Effect of dietary fat saturation on acylcoenzyme A: cholesterol acyltransferase activity of Ehrlich cell microsomes

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Abstract Ehrlich cells grown in mice fed coconut oil diets (highly saturated) contain about twice as much cholesteryl ester as those grown in mice fed sunflower oil diets (highly polyunsaturated). Acylcoenzyme A: cholesterol acyltransferase (ACAT) activity was 30–100% higher in microsomes prepared from the cells grown on coconut oil (M_c) than in those prepared from the cells grown on sunflower oil (M_s). Increased ACAT activity was noted in M_c with either [1-^{14}C]palmitoyl CoA or [1,2-^{3}H]cholesterol as the labeled substrate. This occurred at all acyl CoA concentrations tested and, in the [1,2-^{3}H]cholesterol assay, with palmitoyl, oleoyl, or linoleoyl CoA as the substrate. The pH optimum for ACAT activity was the same with M_c and M_s, pH 7.0. ACAT activity obeyed Michaelis–Menten kinetics at palmitoyl CoA concentrations between 1 and 10 μM. Substrate inhibition occurred at higher concentrations. Kinetic analysis with [1-^{14}C]palmitoyl CoA as the substrate indicated that the apparent K_m for M_c was 33% smaller than for M_s. There was no difference, however, in apparent V_{max} values. The cholesterol and phospholipid contents of M_c and M_s were similar, but their fatty acid compositions differed considerably. M_c contained 2.7 times more monoenoic fatty acid and only half as much polyenoic fatty acid as M_s. Our results indicate that dietary modification of the microsomal fatty acid composition is associated with alterations in the activity of ACAT, an enzyme that is tightly bound to the microsomes. These changes in ACAT activity may be partly responsible for the differences in cholesteryl ester contents of Ehrlich cells grown in mice fed the coconut and sunflower oil diets.

Supplementary key words cholesteryl esters · fatty acids · phospholipids · membranes.

Acylcoenzyme A: cholesterol acyltransferase (ACAT) catalyzes the synthesis of cholesteryl esters in mammalian cells (1). ACAT is tightly bound to membranes, and it is recovered predominantly in the microsomes when subcellular fractions are prepared from homogenates of animal tissues (1, 2). Long-chain acylcoenzyme A thioesters supply the fatty acids utilized for cholesteryl ester synthesis in the ACAT reaction (1, 3, 4). This is in contrast to the acyltransferase that catalyzes the synthesis of cholesteryl esters in plasma lipoproteins, which directly transfer the fatty acyl group from lecithin to cholesterol (5, 6). Studies with human skin fibroblasts indicate that ACAT catalyzes the esterification of the cholesterol that is taken up as a result of low density lipoprotein binding to cell membrane receptors (7). The ACAT activity in these fibroblasts is subject to regulation and is increased markedly when the cells are exposed to oxygenated sterols (8).

Similar to other cell lines (9), Ehrlich ascites tumor cells exhibit a rapid turnover of membrane phospholipids, particularly of the choline and ethanolamine phosphoglycerides (10). In addition, de novo fatty acid synthesis in Ehrlich cells is depressed when the cells are exposed to either fatty acids bound to albumin or triglyceride-rich lipoproteins (11–13). This suggested that the fatty acid composition of Ehrlich cell membranes might be susceptible to alteration by exposing the cells during rapid growth to different types of fatty acids. Since the Ehrlich cells grow as a suspension in the peritoneal cavity of mice, the simplest way to achieve this was to feed the animals diets containing different fats (14,15). In the course of these studies, we made a preliminary observation that the cholesteryl ester content was increased in cells containing a higher percentage of saturated and monoenoic fatty acids (16). Because of the potential importance of this finding to the general problem of intracellular cholesteryl ester accumulation, we felt that this observation warranted additional exploration.

Recent evidence indicates that the activity of certain membrane-bound enzymes can be modified by changes in the lipid composition of the membrane

Abbreviations: ACAT, acylcoenzyme A: cholesterol acyltransferase; M_c, microsomes isolated from the cells grown on coconut oil; M_s, microsomes isolated from the cells grown on sunflower oil.

in which they are embedded (17–20). In this context, we noted that the activation energy of the Na^+/K^+-dependent ATPase of the Ehrlich cell was associated with changes in the fatty acid composition of the Ehrlich cell plasma membrane (21). Because of the differences observed in cellular cholesteryl ester content (16), we examined the question of whether changes in the microsomal fatty acid composition might be associated with similar types of changes in ACAT activity. This communication describes our finding that changes in ACAT activity accompany dietary modification of the fatty acid composition of the Ehrlich cells. A preliminary report of these observations has been presented (22)[1].

MATERIALS AND METHODS

Animals, diets, and cell preparation

Male CBA mice weighing approximately 15 g were placed on a semisynthetic diet containing 26% casein, 10% corn starch, 43% sucrose, 4% mineral mix, and 1% vitamin mix (Teklad, Madison, WI). This nutrient mixture was supplemented with either 16% coconut or 16% sunflower oil and blended. The fatty acid composition of these two diets has been reported (15). Mice were fed one of these diets for 4 weeks prior to inoculation of the Ehrlich cells and were maintained on the diet during the subsequent 14-day period of tumor growth. The contents of the peritoneal cavity were then removed by aspiration, and the Ehrlich cells were isolated by centrifugation (11). After the cells were washed three times (11), they were suspended in a solution adjusted to pH 7.4 containing 250 mM sucrose, 10 mM K_2HPO_4 and 0.05 mM EDTA.

Preparation of microsomes

Homogenates of the Ehrlich cell suspension were prepared by sonification. The sucrose solution, which was adjusted to contain 1×10^9 cells/25 ml, was kept in an ice bath at 0°C. A Branson sonifier fitted with a large probe was employed. It was set at maximum intensity, and four bursts lasting 15 sec each were required to completely disrupt the cell suspension. After dispersion with a Vortex mixer, the homogenate was subjected to two preliminary centrifugations, 10,000 *g* for 10 min at 4°C, followed by 20,000 *g* for 10 min at 4°C, to remove heavier particles. The resulting supernatant solution was filtered through glass wool and then centrifuged at 104,000 *g* for 60 min at 4°C. After the pellet was resuspended in fresh sucrose buffer, the microsomes were sedimented again by centrifugation and then dispersed in a solution containing 0.1 M K_2HPO_4, pH 7.4, and 1 mM dithiothreitol. This solution was adjusted to contain 5 mg/ml of microsomal protein as measured by the biuret method (23).

ACAT activity

Two isotopic methods were employed to measure ACAT activity in the microsomal preparations. One involved [1-^{14}C]palmitoyl CoA of known specific activity as the labeled substrate. The other involved labeling the microsomes with [1,2-^{3}H]cholesterol and then adding unlabeled acyl CoA as the second substrate.

Labeling with cholesterol was accomplished by placing 150 μCi of [1,2-^{3}H]cholesterol (New England Nuclear Corp., Boston, MA) dissolved in hexane into a siliconized flask. After evaporating the hexane under N_2, 50 μl of 95% ethanol and 7 mg of defatted bovine serum albumin dissolved in 10 ml of the K_2HPO_4 buffer were added. This mixture was stirred for 3 hr at 25°C. The freshly prepared microsome solution containing between 50 and 70 mg of protein was added and the mixture was incubated for 20 min at 25°C with the labeled cholesterol solution. Initial experiments revealed that there was no appreciable increase in [1,2-^{3}H]cholesterol incorporation into the microsomes if the incubation was continued for 90 min. After labeling, the microsomes were sedimented by centrifugation at 104,000 *g* for 1 hr at 4°C, washed by recentrifugation, and resuspended in fresh K_2HPO_4 buffer. The distribution of the [1,2-^{3}H]cholesterol radioactivity between the free and esterified cholesterol fractions of the microsomes was determined by TLC (24). In comparative experiments done with ^{3}H-labeled microsomes prepared from cells grown on sunflower or coconut oil (Fig. 2 and Tables 4 and 5), the specific radioactivity of the unesterified cholesterol in the two microsomal preparations varied by no more than 7%.

Unless noted otherwise in the legends of the tables and figures, the incubation mixtures contained 0.5 mg of microsomal protein, 12 μM acyl CoA, 0.1 M K_2HPO_4 adjusted to pH 7.4, and 1 mM dithiothreitol in a total volume of 0.5 ml. When acyl CoA was employed as the isotopic substrate, the medium contained between 7×10^4 and 1.5×10^5 dpm of [1-^{14}C]palmitoyl CoA and 6 nmol of palmitoyl CoA. No cholesterol was added to these assays, and the inherent cholesterol in the microsomes served as the second substrate. When cholesterol was employed as the isotopic tracer, the microsomes contained about 2.5×10^5 dpm of [1,2-^{3}H]cholesterol

[1] Presented to the Council on Arteriosclerosis, American Heart Association, Miami Beach, Florida on November 17, 1976.

per 0.5 mg of protein. Incubation usually was carried out for 5 min at 37°C with shaking. It was terminated by the addition of 2 ml of a chloroform–methanol mixture 2:1 (v/v) followed by vigorous agitation using a Vortex mixer. The mixture was transferred quantitatively to a separatory funnel and the chloroform phase was isolated following addition of 0.04 N HCl. An aliquot of the chloroform solution was taken for measurement of lipid radioactivity, and additional aliquots of the chloroform solution were taken for thin-layer chromatography on silica gel G in order to separate the lipid components (24). A solvent system consisting of hexane–diethyl ether–methanol–acetic acid 180:40:4:6 was used, and standards obtained from Applied Science Laboratories (State College, PA) were added to each chromatogram. Lipids were visualized by exposure of the chromatographic plate to I_2 vapor.

The outlined segments of silica gel were scraped directly into liquid scintillation vials containing 15 ml of a dioxane-based scintillator solution (25). Measurements of radioactivity were made in a Packard Tri-Carb model 2425 refrigerated spectrometer (Packard Instruments, Downers Grove, IL), and quenching was monitored with a ^{226}Ra external standard as well as by channels ratio counting. These tests indicated that it was not necessary to correct for quenching in any of the experiments. In experiments with [1-^{14}C]palmitoyl CoA as the tracer, the synthesis of cholesteryl esters was expressed as pmol/mg microsomal protein using the specific activity of the added palmitoyl CoA substrate. When [1,2-^{3}H]cholesterol served as the isotopic tracer, an aliquot of the microsomal preparation was taken for analysis prior to incubation in order to determine the amount of radioactivity present as cholesteryl ester before the start of the incubation with unlabeled acyl CoA. This always amounted to less than 0.1% of the cholesterol radioactivity contained in the microsomes at the beginning of the incubation. These small amounts of baseline cholesteryl ester radioactivity were subtracted from the values obtained after the microsomes were incubated with acyl CoA. Control incubations without added acyl CoA also were carried out and indicated little increase in radioactive cholesteryl ester formation over the baseline values.

Lipid analyses

The fatty acid composition of the microsomal preparations was determined by gas–liquid chromatography. After extraction with the chloroform–methanol mixture, the microsomal lipids were saponified and methylated (12). The fatty acid methyl esters were isolated and chromatographed on a 6 ft × 0.4 mm ID glass column containing 10% Silar 10C on 100–200 mesh Gas Chrom Q obtained from Applied Science Laboratories. A Hewlett Packard (Palo Alto, CA) 5710 A gas chromatograph equipped with flame ionization detector and a 3380 A integrator was used. N_2 served as the carrier gas, the flow rate being 60 ml/min. The fatty acid methyl ester standards employed were obtained from Applied Science Laboratories and Supelco (Bellefonte, PA).

Total cholesterol was measured by the method of Abell et al. (26), and the unesterified fraction was determined as described by Schoenheimer and Sperry (27). Phospholipids were measured by the method of Raheja et al. (28). Triglycerides were measured with the Technicon Auto Analyzer II method developed by the Lipid Research Clinics Program (29).

RESULTS

Cell lipid content

Earlier preliminary results indicated that Ehrlich cells grown in mice fed diets high in saturated fat contained more cholesteryl esters than those grown in mice fed diets high in polyunsaturated fat (16). More detailed studies with the coconut and sunflower oil diets confirmed this observation. As shown in **Table 1**, the cholesteryl ester content of the cells grown on the coconut oil diet was about twice as large as that of the cells grown on the sunflower oil diet. Similar changes were observed when the cells were grown in the mice for either 11 or 14 days. By contrast, there were no comparable changes in the unesterified cholesterol, phospholipid, or triglyceride contents of the cells.

Microsomal lipids

Table 2 shows the difference in fatty acid composition of the microsomal fractions isolated from the cells grown in mice fed either the coconut oil or sunflower oil diets. Large differences were observed in their monoenoic and polyenoic fatty acid contents. The microsomes from the cells grown on coconut oil (M_c) contained 2.7 times more monoenoic fatty acid but only half as much polyenoic fatty acid as the microsomes from the cells grown on sunflower oil (M_s). Although small percentage differences occurred in many of the unsaturated fatty acids, the major changes were in the 18:1 and 18:2 fractions[2]. In spite of the large increases in 18:2, 20:2, and 22:4

[2] The fatty acids are abbreviated as chain length: number of double bonds.

TABLE 1. Effect of dietary fat on the lipid content of Ehrlich cells[a]

Dietary Fat	Days After Transplantation	Cell Lipid Content[b]			
		Cholesteryl Esters	Unesterified Cholesterol	Phospholipids	Triglycerides
		μg/10⁸ cells			
Sunflower oil	11	116 ± 8	314 ± 12	2140 ± 63	1350 ± 63
Coconut oil	11	254 ± 34[c]	325 ± 17	2480 ± 65[c]	1400 ± 83
Sunflower oil	14	135 ± 15	313 ± 17	2400 ± 93	1510 ± 120
Coconut oil	14	252 ± 18[c]	366 ± 18	2580 ± 71	1370 ± 63

[a] These analyses were made on the chloroform–methanol extracts of intact Ehrlich cells.
[b] Each value is the mean ± SE of six separate cell preparations.
[c] The difference in the mean values on this day are significantly different at the $P < 0.01$ level.

in M_s, their 20:4 content was about the same as that of the M_c. There was very little difference in the saturated fatty acid contents of the two microsomal preparations; if anything, M_c contained slightly less total saturated fatty acids than M_s. The average number of double bonds per fatty acid was larger in M_s, 1.65 as compared with 1.29, a 28% increase. Although the average chain length of a fatty acid also was greater in M_s, 18.3 as compared with 17.7, the increase was only 3.4%.

As shown in **Table 3**, these changes in microsomal fatty acid composition were not accompanied by any changes in the lipid content of the microsomes. Most of the cholesterol in both M_c and M_s was unesterified. Although the mean unesterified cholesterol value was 7% greater in M_c, the increase was not statistically significant. Only trace amounts of triglycerides were contained in these microsomal preparations.

TABLE 2. Fatty acid composition of microsomes

Fatty Acid	Percentage Composition[a]	
	M_c[b]	M_s
	%	
Classes		
Saturated	30.5	34.2
Monoenoic	43.2	16.0
Polyenoic	26.3	50.0
Major individual acids		
14:0	1.0 ± 0.8	0.4 ± 0.03
16:0	14.0 ± 0.1	14.6 ± 0.2
18:0	15.5 ± 0.5	19.2 ± 0.4
16:1	3.9 ± 0.2	1.5 ± 0.03
18:1	37.7 ± 0.9	14.0 ± 0.3
18:2	7.7 ± 0.3	28.1 ± 0.4
20:2	0.3 ± 0.1	2.5 ± 0.05
18:3	1.6 ± 0.1	0.5 ± 0.1
20:3	4.9 ± 0.5	0.8 ± 0.1
20:4	7.9 ± 0.9	9.4 ± 0.1
22:4	0.8 ± 0.2	3.8 ± 0.1
22:5	1.2 ± 0.6	1.2 ± 0.6
22:6	1.9 ± 0.3	4.2 ± 0.2

[a] Mean ± SE of four separate microsomal preparations.
[b] M_c refers to the microsomal preparations isolated from cells grown in mice fed the coconut oil diet; M_s refers to the microsomal preparations isolated from cells grown in mice fed the sunflower oil diets.

TABLE 3. Lipid composition of microsomes

Lipid Class	Content[a]	
	M_c	M_s
	μg/mg protein	
Phospholipids	105.0 ± 11.0	106.0 ± 9.0
Total cholesterol	19.5 ± 0.9	18.3 ± 0.9
Unesterified cholesterol	18.9 ± 1.1	17.6 ± 1.1

[a] Mean ± SE of seven separate microsomal preparations. None of the differences are statistically significant ($P > 0.1$).

Comparison of ACAT activity

Fig. 1 shows the time course of the ACAT reaction in M_c and M_s preparations with [1-^{14}C]palmitoyl CoA as the labeled substrate. In both cases, the reaction was linear over 10 min. At each time, greater ACAT activity was observed with the M_c preparation, the increases varying between 29 and 97%.

As seen in **Fig. 2**, similar results were obtained when [1,2-^{3}H]cholesterol served as the labeled substrate. The reactions were linear over 10 min, and higher ACAT activity occurred with M_c at each time point, the increases varying from 47 to 82%. Since the cholesterol specific activity was almost identical in both microsomal preparations, these results with labeled cholesterol also indicate that the ACAT activity is higher in M_c. These data also were expressed in terms of pmol of cholesterol esterified, using the microsomal free cholesterol content to calculate the specific radioactivity. The values for M_c and M_s were from 25% to 60% greater than the respective pmol incorporation calculated from the [1-^{14}C]palmitoyl

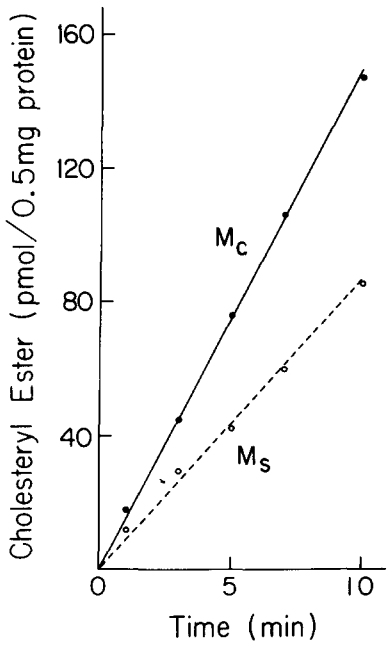

Fig. 1. Time course of the ACAT reaction with [1-^{14}C]palmitoyl CoA as the labeled substrate. Each point is the average of two closely agreeing determinations.

CoA assays. This difference probably is due to compartmentation of the microsomal cholesterol, with incomplete mixing of the added [1,2-^{3}H]cholesterol and the inherent cholesterol of the microsomes (30, 31).

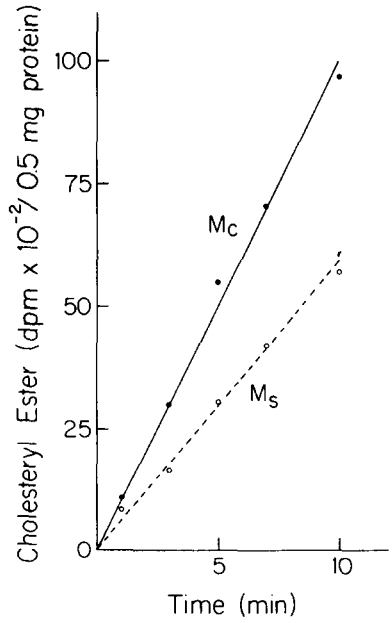

Fig. 2. Time course of the ACAT reaction with [1,2-^{3}H]cholesterol as the labeled substrate. Both microsomal preparations contained almost the same quantities of cholesterol mass and radioactivity at the start of the incubation; M_c contained 2.11 nmol and 1.81×10^5 dpm, and M_s contained 2.25 nmol and 1.83×10^5 dpm. Each point is the average of two closely agreeing determinations.

The effect of microsomal protein concentration on ACAT activity in the [1-^{14}C]palmitoyl CoA assay system is shown in **Fig. 3**. With both M_c and M_s, ACAT activity was linearly related to protein concentration. Higher activities were noted with M_c at each protein concentration, the differences varying from 53 to 120%.

The effect of pH on ACAT activity is shown in **Table 4**. [1,2-^{3}H]cholesterol served as the labeled substrate in this experiment. With both M_c and M_s, the maximal activity occurred at pH 7.0, but there was little difference between the pH 7.0 and 7.5 values. At each pH tested, the value with M_c was greater than that with M_s, the increases varying from 45 to 110%. This broad pH optimum around pH 7 is similar to that observed with rat liver ACAT (1), and there is no peak of activity in the pH 5 range as might be expected if appreciable cholesteryl ester synthesis had occurred through a reversal of the lysosomal cholesteryl esterase reaction (3, 4, 32).

All of the experiments reported to this point were done with palmitoyl CoA, either radioactive or unlabeled, as the substrate. In order to examine the effects of various acyl CoA substrates, experiments were done with [1,2-^{3}H]cholesterol-labeled microsomes to which palmitoyl, oleoyl, or linoleoyl CoA were added. The results are shown in **Table 5**. With each of the acyl CoA substrates, higher ACAT activity was observed with M_c as compared with M_s at every concentration tested. When palmitoyl or

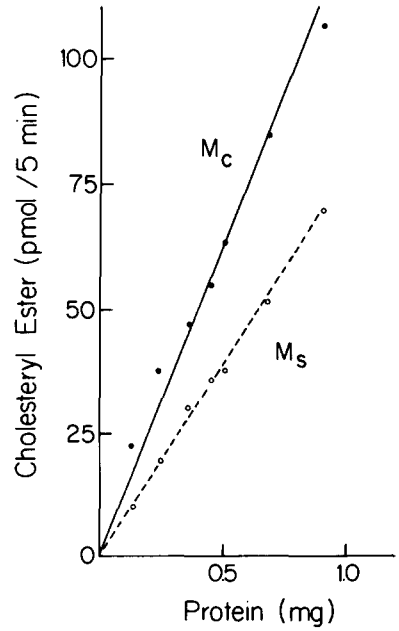

Fig. 3. Effect of microsomal protein concentration on ACAT activity in the [^{14}C]palmitoyl CoA assay system. A 5-min incubation was employed. Each point is the average of two closely agreeing determinations.

oleoyl CoA were present, the incorporation of [1,2-^{3}H]cholesterol into cholesteryl esters increased as the acyl CoA concentration was raised between 1.2 and 6.0 μM. Inhibition was observed at 9 μM with oleoyl CoA and at 15 μM with palmitoyl CoA. By contrast, inhibition began to occur between 3.0 and 6.0 μM with linoleoyl CoA. The maximum incorporations with oleoyl and linoleoyl CoA were in most cases higher than those noted with palmitoyl CoA. At the 6 μM concentration, oleoyl CoA produced the largest incorporation, in agreement with previous studies with rat liver ACAT (1). The maximum incorporations determined in this assay probably are influenced by the inhibitory effects of the various acyl CoA on the reaction. Therefore, they should not be interpreted as maximum velocity measurements for the respective acyl CoA substrates.

ACAT activity also was observed in microsomal preparations labeled with [1,2-^{3}H]cholesterol without addition of acyl CoA to the incubation medium. Maximum activity was achieved in this assay when 1 μM CoA, 10 μM ATP, and 5 μM $MgCl_2$ were added, and very little activity occurred when ATP and CoA were omitted. Apparently, sufficient free fatty acid either was present initially in the microsomal preparations or was generated during the in vitro incubations to permit some acyl CoA synthesis. With M_c, the cholesterol radioactivity that was incorporated into cholesteryl esters in this assay was 1180 ± 24 dpm; it was 670 ± 17 dpm with M_s (mean ± SE of three determinations). The greater ACAT activity noted with M_c is consistent with the previous experiments in which preformed acyl CoA was added as the substrate.

Apparent kinetic parameters

Studies with [1-^{14}C]palmitoyl CoA indicated that ACAT activity increased hyperbolically with respect to acyl CoA concentration until the concentration exceeded 10 μM. As shown in **Fig. 4**, a linear relationship was obtained in a double reciprocal plot in the range of 1.2–10 μM palmitoyl CoA. At 15 μM palmitoyl CoA, substrate inhibition was observed. In an attempt to gain some insight into the mechanism responsible for the greater ACAT activity in M_c, we repeated the concentration dependence experiment five times using palmitoyl CoA concentrations between 1.2 and 10 μM. The double reciprocal plot for each experiment was similar qualitatively to that shown in Fig. 4. Curve fitting was done with a linear regression least squares procedure. In each case, the M_c and M_s curves intersected the vertical axis at approximately the same point and the horizontal axis at different points. The calculated kinetic parameters for palmitoyl CoA are listed in **Table 6**. The average apparent V_{max} values for M_c and M_s were not significantly different. However, the average

TABLE 4. Effect of pH on ACAT activity[a]

pH[b]	Cholesteryl Ester Formation[c]	
	M_c	M_s
	dpm	
5.0	308	212
5.5	664	316
6.0	1300	862
6.5	2060	1380
7.0	2630	1730
7.5	2520	1600
8.0	2330	1370

[a] Incubations were for 5 min, and each tube contained 0.5 mg of microsomal protein. Activity was determined by measuring the incorporation of [1,2-^{3}H]cholesterol into cholesteryl esters. Prior to incubation, the cholesterol and isotope content of each microsomal preparation was similar; the sp act of cholesterol was 10,500 dpm/nmol in M_c and 9,740 dpm/nmol in M_s, a difference of only 7.8%.

[b] Each of the incubation media contained 0.1 M K_2HPO_4 and was adjusted to the required pH with 1 N HCl. There was no measurable difference in pH after the 5 min incubation.

[c] Each value is the average of two closely agreeing determinations.

TABLE 5. Effect of acyl CoA structure on ACAT activity[a]

Acyl CoA Concentration	Cholesteryl Ester Formation[b]					
	Palmitoyl CoA		Oleoyl CoA		Linoleoyl CoA	
	M_c	M_s	M_c	M_s	M_c	M_s
μM	*dpm*					
1.2	729 ± 11	385 ± 7	1340 ± 52	720 ± 12	1430 ± 84	824 ± 20
3.0	1780 ± 30	1120 ± 41	2520 ± 120	1520 ± 28	2740 ± 48	1410 ± 16
6.0	2370 ± 55	1410 ± 58	2900 ± 76	1570 ± 40	2460 ± 24	1280 ± 44

[a] The incubations were for 5 min, and each tube contained 0.5 mg of microsomal protein. The microsomes were labeled with [1,2-^{3}H]cholesterol, the specific activity being 10,500 dpm/nmol cholesterol in M_c and 9,740 dpm/nmol cholesterol in M_s.

[b] Each value is the mean ± SE of four determinations.

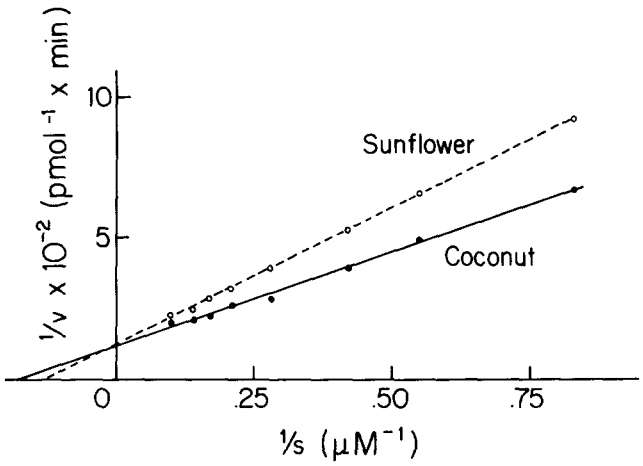

Fig. 4. Double reciprocal plot of microsomal ACAT activity with [1-^{14}C]palmitoyl CoA as the labeled substrate. The palmitoyl CoA concentration was varied between 1.2 and 10 μM, and the microsomal protein content was 0.5 mg. Incubations were for 5 min at 37°C. Each point is the average of two closely agreeing determinations.

apparent K_m value was 33% smaller for the M_c preparations. These are apparent V_{max} and K_m values because the true unbound palmitoyl CoA concentrations are unknown, and there is no certainty that the cholesterol is present in the microsomes in saturating concentrations for ACAT activity.

DISCUSSION

ACAT is thought to occupy a key role in cellular cholesterol metabolism, particularly in the utilization of cholesterol contained in plasma lipoproteins. Studies with cultured human skin fibroblasts indicate that ACAT is involved in the utilization of cholesterol present in low density lipoproteins (33, 34). In this experimental system, low density lipoproteins bind to specific receptors on the cell surface and are taken up by endocytosis (35). The lipoprotein cholesteryl esters then undergo lysosomal hydrolysis, and the released cholesterol is reesterified by the ACAT reaction (7). A similar process appears to be operative in human lymphoid cell lines (36) as well as in freshly isolated human lymphocytes (37) and leukocytes (38). ACAT probably also is involved in the uptake of cholesterol by hepatoma cells in culture (39, 40). These cells accumulate cholesteryl esters when they are exposed to hyperlipemic rabbit serum (39). Much of the accumulated cholesteryl ester is derived from the serum cholesteryl esters, these being hydrolyzed in the uptake process and the released cholesterol then reesterified intracellularly (40). Finally, increased ACAT activity has been reported in the atherosclerotic rabbit aorta (41). Because of its important function in intracellular cholesterol accumulation and possible role in the etiology of atherosclerosis, information concerning the regulation of ACAT activity is of potential interest.

TABLE 6. Apparent kinetic parameters for ACAT activity[a]

Experiment	V'_{max}		K_m'	
	M_c	M_s	M_c	M_s
	pmol/0.5 mg protein × 5 min.		*μM*	
1	86.5	98.1	6.69	10.00
2	70.4	80.4	4.53	8.07
3	99.7	97.1	4.68	6.00
4	48.6	50.4	4.86	6.93
5	84.8	83.7	5.63	8.13
Mean ± SE	78.0 ± 8.7	81.9 ± 8.6	5.27 ± 0.40	7.83 ± 0.67[b]

[a] The experimental procedure was the same as that described in Fig. 4.
[b] $0.01 < P < 0.02$.

The present studies indicate that the type of lipid contained in the diet of the host animal can alter the activity of ACAT in a transplantable ascites tumor cell. Since the coconut and sunflower oil diets were prepared using the same "fat-free" basal ingredients and both contained 16% fat, it appears that the difference in fatty acid saturation was the factor responsible for the change in ACAT activity. Our preliminary studies with intact cells indicated that saturated dietary fats, in addition to producing an increase in intracellular cholesteryl esters, also altered the fatty acid composition of the cholesteryl esters (16). Their oleic acid content increased from 23% to about 35%, and their linoleic acid content decreased from 23% to about 7% when either tristearin or coconut oil was the only dietary source of fat (16).

Work in other systems indicated that oleoyl CoA produced higher rates of ACAT activity than other acyl CoA substrates (1). Studies with intact Ehrlich cells also indicated that oleate was incorporated into cholesteryl esters at slightly higher rates than other long chain fatty acids (24). These data suggested that the increase in ACAT activity when coconut oil was fed might be due simply to the higher percentage of oleate potentially available as substrate, for saturated fat diets increase the oleate content of all of the cell lipids (14). The long chain fatty acyl CoA content of Ehrlich cells is only about 2 nmol/10^8 cells (12). Although this value does not indicate the content in washed microsomes, it suggests that the amount is very small relative to the quantities added for the in vitro assays. This is supported by the observation that very little activity was obtained in these assays when either acyl CoA or ATP and CoA were omitted. Any differences in the inherent acyl CoA

composition of the microsomes would have been masked by the large amount of added acyl CoA. Because of this, it is possible that the cholesteryl ester differences noted in the intact cells (Table 1) could be due, at least in part, to changes in intracellular acyl CoA composition produced by the diets. On the other hand, studies with added acyl CoA substrates indicated that while the highest ACAT activities occurred with oleoyl CoA (Table 5), differences in ACAT activity between M_c and M_s were observed with all of the acyl CoA substrates tested. Therefore, the dietary differences probably produce some change in the ACAT activity itself rather than exerting their effects entirely by altering the composition of the available acyl CoA substrate.

How the changes in dietary fat altered the Ehrlich cell ACAT activity is unknown at this time. One possibility is that greater amounts of enzyme were present in the microsomes of cells grown in the mice fed the coconut oil diet. Based on the kinetic analysis with [1-^{14}C]palmitoyl CoA (Fig. 4 and Table 6), however, there is no difference in the apparent V_{max} between M_c and M_s. This suggests that the total amount of enzyme probably is not greater in M_c. These are apparent V_{max} and K_m values and must be interpreted cautiously. One reason is that our assay utilized the cholesterol inherent in the microsomal preparations as the second substrate for ACAT. Even when [1,2-^{3}H]cholesterol was used, no carrier was added so that only trace amounts of cholesterol were incorporated into the microsomes. Therefore, it is possible that cholesterol was not present in saturating amounts over the entire range of acyl CoA concentrations tested.

Another reason for caution in interpreting the kinetic data is that the palmitoyl CoA substrate, even in the 1–10 μM range, probably exists to some extent in micellar form. Therefore, the monomer concentrations of acyl CoA available to ACAT, if indeed this is the substrate form for the enzyme, are uncertain. A protein carrier was not used in these assays in order to avoid the further complication of bound and free acyl CoA concentrations, but the true substrate concentration of acyl CoA for the ACAT reaction still cannot be determined with certainty.

Finally, Ehrlich cells are known to hydrolyze cholesteryl esters contained in plasma lipoproteins (24). The cholesteryl esterase activity of the cells has not been characterized, and it is possible that the microsomes may have contained some hydrolytic activity operative in the neutral pH range (42). If hydrolysis occurred, our measurements would be in error. We believe that this probably is not a serious problem because the synthetic rates remained linear throughout the brief period of incubation that we employed (Figs. 1 and 2).

We suggest that the K_m effect is related in some way to the difference in fatty acid composition of the two microsomal preparations (Table 2). This might be thought of as a solvent effect of the surrounding phospholipids on ACAT, which is very tightly bound to the microsomes and probably is an integral membrane protein. If this model is correct, the lesser unsaturation of the M_c membrane might in some way optimize the active site and facilitate acyl CoA binding. Fluidity measurements are not available for these microsomes. It is reasonable to assume, however, that the M_s preparation has greater fluidity. Although there is no strict relationship between fluidity and enzyme activity, one might predict that a more fluid membrane structure would permit greater activity (43). This is opposite from what we observed, suggesting that the relationship between membrane lipid structure and ACAT activity probably is very complex. A second possibility is that the approach of acyl CoA to ACAT is somehow facilitated by the lesser unsaturation of the M_c membrane, i.e., acyl CoA may bind more readily to these membranes because of the differences in fatty acyl chain structure. We believe that this latter explanation is less likely because enhanced activity was noted with M_c even when no acyl CoA substrate was added. The concept that membrane lipid structure can influence the activity of enzymes embedded in the lipid bilayer is well established (17–20), and it has been shown to be operative in the Ehrlich cell in the case of the plasma membrane Na^+/K^+-ATPase (21).

Because the cholesteryl ester content of the Ehrlich cell also increases when the mice are fed diets high in saturated fat (Table 1), one may speculate that this is due in part to the increase in ACAT activity. There are, however, several other possible contributing factors. As described earlier, the oleic acid content is considerably higher in the cells grown in mice fed the saturated fat diets (14, 15). Since oleate appears to be the best substrate for ACAT, part of the cholesteryl ester increase in the intact cell may occur on this basis. In addition, we have preliminary evidence that the lipoprotein content is higher in the ascites plasma when the mice are fed the coconut oil as compared with the sunflower oil diet[3]. Since studies with rat hepatoma cells indicate that hyperlipemic serum can produce intracellular cholesteryl ester accumulation (39, 40), the increased lipid con-

[3] Mathur, S. N., and A. A. Spector. Unpublished observations.

tent in the ascites fluid of the mice fed coconut oil also might contribute to the cholesteryl ester increase in the intact cell. Finally, there is no information as to what effect the different lipid diets may have on cholesterol synthesis in Ehrlich cells. For these reasons, it is not possible to establish that the changes in ACAT activity are responsible for the observed differences in intracellular cholesteryl ester content. Independently of the precise molecular mechanism or the actual relation to cell cholesteryl ester content, however, the observation that ACAT activity may be subject to dietary regulation appears of itself a potentially important finding that is worthy of further exploration.

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