal FC concentrations and there was no interaction between fat type and cholesterol level.

Incubation of microsomes with C/PC liposomes for 1 hour resulted in an increase in microsomal FC concentrations, with an average threefold increase in the basal and low cholesterol diet groups and a twofold increase in the medium and high cholesterol groups ($P < 0.0001$; *Figure 1A*). There was no fat effect for FC concentrations; however, there was an interactive effect ($P < 0.01$) because microsomes from guinea pigs fed the high cholesterol corn oil diet had the highest values of FC.

Both dietary cholesterol ($P < 0.001$) and fat type ($P < 0.001$) affected microsomal PC concentrations (*Figure 1B*).

Table 3 Hepatic free and esterified cholesterol of guinea pigs fed semi-purified diets containing 15 g/100 g corn oil, olive oil, or lard with 0.01% (basal), 0.08% (low), 0.17% (medium), or 0.33% (high) dietary cholesterol*

	Free (μmol/g)			Esterified (μmol/g)		
	Corn oil	Olive oil	Lard	Corn oil	Olive oil	Lard
Basal	4.70 ± 1.04	5.40 ± 0.91	4.00 ± 0.34	0.93 ± 0.28	1.14 ± 0.38	0.35 ± 0.29
Low	7.60 ± 0.59	11.1 ± 0.32	8.10 ± 0.98	2.84 ± 0.52	7.37 ± 3.72	1.03 ± 0.32
Medium	12.7 ± 3.01	14.2 ± 0.91	9.00 ± 0.62	6.85 ± 2.57	19.8 ± 3.66	1.49 ± 0.53
High	25.8 ± 3.02	17.9 ± 3.39	12.2 ± 1.50	15.4 ± 6.07	19.6 ± 1.39	4.91 ± 0.66
Two-way ANOVA†						
Fat		$P < 0.001$			$P < 0.001$	
Cholesterol		$P < 0.001$			$P < 0.001$	
Interaction		$P < 0.001$			$P < 0.001$	

*Values are means ± SD for $n = 4$ guinea pigs per dietary group.
†Differences due to dietary cholesterol, fat type, and interactions were determined by two-way analysis of variance (ANOVA).

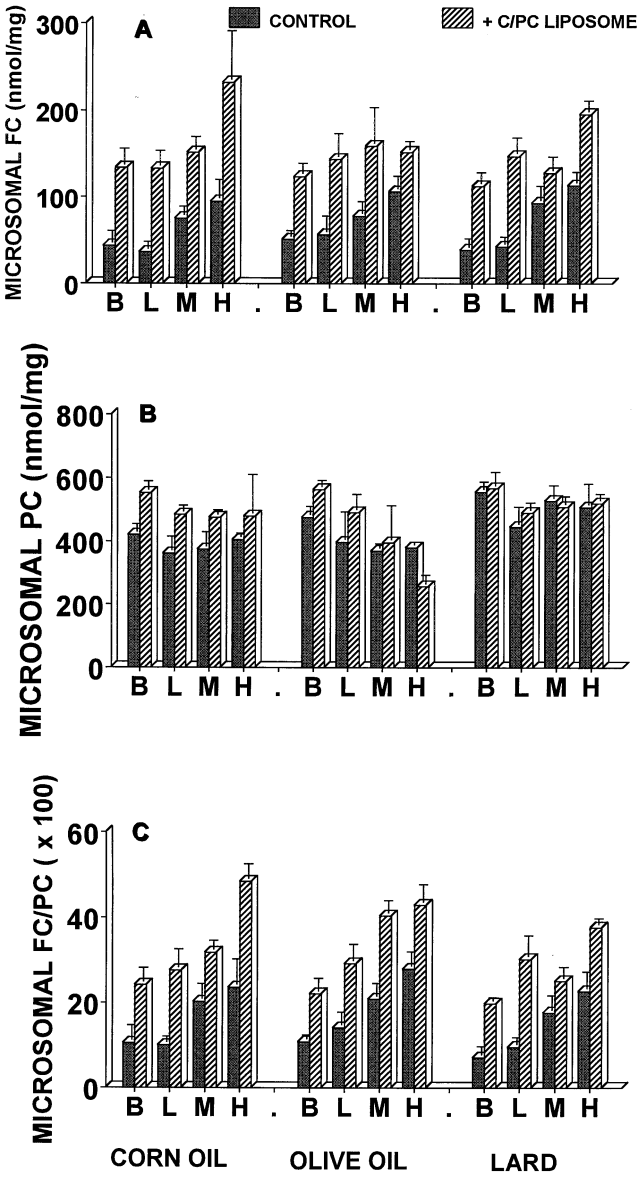

Figure 1 Hepatic microsomal lipid concentrations of guinea pigs fed the 12 test diets with (+ C/PC LIPOSOME) and without (CONTROL) cholesterol/phosphatidylcholine (C/PC) liposome treatment. Semi-purified diets contained either corn oil, olive oil, or lard with 0 (basal, B), 0.08% (low, L), 0.17% (medium, M), or 0.33% (high, H) cholesterol. Microsomes were incubated with C/PC liposomes for 1 hour and the re-isolated microsomes were used to determine lipid content. Values are means ± SD for four guinea pigs per diet group. *(A)* Microsomal free cholesterol (FC) concentrations (nmol/mg). Cholesterol effect for both groups ($P < 0.0001$); interaction effect for the C/PC LIPOSOME groups ($P < 0.01$). *(B)* Microsomal PC concentrations (nmol/mg). For both groups, cholesterol effect ($P < 0.001$) and fat effect ($P < 0.001$); interaction for the C/PC LIPOSOME group ($P < 0.05$). *(C)* Microsomal FC/PC molar ratios. For both groups: cholesterol effect ($P<0.0001$) and fat effect ($P<0.001$); interaction effect for C/PC LIPOSOME group ($P < 0.001$).

Basal dietary cholesterol resulted in the highest PC concentration for all fat groups, with the olive oil and lard groups having the greatest values. Guinea pigs fed lard had the highest microsomal PC concentrations ($P < 0.001$). No interactive effects were present.

Table 4 Hepatic ACAT activity of guinea pigs fed semi-purified diets containing 15% (wt/wt) fat, either corn oil, olive oil, or lard with 0.01% (basal), 0.08% (low), 0.17% (medium), or 0.33% (high) cholesterol*

	Corn oil	Olive oil	Lard
Cholesterol			
Basal	16.1 ± 3.1	33.0 ± 9.7	11.6 ± 1.2[a†]
Low	29.3 ± 10.0	117.6 ± 71.5	35.0 ± 14.8[a†]
Medium	132.3 ± 58.7	191.9 ± 31.2	91.6 ± 46.0[b†]
High	218.5 ± 43.7	219.9 ± 34.0	211.2 ± 39.2[c]
Two-way ANOVA†			
Fat saturation		$P = 0.001$	
Cholesterol		$P < 0.001$	
Interaction		NS	

ACAT–acyl-coenzyme: cholesterol acyltransferase.
*Values, measured in pmol/min/mg, are means ± SD for $n = 4$ guinea pigs per diet group.
†Differences due to dietary cholesterol, dietary fat type, and interaction of dietary cholesterol level and fat type were determined by two-way analysis of variance (ANOVA).

Incubation of microsomes with C/PC liposomes increased microsomal PC concentrations (*Figure 1B*) except in the olive-high and lard-medium diet groups (interactive effect, $P < 0.05$). PC concentrations were greater with lower amounts of dietary cholesterol ($P < 0.0001$) and lowest for the olive oil group ($P < 0.01$).

The FC/PC ratios increased as the amount of cholesterol in the diets increased ($P < 0.001$), with guinea pigs fed the olive oil diets generally having the highest FC/PC molar ratios compared with those fed corn oil or lard diets ($P < 0.001$; *Figure 1C*). Ratios were increased after incubation with C/PC liposomes.

Hepatic ACAT activity

Guinea pigs fed increasing amounts of dietary cholesterol had significant increases in hepatic microsomal ACAT activity in a dose response manner for all three dietary fat groups (*Table 4*). Significant increases in hepatic ACAT activity were found in guinea pigs fed the medium or higher levels of cholesterol with corn oil and lard diets, and at the low cholesterol intake in animals fed olive oil. High cholesterol intakes resulted in 14-, 7-, and 18-fold increases in hepatic microsomal ACAT activity in animals fed corn oil, olive oil, and lard, respectively, compared with the basal cholesterol diet. Guinea pigs fed the olive oil diet had the highest hepatic ACAT activity for all but the high level of dietary cholesterol ($P < 0.001$). No interactions of dietary fat type and dietary cholesterol amount were detected in determining hepatic ACAT activity.

Microsomal ACAT activities were decreased 24 to 60% when incubated with buffer, although there were no changes in microsomal lipid concentrations (data not shown). The percent decreases were highest for microsomes isolated from guinea pigs fed the highest levels of cholesterol. Based on these data, it is evident that the use of a buffer control is needed to make comparisons of ACAT activities between control and C/PC liposome treatment. ACAT activities following incubation of microsomes with either buffer or C/PC liposome are presented in *Figure 2*. C/PC liposome

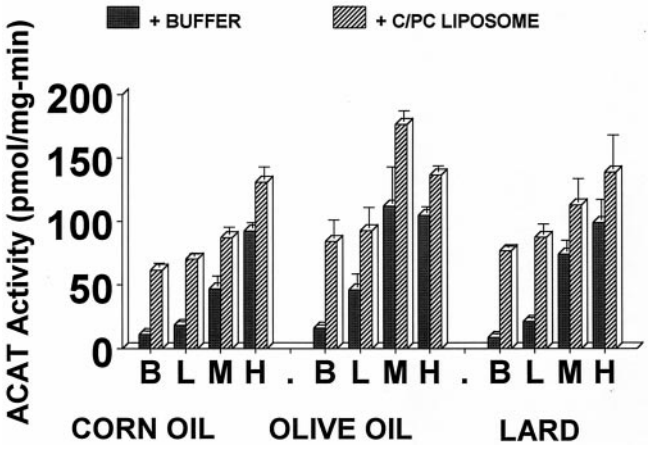

Figure 2 Hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activities following treatment of microsomes with buffer or cholesterol/phosphatidylcholine (C/PC) liposomes. Semi-purified diets contained either corn oil, olive oil, or lard with 0 (basal, B), 0.08% (low, L), 0.17% (medium, M), or 0.33% (high, H) cholesterol. Microsomes were incubated with either C/PC liposomes or isolation buffer for 1 hour and the reisolated microsomes were used to determine ACAT activity. Values are mean ± SD for four guinea pigs per diet group.

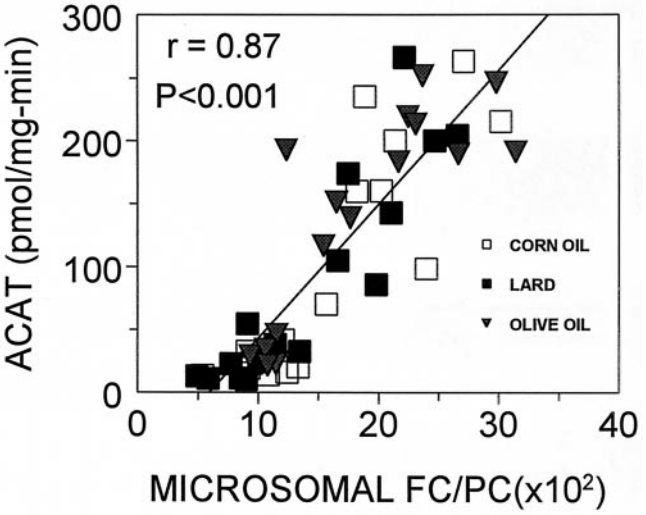

Figure 3 Correlation between hepatic microsomal free cholesterol/phosphatidylcholine molar ratios and hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activities of guinea pigs fed corn oil, olive oil, or lard with 0 (basal), 0.08% (low), 0.17% (medium), or 0.33% (high) cholesterol ($r = 0.87$, $N = 48$, $P < 0.001$, $y = 10.6x - 64.0$).

treatment resulted in significant increases in hepatic microsomal ACAT activity compared with controls for all diet groups ($P < 0.001$). Higher percentage increases in ACAT activities with C/PC liposome treatment were found in all low cholesterol groups. Dietary cholesterol amount resulted in a gradual increase in ACAT activity in both control and C/PC liposome treated groups ($P < 0.001$). Guinea pigs fed the olive oil diets had the highest expressed ACAT activity under all test conditions ($P < 0.001$).

Correlations

Correlation between microsomal lipid concentrations and ACAT activities were measured to determine the effects of substrate availability, specifically microsomal FC, on ACAT activities. Microsomal FC concentrations were positively correlated with hepatic ACAT activities in guinea pigs fed the 12 test diets ($r = 0.81$, $N = 48$, $P < 0.001$). Interestingly, there was a slightly higher correlation between the microsomal FC/PC molar ratios and ACAT activities ($r = 0.87$, $N = 48$, $P < 0.001$; *Figure 3*).

After C/PC liposome treatment, microsomal FC concentrations remained significantly correlated with ACAT activities ($r = 0.55$, $N = 48$, $P < 0.001$), and again a higher correlation was found between microsomal FC/PC molar ratios and ACAT activities ($r = 0.70$, $N = 48$, $P < 0.001$) (*Figure 4*). All correlations exhibited linear relationships and no effects of dietary fat type on these correlations were noted.

Discussion

In this and our previous studies,[6,7] increasing dietary cholesterol within a physiologic range resulted in modest increases in plasma LDL cholesterol concentrations whereas significant increases occur with pharmacologic levels of dietary cholesterol (0.33%). These data have been interpreted as a demonstration that plasma LDL homeostasis is well regulated, with the limitation being that absorbed dietary cholesterol input remains below the mass of endogenous cholesterol synthesis.[7] The dose-response increases in plasma LDL levels with increases in dietary cholesterol are associated with decreased expression of hepatic LDL receptors.[7,29]

Carr et al.[30] have postulated a role of ACAT in incorpo-

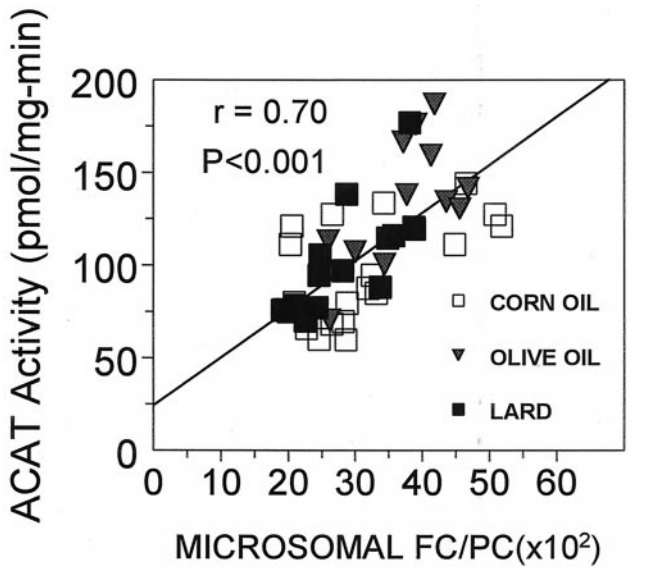

Figure 4 Correlation between hepatic microsomal free cholesterol/phosphatidylcholine (FC/PC) molar ratios and hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activities of guinea pigs fed corn oil, olive oil, or lard with 0 (basal), 0.08% (low), 0.17% (medium), or 0.33% (high) cholesterol after C/PC liposome treatment ($r = 0.70$, $N = 48$, $P < 0.001$, $y = 2.6x + 24.0$).

rating cholesteryl ester into newly secreted apo-B containing lipoproteins in African green monkeys. In agreement with this hypothesis, increases in hepatic ACAT activity with increasing doses of dietary cholesterol were significantly correlated ($r = 0.85$, $P < 0.01$) with increases in plasma LDL cholesterol concentrations in this study. On the other hand, it has been proposed by Daumerie et al.[31] that dietary fat saturation and dietary cholesterol have differential effects on hepatic LDL catabolism due to specific effects on the availability of FC for the putative cholesterol regulatory pool.[7,29] As ACAT activity changes with dietary fat saturation, the availability of FC to equilibrate with the regulatory pool either increases or decreases, which results in modifications of hepatic apo-B,E receptor-mediated LDL catabolism. With dietary cholesterol the regulatory pool is increased and LDL receptor expression is suppressed, resulting in decreased LDL catabolism and higher plasma cholesterol levels.[7,29]

The present study is an extension of our previous observations that sought to define the interactions between dietary cholesterol and fat saturation on the regulation of hepatic ACAT activity and how changes in enzyme activity are related to microsomal lipid composition. Modifications of microsomal lipid concentrations with C/PC liposome treatment also were measured in an attempt to differentiate whether observed ACAT activities are due to changes in enzyme levels or changes in substrate availability.

Dietary cholesterol and hepatic ACAT activity

ACAT is an integrated enzyme in the rough endoplasmic reticular membrane,[32] and the evidence that increments in ACAT activity are associated with high cholesterol diets and with addition of exogenous cholesterol to the microsomal fraction support the hypothesis that microsomal ACAT is not saturated with its cholesterol substrate under physiologic conditions.[33,34] Therefore, it is possible that the role of the cholesterol substrate pool in regulating ACAT activity could be as significant as actual changes in the amount of ACAT enzyme.

In the present study we found that a range of dietary cholesterol intakes increased the microsomal FC concentration in a dose-dependent manner. As predicted, there was a significant increase in hepatic ACAT activity with increasing amounts of dietary cholesterol as well as a significant positive correlation between microsomal FC and hepatic ACAT activities. Nonesterified cholesterol and phospholipids are the major lipid constituents for biological membrane structure, with nonesterified cholesterol accounting for 10 to 12% of lipids in endoplasmic reticulum membranes,[35] and PC is the major membrane phospholipid. Therefore, the microsomal FC/PC molar ratio can be used as an indicator of cholesterol substrate availability for ACAT. Our data suggest that the hepatic ACAT cholesterol substrate pool is influenced by dietary cholesterol over a wide physiologic range in a dose-dependent manner, presumably due to increases in the influx of dietary cholesterol to the liver.[36] These data support the concept that hepatic ACAT activity is regulated by the availability of FC substrate even when expressed as a microsomal FC/PC molar ratio.

Dietary fat saturation and hepatic ACAT activity

Dietary fat saturation exhibited independent effects on both hepatic microsomal cholesterol content and ACAT activity. Intake of the olive oil diet resulted in the highest microsomal FC/PC molar ratio and hepatic ACAT activity as compared with intake of the corn oil and lard diets, a finding that is consistent with reports of hepatic cholesterol accumulation in guinea pigs,[27] rats,[37] and hamsters[38] fed diets containing olive oil. A previous study demonstrated that excretion of fecal acidic sterols was decreased significantly in guinea pigs fed an olive oil diet compared with corn oil or lard diets,[18] which could partially explain the accumulation of cholesterol in the liver.

Polyunsaturated corn oil versus saturated lard diets with a range of dietary cholesterol levels did not cause differences in the microsomal FC/PC molar ratio or hepatic ACAT activities, and there was no interaction between dietary cholesterol and fat in determining hepatic ACAT activity. These observations suggest that dietary fat saturation can regulate hepatic ACAT activity through changes in substrate availability when estimated as microsomal FC/PC molar ratio.

It has been reported that rats fed diets enriched with PUFA had higher ACAT activity than those fed SFA.[14,39,40] However, the opposite effect was observed in guinea pigs: Intake of saturated palm kernel oil resulted in higher hepatic ACAT activities than intake of a polyunsaturated corn oil diet,[25] which is in agreement with the observations in African green monkeys in which higher hepatic ACAT activity correlated with larger, cholesteryl ester-enriched particles associated with atherosclerosis.[30] These different results could be due to species specificity in that the rate of cholesteryl ester synthesis is higher in rats than in guinea pigs or African green monkeys.[11,25,30]

In the present study, the higher hepatic cholesterol concentrations found in guinea pigs fed polyunsaturated corn oil with cholesterol compared with saturated lard could not be due to changes in hepatic ACAT activity but rather to a combination of decreased VLDL production and increased LDL cholesterol input[5] or possibly to decreased cholesteryl ester hydrolysis by the action of cytoplasmic cholesteryl ester hydrolase.[41]

C/PC liposome treatment and hepatic ACAT activity

Incubation of hepatic microsomes with C/PC liposome resulted in significant increases in ACAT activities compared with buffer treatment as a control. Higher ACAT activities with C/PC liposome treatment were found for all low cholesterol fed groups, which could be related to the addition of exogenous cholesterol from C/PC liposome to microsomes. A significant correlation between the microsomal FC/PC molar ratios and hepatic ACAT activities after C/CP liposome treatment is consistent with the regulation of ACAT activity by cholesterol substrate availability.

Increases in microsomal PC content with PC liposome treatment have been shown to be associated with a decrease in ACAT activity.[10,12] This decrease in ACAT activity may result from a decrease in the FC/PC molar ratio and thus a decrease in substrate availability. The other possible expla-

nation could be modulation of ACAT activity by changes in membrane phospholipid composition.[42]

The modification of microsomal lipid compositions with C/PC liposome has been used in this study to determine the molecular mechanism regulating microsomal ACAT activity. This method, however, could have some shortcomings. For example, the high microsomal FC/PC molar ratio up to 1:2 after C/PC liposome treatment may never occur in vivo under physiologic conditions. In addition, microsomal ACAT activities decreased by 24 to 60% after incubation with microsomal isolation buffer for 1 hour.

One study in rats also reported decreases in ACAT activity after incubation with buffer[43]; however, in another study rat hepatic microsomal ACAT activity increased up to threefold following incubation with 0.15 M NaCl for 50 minutes.[12] These discrepancies might be related to the various buffer components in these assays.

In this study, a higher relative decrease in microsomal ACAT activities after buffer treatment was found, especially in the high cholesterol groups. Possible explanations are that these procedures could change the microsomal ACAT environment or the enzyme itself and influence substrate availability by altering the supply of FC to the enzyme. It is also possible that degradation of ACAT occurred during these treatments through the action of proteases.[44]

Dietary fat saturation and amount of ACAT enzyme

Jackson et al.[36] found that addition of cholesterol with liposome treatment to microsomes from hamsters fed 20% coconut oil did not increase ACAT activity to the level of a control group fed regular chow, suggesting the possibility of a lower amount of ACAT enzyme related with the coconut oil diet. However, coconut oil has a high percentage of lauric acid (12:0) and myristic acid (14:0) and microsomal membranes from animals fed coconut oil presumably are more rigid than those from the chow-fed group. Thus, less cholesterol from cholesterol-rich liposome could incorporate into the microsomes. Therefore, without information of microsomal lipid content of the membranes from the coconut oil group, it is difficult to distinguish the effects of substrate availability versus enzyme levels on ACAT activity.

In our study, we could not find significant effects of dietary fat on the correlation between microsomal FC/PC molar ratio and hepatic ACAT activity even after we modified the microsome lipid composition with C/PC liposome treatment. These findings suggest that posttranscriptional regulation, especially the availability of cholesterol, rather than changes in the amount of enzyme, is the major determinant of ACAT activity under these conditions.

Conclusion

Our findings do not support the hypotheses that the stimulation of hepatic ACAT activity with cholesterol intake is enhanced by PUFA intake or that the amount of hepatic microsomal ACAT enzyme is induced by dietary fat saturation with a range of dietary cholesterol. The data do demonstrate that hepatic ACAT activity is affected by dietary intervention, and that whereas dietary fat type and cholesterol amount have differential effects on hepatic ACAT activity, substrate availability, expressed as microsomal FC/PC molar ratio, serves a major regulator of hepatic microsomal ACAT activity.

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