Homoeostatic control of membrane cholesterol and fatty acid metabolism in the rat liver

Manohar L. GARG* and John R. SABINE†

Department of Animal Sciences, Waite Agricultural Research Institute, Glen Osmond, S.A., 5064, Australia

Experiments were designed to assess the effect of cholesterol feeding, with or without high levels of either saturated (coconut oil) or unsaturated (sunflower-seed oil) fat on the fatty acid composition of hepatic microsomal membrane lipids, as well as on the activities of several membrane-bound enzymes of cholesterol synthesis and metabolism. Administration of 2% (w/w) cholesterol in the rat diet inhibited hydroxymethylglutaryl-CoA reductase activity, and this inhibition was much more pronounced when cholesterol was fed in combination with unsaturated rather than with saturated fat. Cholesterol 7 α -hydroxylase activity was increased by all the high-cholesterol diets and inhibited by both the high-fat diets. Cholesterol esterification, as assessed by acyl-CoA:cholesterol acyltransferase (ACAT) activity, was enhanced after unsaturated-fat feeding. Cholesterol supplement, without any added fat, failed to elicit any significant increase in ACAT activity, whereas consumption of cholesterol in combination with unsaturated fat led to the greatest increase in ACAT activity. After cholesterol feeding, C_{18:1} and C_{18:2} fatty acids in the microsomal phospholipids were increased, with concomitant decreases in C_{18:0}, C_{20:4} and C_{22:6} fatty acids, leading to an overall decrease in membrane unsaturation, irrespective of the particular fat supplement. It can be concluded that the inhibition of cholesterol biosynthesis and the enhancement of cholesterol utilization, either by increased bile formation or by increased cholesterol esterification, after cholesterol feeding, may not be enough to prevent cholesterol accumulation in the microsomal membranes. Then, to compensate for the altered fluidity resulting from cholesterol enrichment, the unsaturation of membrane phospholipids is decreased, which would in turn have an effect on membrane lipid fluidity opposite to that of increased cholesterol.

INTRODUCTION

Fluidity within the lipid bilayer is a central feature of the mosaic model of a biological membrane [1]. The main chemical determinants of membrane lipid fluidity in their presumed order of significance are the cholesterol content, the degree of saturation/unsaturation of phospholipid acyl chains and phospholipid polar head-group composition [2,3]. Alterations in cellular and/or subcellular lipid components can be found related to some pathological processes, such as myocardial infarction and stress [4], atherosclerosis [5], diabetes [6] and carcinogenesis [7–9]. The concept of membrane homoeostasis [10] implies that such change in lipid composition, and thus in the fluidity of cellular or subcellular membranes, may be a major event occurring in such pathology, under the influence of a variety of etiological factors. Then, with an impaired tendency to restore native fluidity, these membranes may exhibit properties distinctly different from normal membranes, and thus the cell may start showing abnormal metabolic behaviour and loss of structural integrity.

The enquiry described in the present paper is concerned primarily with this question of membrane homoeostasis, i.e. whether or not a biological membrane does possess the tendency to maintain a certain constancy of the physico-chemical status (fluidity) of its lipids in the face of specific external stresses, namely, in this instance,

dietary cholesterol and/or fatty acids. The effect of such treatments on membrane lipid profiles and on the activity of membrane-bound enzymes has been examined. Microsomal membranes have been selected for this purpose, mainly because many of the enzymes of cholesterol and fatty acid metabolism (e.g. HMGR, C7H, ACAT and fatty acyl desaturases) are bound to these membranes.

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MATERIALS AND METHODS

3-Hydroxy-[3-¹⁴C]3-methylglutaryl-CoA was purchased from Amersham International, Amersham, Bucks., U.K., whereas [1-¹⁴C]palmitoyl-CoA and [7-³H(n)]cholesterol were obtained from New England Nuclear, Boston, MA, U.S.A. All the radiochemicals were more than 98% pure and were used without further purification. Unlabelled chemicals and other biochemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Animals and diets

Male, Buffalo-strain (Wistar origin) rats weighing between 200 and 500 g, bred and raised at this Institute, were used throughout the course of this investigation. Six

Abbreviations used: HMGR, hydroxymethylglutaryl-CoA reductase; C7H, cholesterol 7 α -hydroxylase; ACAT, acyl-CoA:cholesterol acyltransferase; REF, reference diet; CH, cholesterol; SSO, sunflower-seed oil; CO, coconut oil; ACH, acyl-CoA hydrolase.

* Present address: Nutrition and Metabolism Research Group, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada.

† To whom correspondence and reprint requests should be addressed.

groups of animals were fed either a standard laboratory chow (reference diet, REF) or this same diet supplemented with either 2% (w/w) cholesterol (REF + CH), 15% (w/w) sunflower-seed oil (SSO), 15% (w/w) sunflower-seed oil plus 2% (w/w) cholesterol (SSO + CH), 15% (w/w) coconut oil (CO) or 15% (w/w) coconut oil plus 2% (w/w) cholesterol (CO + CH). Unless noted otherwise, all rats were fed the diets for a 4-week period, and food and water were provided *ad libitum*. The animals were housed in a well-ventilated room maintained at $22 \pm 2^\circ\text{C}$, with lights on from 0700 to 1900 h. The preparation and fatty acid compositions of these diets have been presented elsewhere [11]. After 4 weeks on experimental diets, the animals were killed (between 0800 and 1000 h), their livers excised quickly and microsomes prepared as explained previously [12] and dispersed in appropriate buffer for enzyme assays. Protein was assayed by the method of Lowry *et al.* [13], with bovine serum albumin as standard.

HMGR assay

HMGR activity was measured essentially as described by Shapiro *et al.* [14]. Microsomal protein (125 μg) was pre-incubated for 5 min at 37°C in 50 μl of 0.25 M-sucrose/5 mM-potassium phosphate/1 mM-EDTA buffer, pH 6.8, containing 0.01 M-dithioerythritol. Then 30 μl of 0.02 M-EDTA/KOH, pH 6.8, containing 5 μmol of glucose 6-phosphate, 0.5 μmol of NADP⁺ and 1 unit of glucose-6-phosphate dehydrogenase was added. After another 5 min, 20 μl of 1 mM-DL-hydroxy[3-¹⁴C]-methylglutaryl-CoA (400 d.p.m./ μmol) was added and the incubations were continued for another 30 min. The reaction was stopped with 25 μl of 4 M-HCl containing 4 μmol of [5-³H]mevalonic acid (500 d.p.m./ μmol) as an internal standard. The mixture was further incubated for at least 15 min at 37°C to permit the mevalonic acid to form its lactone derivative, and then centrifuged for 5 min at 2000 *g* to sediment denatured proteins. A 100 μl portion of this protein-free supernatant was applied directly to activated silica-gel G on t.l.c. plates and developed in a solvent system comprising benzene/acetone (1:1, v/v) and air-dried. The region (R_f 0.5–0.8) containing the mevalonolactone was scraped directly into a scintillation vial and counted for radioactivity with 10 ml of scintillation fluor [5 g of PPO and 0.1 g of POPOP/litre of toluene). Enzyme activity was expressed as pmol of mevalonic acid synthesized/min per mg of microsomal protein.

C7H assay

The microsomal pellet was resuspended in 0.1 M-potassium phosphate buffer, pH 7.3, and C7H was assayed as described by Van Cantfort *et al.* [15]. The final incubation mixture, in 1 ml, contained: 2 mg of microsomal protein, 4 mM-MgCl₂, 1 mM-NADP⁺, 10 mM-glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase, 20 mM-cysteamine and 300 μM (0.5 μCi) of [7(n)-³H]cholesterol. A detergent (Tween 80), 1 mg/assay, was used to solubilize the [7(n)-³H]cholesterol in the incubation medium. After 30 min at 37°C in a shaking water-bath, the reaction was stopped with 0.2 ml of 50% (w/v) trichloroacetic acid.

³H₂O, formed in the above reaction, was isolated by distillation under reduced pressure as described by Hutton *et al.* [16]. A portion of this ³H₂O was counted for radioactivity in 10 ml of Bray's scintillation fluor

containing 5 g of PPO, 0.1 g of POPOP, 80 g of naphthalene in 1 litre of toluene, dioxane and ethanol in the ratio 7:7:4 (by vol.). Enzyme activity was expressed as pmol of cholesterol hydroxylated/min per mg of microsomal protein.

ACAT and ACH assays

ACAT and ACH were assayed simultaneously in the same reaction mixture, essentially by the method of Spector *et al.* [17]. The mixture contained, in a final volume of 0.5 ml: 100–200 μg of microsomal protein, 0.1 M-potassium phosphate buffer, pH 7.2, and 1 mM-dithiothreitol. The reaction was started by the addition of 10 nmol of [1-¹⁴C]palmitoyl-CoA (0.05 μCi). Incubations were carried out for 5 min at 37°C with shaking, and the reaction was terminated with 2 ml of chloroform/methanol (2:1, v/v) containing 0.005% (w/v) butylated hydroxytoluene as an antioxidant.

Lipids from these samples were recovered in the chloroform phase, the extraction method of Folch *et al.* [18] being used. Cholesterol esters and non-esterified-fatty-acid fractions, produced as a result of ACAT and ACH actions respectively, were separated by t.l.c. on silica-gel G plates, using a solvent system comprising light petroleum (b.p. 60–80 $^\circ\text{C}$), diethyl ether, acetic acid (80:20:1, by vol.) as described previously [19]. The zones across the chromatographic plates corresponding to cholesterol esters and non-esterified fatty acids were scraped directly into scintillation vials containing 10 ml of toluene fluor (5 g of PPO, 0.1 g of POPOP in 1 litre of toluene). From the known specific activity of the [1-¹⁴C]palmitoyl-CoA substrate, ACAT activity was expressed as pmol of cholesterol palmitate formed/min per mg of microsomal protein, and ACH activity as pmol of palmitic acid formed/min per mg of microsomal protein.

Fatty acid analysis

Total lipids from the microsomal preparations were extracted by the method of Folch *et al.* [18], and the phospholipids were separated from neutral lipids as described by Nichols [9]. Fatty acid methyl esters were prepared and were analysed by g.l.c., using a 25-metre glass capillary column (LC2/BP1) as described previously [20]. Peak areas were measured automatically with a g.c. terminal (Hewlett-Packard 5840 A) with built-in integrating capacity. Identification of individual fatty acids was made by comparison of their retention times with those of authentic standards.

Statistical analysis

The data are presented as means $\pm$ S.D., and the experimental groups were compared for significance by using Student's *t* test pair-wise.

RESULTS

All the animals appeared healthy after the 4-week experimental period, and there were no significant differences in the mean body weights of rats on different diets, although the rats on the SSO + CH diet had some tendency to gain weight at a higher rate than did all other dietary groups. All the dietary groups studied had similar liver weights, except for the group fed the SSO + CH diet, which had a significantly higher average liver weight

Table 1. HMGR activity in liver microsomes from rats fed various lipid-supplemented diets

Values are the means $\pm$ S.D. for triplicate measurements on liver microsomal preparations from five rats in each dietary group. Statistical significance: ^a, significantly different from the REF-diet or CO-diet animals ($P < 0.05$); ^b, significantly different from each respective group of low-cholesterol-diet animals ($P < 0.001$); ^c, significantly different from the CH + CO-diet animals ($P < 0.05$).

Cholesterol (2%)	Diet...	HMGR activity (pmol of mevalonate formed/min per mg of protein)		
		REF	SSO	CO
—		1120 $\pm$ 126	860 $\pm$ 49 ^a	1068 $\pm$ 99
+		588 $\pm$ 88 ^b	426 $\pm$ 52 ^{b,c}	702 $\pm$ 86 ^b

Table 2. C7H activity in liver microsomes from rats fed various lipid-supplemented diets

Values are the means $\pm$ S.D. for triplicate measurements on liver microsomal preparations from five rats in each dietary group. Statistical significance: ^a, significantly different from the REF diet animals ($*P < 0.05$; $**P < 0.01$); ^b, significantly different from each respective low-cholesterol-diet animals ($P < 0.001$).

Cholesterol (2%)	Diet...	C7H activity (pmol of cholesterol hydroxylated/ min per mg of protein)		
		REF	SSO	CO
—		66.4 $\pm$ 3.2	52.1 $\pm$ 1.7 ^{***}	52.8 $\pm$ 3.4 ^{a*}
+		114.9 $\pm$ 10.3 ^b	99.2 $\pm$ 4.9 ^b	111.6 $\pm$ 12.9 ^b

when compared with its respective low-cholesterol (SSO) diet group.

HMGR

The activities of microsomal HMGR from livers of rats fed low- and high-cholesterol diets, with and without a high level of either saturated or unsaturated fat, are presented in Table 1. HMGR activity was significantly decreased in microsomal preparations from rats fed on the SSO diet, i.e. relative to preparations from rats fed on the CO or REF diet (Table 1). The addition of 2% cholesterol inhibited enzyme activity, irrespective of the amount or type of fat fed in the diet. The level of suppression, however, was greatest when cholesterol was added in combination with SSO (Table 1).

C7H

The addition of either 15% CO or 15% SSO to the REF diet resulted in a lowering of C7H activity (Table 2). Inclusion of 2% cholesterol into these diets enhanced the rate of cholesterol hydroxylation approx. 2-fold, regardless of the quantity or quality of fat also fed in the diet (Table 2).

ACAT and ACH

ACAT and ACH activity in liver microsomes as affected by the lipid-supplemented diets is shown in Table 3. The rate of cholesterol ester formation, as assessed by ACAT activity, was significantly elevated in the liver microsomes from rats fed on 15% SSO, whereas 15% CO had no effect, i.e. in comparison with the low-fat REF diet. Addition of 2% cholesterol to the REF diet failed to produce any significant change in ACAT activity. On the other hand, the feeding of 2% cholesterol

along with SSO or CO significantly raised ACAT activity (Table 3). Within the high-cholesterol high-fat diet groups, the animals on the SSO + CH diet exhibited higher ACAT activity than those on the CO + CH diet, but the difference did not reach a significant level ($P > 0.05$) (Table 3).

ACH activity in the liver microsomes (microsomal fractions) was not significantly affected by any of the experimental diets (Table 3).

Fatty acid composition of microsomal phospholipids

Addition of 2% cholesterol to the otherwise low-cholesterol diets resulted in several distinct alterations in the fatty acid composition of liver microsomal phospholipids (Table 4). Cholesterol feeding led to the accumulation of C_{18:1} fatty acid, with an accompanying depletion of C_{18:0} fatty acid. The total saturated-fatty-acid content of microsomal phospholipids, however, remained the same in all the dietary groups (Table 4). On the other hand, 2% cholesterol feeding significantly increased the C_{18:2}-fatty-acid content, at the expense of C_{20:4, n-6} and C_{22:6, n-3}. These changes in the fatty acid composition of the microsomal membranes resulted in a lowering of overall unsaturation, as assessed by the unsaturation index, of the phospholipids when 2% cholesterol was added to the diets (Table 4).

DISCUSSION

The aim of the present study was to examine the validity of the concept of membrane lipid homoeostasis [10]. The dietary cholesterol and/or fatty-acid-induced alterations in the activity of those enzymes responsible for producing changes in membrane cholesterol content

Table 3. ACAT and ACH activities in liver microsomes from rats fed various lipid-supplemented diets

Values are means $\pm$ S.D. for triplicate measurements on liver microsomal preparations from five rats in each dietary group. Statistical significance: ^a significantly different from REF- or CO-diet animals ($P < 0.001$); ^b significantly different from the respective low-cholesterol-diet animals ($P < 0.001$).

Enzyme	Cholesterol (2%)	Diet...	Enzyme activity [pmol of cholesterol palmitate (ACAT) or palmitic acid (ACH)/min per mg of protein]		
			REF	SSO	CO
ACAT	—		46.8 $\pm$ 5.4	75.9 $\pm$ 2.7 ^a	53.7 $\pm$ 3.9
	+		55.0 $\pm$ 4.5	99.7 $\pm$ 3.8 ^b	91.2 $\pm$ 2.9 ^b
ACH	—		624 $\pm$ 86	709 $\pm$ 60	684 $\pm$ 20
	+		551 $\pm$ 33	650 $\pm$ 62	736 $\pm$ 73

Table 4. Effect of dietary cholesterol on the percentage composition of major fatty acids of liver microsomal phospholipids

Values are means $\pm$ S.D. for five liver microsomal preparations from each dietary group. Values that are significantly different from their respective low-cholesterol controls are indicated thus: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Fatty acid	Diet...	Percentage composition					
		REF	(REF + CH)	SO	(SO + CH)	CO	(CO + CH)
C _{16:0}		22.4 $\pm$ 0.3	23.8 $\pm$ 0.4	14.7 $\pm$ 0.2	15.4 $\pm$ 0.3	19.8 $\pm$ 0.2	19.4 $\pm$ 0.2
C _{18:0}		21.7 $\pm$ 0.2	18.1 $\pm$ 0.0**	28.6 $\pm$ 0.3	24.7 $\pm$ 0.1**	27.0 $\pm$ 0.4	23.4 $\pm$ 0.3*
C _{18:0, n-9}		7.9 $\pm$ 0.0	11.2 $\pm$ 0.1***	5.8 $\pm$ 0.1	8.5 $\pm$ 0.0***	6.6 $\pm$ 0.0	11.6 $\pm$ 0.0***
C _{18:2, n-6}		18.1 $\pm$ 0.1	22.5 $\pm$ 0.2***	21.6 $\pm$ 0.2	28.5 $\pm$ 0.2***	18.1 $\pm$ 0.1	23.0 $\pm$ 0.3**
C _{20:4, n-6}		24.9 $\pm$ 0.3	21.3 $\pm$ 0.5*	25.4 $\pm$ 0.3	20.5 $\pm$ 0.4**	21.8 $\pm$ 0.3	19.6 $\pm$ 0.1*
C _{22:6, n-3}		4.8 $\pm$ 0.1	3.6 $\pm$ 0.3**	3.7 $\pm$ 0.0	2.5 $\pm$ 0.1***	6.6 $\pm$ 0.1	4.8 $\pm$ 0.2**
Saturated		44.1	41.9	43.3	40.1	46.8	42.8
Monounsaturated		7.9	11.2	5.8	8.5	6.6	11.6
Polyunsaturated		47.8	47.4	50.7	51.5	46.5	47.4
n-6/n-3...		8.96	12.17	12.70	19.60	6.04	8.88
Unsaturation index...		172.5 $\pm$ 2.0	163.0* $\pm$ 4.0	172.8 $\pm$ 1.7	162.5** $\pm$ 3.0	169.6 $\pm$ 2.0	164.8* $\pm$ 2.2

and fatty acid composition, when considered in unison, clearly underline this validity, as least for the microsomal membranes of the rat liver.

Under a high dietary load of cholesterol, the microsomal membranes exhibited an inhibition of HMGR activity and an increase of C7H and ACAT activities. Other workers have reported a similar decrease, but of larger magnitude, in HMGR activity after the consumption of unsaturated fat [21]. This difference in the degree of change in enzyme activity may well be due to differences in the length of the feeding period. Mitropoulos *et al.* [21] fed the fat-supplemented diets for only 12 h, whereas the feeding time in the present study was 28 days. A short-term feeding of only 12 h may not be enough time to produce any adaptive changes to compensate for this external stress. Some adaptations in the parameters related to physico-chemical properties of microsomal membrane do occur, however, after 28 days on unsaturated fat, e.g. changes in cholesterol content and fatty acid composition [11]. In like manner the feeding of 2% cholesterol for 12 h would put sudden pressure on the liver to inhibit cholesterol synthesis, and thus a greater decrease in HMGR activity, a rate-limiting enzyme in the cholesterol-biosynthetic pathway. On the

other hand the consumption of high-cholesterol diets for 28 days does indeed produce some adaptive changes, such as in fatty acid desaturase activity, which may relieve some pressure from the liver to inhibit its HMGR activity [22]. Finally, the source and amount of saturated fat (tristearoylglycerol; 10%, w/w) used by Mitropoulos *et al.* [21] was different from that of the present study (CO; 15% w/w), which may also affect the magnitude of differences in HMGR activity between the two experiments.

Loading of microsomes with cholesterol *in vitro*, by incubation with reconstituted human serum, has been shown to inhibit HMGR and increase ACAT activity [23]. In contrast with this, others [15,24], using different techniques for loading microsomal membranes with cholesterol, have demonstrated no change in HMGR activity after such treatment.

We have recently shown that dietary cholesterol treatment results in the accumulation of cholesterol, mainly as cholesterol esters, in hepatic microsomal membranes, and significantly more so when the cholesterol is fed in combination with unsaturated fat [11]. In the liver an accumulation of cholesterol in microsomal membranes may be responsible for the inhibition of

cholesterol synthesis by feedback mechanism [21]. The greater inhibition of cholesterol synthesis when cholesterol was fed with unsaturated (SSO) fat is also consistent with the greater accumulation of cholesterol after feeding cholesterol and SSO together. SSO diet, even without added cholesterol, resulted in both the accumulation of cholesterol in the microsomal membranes [11] and the inhibition of activity (the present paper, Table 1).

Both saturated (CO) and unsaturated (SSO) fat-enriched diets, without added cholesterol, inhibited the rate of cholesterol metabolism, i.e. as assessed by C7H activity. The SSO diet was enriched with respect to long-chain unsaturated fatty acids ($> 55\%$ of $C_{18:2,n-6}$) and the CO diet was enriched with medium-chain saturated fatty acids ($> 55\%$ of $C_{8:0} + C_{10:0} + C_{12:0}$), whereas the REF diet contained no medium-chain fatty acids and a relatively smaller amount of long-chain unsaturated fatty acids [11,20]. It has been demonstrated previously that the rate of absorption of medium-chain saturated and long-chain unsaturated fatty acids is much higher than that of long-chain saturated fatty acids [25,26]. Therefore it is likely that the fatty acids of both the high-fat diets in the present study were efficiently absorbed, which can be associated with an increased number of circulations of bile acid from the intestine. An increased enterohepatic circulation of bile acids may have been the factor primarily responsible for the inhibition of C7H activity in the liver microsomes by some form of feedback inhibition, since this enzyme catalyses the rate-limiting step in the synthesis of bile acids from cholesterol, as suggested by Bjorkhem *et al.* [25]. Inclusion in the diet of 2% cholesterol, irrespective of the quality and quantity of fat fed, accelerated the apparent rate of cholesterol metabolism to bile acids, as indicated by increased C7H activity in the liver microsomes.

The alternative route of cholesterol clearance from the liver is its esterification, along with a long-chain fatty acid, by the microsomal ACAT enzyme. The rate of cholesterol esterification was increased by the ingestion of SSO, whereas CO had no significant effect. These results are in agreement with the observations of Spector *et al.* [17]. The rate of cholesterol ester formation was highest when cholesterol was fed along with SSO, rather than with CO. This increase of ACAT is consistent with the previously observed greater accumulation of cholesterol in the liver microsomes after the ingestion of cholesterol and unsaturated fat [11]. Alternatively, it is possible that different fatty acid and/or cholesterol feeding resulted in different amounts of non-esterified fatty acids in the liver microsomes, which in turn have been shown to be inhibitory for ACAT [27]. This seems perhaps unlikely, however, as palmitoyl-CoA hydrolase activity was unaffected by the dietary lipid treatments (Table 3).

We have recently shown that dietary cholesterol enrichment results in an increase in microsomal Δ^9 -desaturase activity [22], which is consistent with the accumulation of microsomal monounsaturated fatty acids observed in the present study. On the other hand, depletion of 50% of sterols from mouse LM cells has been shown to increase Δ^9 -desaturation and monounsaturated fatty-acid content [28]. Thus it would appear that there is an optimum level of membrane cholesterol [10] that governs Δ^9 -desaturation activity. When cholesterol level is increased or decreased, Δ^9 -desaturation is

affected in such a way as to compensate for the altered physico-chemical properties caused by the cholesterol depletion or enrichment.

Dietary cholesterol resulted in a decrease of $C_{20:4,n-6}$ fatty acid, with an accompanying increase in $C_{18:2,n-6}$ fatty-acid content, regardless of the amount and type of fat fed. Dietary cholesterol could be expected to put higher demands on the liver to make cholesterol esters, for synthesis and secretion of very-low-density lipoproteins. Thus the decreased $C_{20:4,n-6}$ fatty-acid content in liver microsomal membranes, after dietary cholesterol treatment, may be due to an increased utilization of $C_{20:4,n-6}$ fatty acid for cholesterol esterification. In a recent study we demonstrated that dietary cholesterol inhibits microsomal Δ^6 - and Δ^5 -desaturase enzyme activities [22]. Alternatively, therefore, the decreased content of $C_{20:4,n-6}$ may partly be due to an inhibition of $C_{20:4,n-6}$ synthesis from $C_{18:2,n-6}$.

Medium-chain fatty acids ($C_{8:0}$ – $C_{14:0}$) from the CO diet were not incorporated into microsomal phospholipids. We have previously shown, however, that these medium-chain fatty acids do get incorporated into the neutral lipid (mainly triacylglycerols) fractions [11,20]. Thus the chain-elongation mechanisms of microsomal membranes seem to be effective enough to prevent medium-chain-fatty-acid build-up, in spite of a large dietary load. These results are in agreement with the observations of Mayorek & Jacob [29], who demonstrated that only fatty acids longer than myristic acid ($C_{14:0}$) are incorporated into phospholipids of cultured rat hepatocytes. The medium-chain fatty acids ($C_{8:0}$ – $C_{14:0}$) would be substrates for diacylglycerol acyltransferase, which leads to the synthesis of triacylglycerols [29].

In conclusion, it appears that the accumulation of microsomal cholesterol is a primary event occurring under the influence of a high-cholesterol diet. Then, in an attempt to nullify the effects of high cholesterol intake, the liver microsomes exhibit an inhibition of HMGR and an increase in both C7H and ACAT activity. This decreased endogenous synthesis and increased removal of cholesterol, however, may not be effective enough to prevent an enrichment of these membranes with cholesterol. Thus, apparently in order to compensate for this accumulated cholesterol, the fatty acid composition of the microsomal membranes is modified in such a way that the overall unsaturation of the membrane lipids, as assessed by the unsaturation index, is decreased. An increased cholesterol content tends to decrease the transition temperature of membrane lipids, whereas a decreased unsaturation has a tendency to increase the transition temperature [12], all of which is completely consistent with our concept of membrane lipid homoeostasis [10].

The existence of homoeostatic mechanisms for membrane lipids in membranes other than microsomal membranes, in tissues other than liver, and in different pathological processes (such as diabetes, carcinogenesis and cardiovascular disease) remains to be determined.

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