Stimulation of fatty acid biosynthesis by dietary cholesterol and of cholesterol synthesis by dietary fatty acid

Thomas V. Fungwe, James E. Fox, Lauren M. Cagen, Henry G. Wilcox, and Murray Heimberg[1]

Departments of Pharmacology and Medicine, University of Tennessee-Memphis, The Health Science Center, Memphis, TN 38163

Abstract We reported previously that dietary cholesterol produces hypertriglyceridemia in the rat, accompanied by reduced oxidation and increased incorporation of exogenous fatty acid into hepatic triglyceride and increased secretion of very low density lipoprotein. We now report that dietary cholesterol also increases net hepatic fatty acid synthesis and the incorporation of newly synthesized fatty acid into hepatic triglyceride in vivo. Male rats were fed a cholesterol-free, semisynthetic diet (5% [w/w] corn oil) for 7 days, or the same diet supplemented with 0.5% cholesterol. On the day of the experiments, fed animals received 5 mCi ^3H_2O intraperitoneally (i.p.) either at 1200 h (6 h into the light cycle) or at 2400 h (6 h into the dark cycle). Animals were killed 1 h after receiving the radioisotope. Feeding cholesterol increased hepatic triglyceride and cholesteryl ester concentrations, moderately elevated the content of free cholesterol, but did not affect phospholipid levels. Increased net synthesis of fatty acids by livers of animals receiving cholesterol was observed during the dark period; a similar increase during the light period was also observed for incorporation of newly synthesized fatty acid into hepatic phospholipid and cholesteryl ester, although incorporation into triglyceride was of borderline significance ($P < 0.06$). In other experiments male rats were fed similar diets for 3, 7, or 21 days. Fed animals received 10 mCi ^3H_2O, i.p. (900–1000 h), and were killed 24 h later. Duration of feeding did not influence rates of net fatty acid synthesis or the stimulation by cholesterol of incorporation of newly synthesized fatty acid into hepatic triglyceride and cholesteryl ester. Increasing the fat content of the diet to 20% (w/w) and maintaining the cholesterol supplement at 0.5% diminished the net rate of hepatic lipogenesis, but the stimulatory effect of cholesterol was still evident. Biosynthesis of cholesterol was stimulated when the fat content of the diet was increased in the absence or in the presence of added cholesterol. These data indicate that dietary cholesterol stimulates the biosynthesis of fatty acids, while the addition of triglyceride to the diet stimulates synthesis of cholesterol. Fatty acid and cholesterol appear to stimulate each other's biosynthesis in the liver.—**Fungwe, T. V., J. E. Fox, L. M. Cagen, H. G. Wilcox, and M. Heimberg.** Stimulation of fatty acid biosynthesis by dietary cholesterol and of cholesterol synthesis by dietary fatty acid. *J. Lipid Res.* 1994. **35:** 311–318.

Supplementary key words very low density lipoprotein • lipogenesis triglyceride • cholesterol • cholesteryl ester • fatty acid • phospholipid

The importance of triglyceride-rich lipoproteins as atherogenic risk factors is becoming increasingly appreciated (1). Diet plays a significant role in determining plasma concentrations of triglyceride (reviewed in ref. 2). While many studies have related dietary intake of cholesterol to concentrations of cholesterol in the circulation, potential effects of dietary cholesterol on plasma triglyceride levels have received less attention.

We reported previously that, in the rat, increasing the dietary intake of cholesterol increased the concentrations of triglyceride and cholesteryl ester in liver and plasma in vivo (3). Cholesterol-induced hypertriglyceridemia was also recently reported in the hamster (4). In the rat model, perfused livers from cholesterol-fed animals also secreted more triglyceride as well as the other lipid and protein components of the VLDL (3). These data imply that dietary cholesterol stimulates formation of both hepatic triglyceride and cholesteryl ester. Consistent with this, we found that dietary cholesterol decreased the oxidation of exogenous oleic acid in the perfused liver, increased the incorporation of fatty acid into both triglyceride and cholesteryl ester, and reduced the activity of hepatic carnitine palmitoyltransferase (5). The present study was undertaken to determine whether dietary cholesterol increases de novo hepatic synthesis of fatty acids and their subsequent incorporation into neutral lipids.

Abbreviations: CE, cholesteryl ester; FA, fatty acid; FC, free cholesterol; PL, phospholipid; TG, triglyceride; TOFA, 5(tetradecyloxy)-2-furoic acid; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

METHODS

Materials and reagents

Cholesterol (USP), cholesterol-free diet, ^3H_2O, and Ecolume were purchased from ICN Biochemicals, Inc. (Costa Mesa, CA). Mazola corn oil (100% pure, food grade) was obtained locally. Silica-gel G thin-layer plates were purchased from Analtech. Inc (Newark, DE). All other chemicals and reagents were analytical grade.

Animals and diets

Male Sprague-Dawley rats (Harlan Labs, Madison, WI) weighing 150–175 g were fed, ad libitum, a cholesterol-free diet supplemented with 5% corn oil (3). The animals were housed under a normal light–dark cycle, with lights on from 0600 to 1800 h. Animals fed the basal diet for 7 days were then randomly assigned to two dietary groups, and fed the control diet or a cholesterol (0.5% w/w)-supplemented diet for 7 days, as described previously (3). In some experiments, the feeding period was reduced to 3 days or extended to 21 days. In other experiments the corn oil content of the diet was increased to 20% (w/w) and was fed for 7 days. All animals were fed ad libitum.

Measurement of fatty acid synthesis

For short-term labeling experiments, animals received 5 mCi ^3H_2O (in 1 ml 0.9% NaCl) intraperitoneally, either at 1200 or at 2400 h. Animals were killed 1 h after receiving the radioisotope. For long term labeling, each rat was injected as above with 10 mCi ^3H_2O between 0900 and 1000 h and was killed 24 h later. Animals were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg) and killed by exsanguination from the abdominal aorta. The livers were flushed with cold 0.9% NaCl, removed, and weighed. Total lipid extracts were prepared from plasma and liver (6), and hepatic lipid (phospholipid, cholesterol, triglyceride, and cholesteryl esters) concentrations were measured as reported previously (3).

Lipids extracted from 1 ml plasma were saponified by addition of 10 ml of alcoholic KOH (6 ml of 33% KOH and 94 ml of absolute ethanol). Aliquots of total lipid extracts of the liver and adipose tissue were evaporated and the residue was redissolved in 10 ml of alcoholic KOH. The samples were heated at 65–70°C for 2 h and then acidified with 5 ml of 1 N HCl. The reaction mixture was extracted with 10 ml petroleum ether, the petroleum ether was evaporated under a stream of nitrogen, and the residue was redissolved in 0.5 ml chloroform. This extract was spotted onto silica gel thin-layer plates for resolution of cholesterol and fatty acid. Lipid classes in a second aliquot of plasma or tissue extract were resolved by thin-layer chromatography and the individual bands were transferred to saponification tubes and hydrolyzed as described above.

Radioactivity was measured by liquid scintillation spectrometry. Rates of fatty acid and cholesterol synthesis were estimated from incorporation of ^3H from ^3H_2O into fatty acids (7) and cholesterol (8) and the specific activity of plasma ^3H_2O at the time of killing. In the short-term experiments, the calculated radioactivity of total body water 1 h after administration of tracer indicated complete equilibration of the injected dose (5.00 ± 0.08 mCi, n = 15). In experiments in which animals were killed 24 h after injection, the calculated total radioactivity had declined to 86 ± 4% of the 10 mCi injected (n = 39). Neither the cholesterol nor the fat content of the diet significantly affected equilibration or turnover of water, and no correction for the small decline in specific activity is made in reporting data from 24-h pulses. In these calculations, it was assumed that 13.3 microgram atoms of ^3H from ^3H_2O were incorporated per μmol of fatty acid (9) and 14.5 per μmol cholesterol (8).

Statistics

All data were analyzed using Student's *t*-test for unpaired samples. The results are expressed as means ± SE and differences between means were considered significant at the probability level of 0.05.

RESULTS

The average daily consumption of the 5% corn oil diet and the weight gained over the feeding periods (3, 7, or 21 days) were similar to those reported previously (3) and were not significantly different between cholesterol-supplemented and control groups. Weight gain among the rats fed the 20% corn oil diet for 7 days was similarly unaffected by cholesterol (data not shown).

Effects of dietary cholesterol and fat on hepatic lipid concentration

As shown by the data in **Table 1,** cholesterol fed for 7 days to rats consuming the 5% corn oil diet increased hepatic triglyceride content 2-fold. Cholesterol also moderately elevated the concentration of hepatic free cholesterol and increased cholesteryl ester content by 12-fold but had no effect on levels of hepatic phospholipid. These data are consistent with results reported previously (3). No change was observed in the concentration of hepatic lipids between 3 and 21 days, except for a progressive rise in the level of cholesteryl esters in the cholesterol-supplemented group. Raising the fat content of the diet to 20% increased hepatic levels of triglyceride, as expected; cholesterol caused a further 2-fold increase in triglyceride content and increased the concentration of cholesteryl ester and free cholesterol (5).

TABLE 1. Effects of dietary cholesterol on hepatic lipid composition in rats fed diets containing either 5% or 20% corn oil

Treatment	Hepatic Lipid	
	0.0% Cholesterol in Diet (n)	0.5% Cholesterol in Diet (n)
	μmol/g	
5% Corn oil		
3-Day feeding period	(7)	(8)
Phospholipid	28.54 ± 1.28	28.64 ± 0.61
Triglyceride	6.37 ± 0.81	11.14 ± 0.70[a]
Cholesterol	3.68 ± 0.09	4.36 ± 0.19[a]
Cholesteryl ester	0.71 ± 0.04	8.90 ± 0.41[a]
7-Day feeding period	(6)	(7)
Phospholipid	20.64 ± 2.65	20.57 ± 1.18
Triglyceride	4.22 ± 0.94	7.90 ± 0.71[a]
Cholesterol	3.32 ± 0.16	4.06 ± 0.14[a]
Cholesteryl ester	0.69 ± 0.04	11.58 ± 1.68[a]
21-Day feeding period	(7)	(7)
Phospholipid	27.66 ± 0.61	27.20 ± 1.22
Triglyceride	5.88 ± 0.58	11.74 ± 0.72[a]
Cholesterol	3.50 ± 0.06	4.22 ± 0.10[a]
Cholesteryl ester	0.74 ± 0.05	15.97 ± 1.79[a]
20% Corn oil		
7-Day feeding period	(8)	(8)
Phospholipid	32.92 ± 1.30	30.40 ± 1.63
Triglyceride	13.80 ± 1.08	30.41 ± 1.73[a]
Cholesterol	3.77 ± 0.07	4.00 ± 0.15
Cholesteryl ester	2.82 ± 0.34	19.78 ± 2.08[a]

Data are means ± SEM. Figures in parentheses indicate number of animals in each group. All animals received the diets containing 5% (w/w) corn oil for the indicated period.

[a] $P < 0.05$, compared to 0.0% cholesterol.

Effects of cholesterol on net hepatic lipogenesis

Feeding 0.5% cholesterol for 1 week promoted net hepatic synthesis of fatty acid during the dark period, as reflected by rates of incorporation of ^3H_2O into fatty acid contained in hepatic phospholipid (1.55 ± 0.27 vs. 0.87 ± 0.05 μmol fatty acid/g), triglyceride (1.25 ± 0.30 vs. 0.56 ± 0.03 μmol fatty acid/g), cholesteryl ester (0.167 ± 0.046 vs. 0.021 ± 0.002 μmol fatty acid/g) (**Fig. 1**), and into total saponifiable fatty acid (3.02 ± 0.67 vs. 1.32 ± 0.10 μmol fatty acid/g) (**Fig. 2**). Incorporation of ^3H-labeled fatty acid into phospholipid (1.02 ± 0.19 vs. 0.47 ± 0.06 μmol fatty acid/g) and cholesteryl ester (0.067 ± 0.018 vs. 0.009 ± 0.001 μmol fatty acid/g) during the light period was also increased by cholesterol feeding, while the incorporation into triglyceride (0.58 ± 0.14 ± 0.27 ± 0.05 μmol fatty acid/g) and total saponifiable fatty acid (1.63 ± 0.36 vs. 0.84 ± 0.08 μmol fatty acid/g), although increased, showed only borderline statistical significance ($P = 0.062$ and 0.057 for triglyceride and total fatty acid, respectively) (Figs. 1 and 2). The increase in the sum of incorporation of ^3H_2O into phospholipid, triglyceride, and cholesteryl ester fatty acids during the light period in cholesterol fed animals (Fig. 1D) was statistically significant (1.71 ± 0.33 vs. 0.83 ± 0.13 μmol fatty acid/g; $P = 0.034$).

In rats fed the cholesterol-free diet, net hepatic synthesis of fatty acid was, as expected, significantly elevated during the dark period ($P = 0.003$), compared to the light period. A similar trend was observed in the rats fed 0.5% cholesterol, but failed to achieve statistical significance ($P = 0.097$) (Fig. 2).

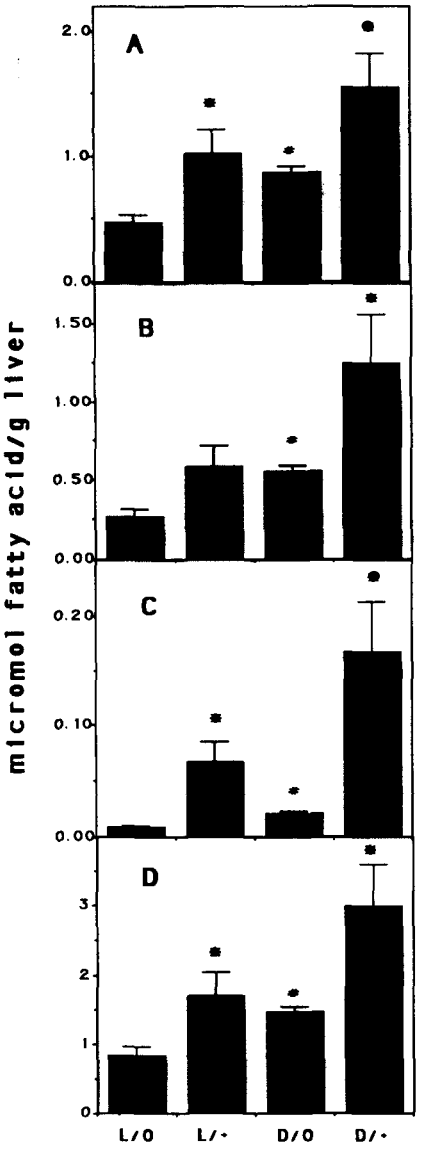

Fig. 1. Effects of dietary cholesterol on biosynthesis of hepatic esterified fatty acids. ^3H_2O was administered for 1 h at the middle of the light (L) or dark (D) periods. Data are expressed as the quantity of product synthesized (μmol fatty acid/g liver per h). Male rats were fed a semipurified diet supplemented with 0.5% cholesterol (+) or cholesterol-free (0) for 7 days. ^3H_2O (5 mCi) was injected i.p. at 1200 or 2400 h, and rats were killed 1 h later. Hepatic lipid classes were separated by thin-layer chromatography, saponified, and the radioactivity in fatty acid liberated from phospholipid (panel A), triglyceride (panel B), and cholesteryl ester (panel C) was determined. Panel D shows the sum of incorporation into the three fractions above. Each bar represents the mean ± SEM for six animals; *different from rats fed control diet and pulsed at the same time ($P < 0.05$); #different from rats fed the same diet and pulsed during the light period ($P < 0.05$).

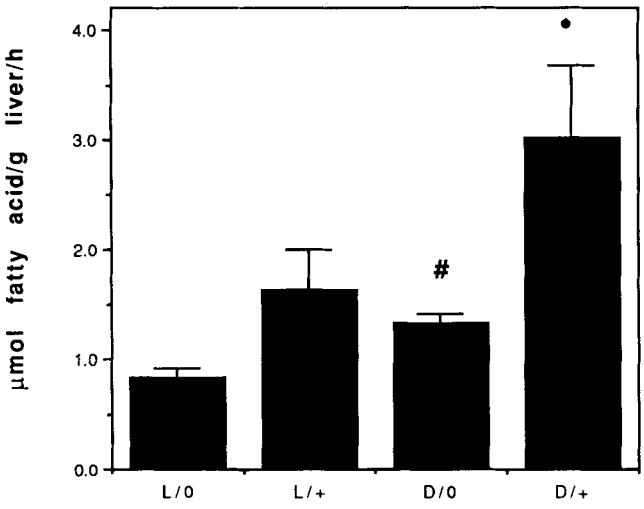

Fig. 2. Effects of dietary cholesterol on biosynthesis of total hepatic esterified fatty acids. ^3H_2O was administered for 1 h at the middle of the light (L) or dark (D) periods. Male rats were fed a semipurified diet supplemented with 0.5% cholesterol (+) or cholesterol-free (0) for 7 days. ^3H_2O (5 mCi) was injected i.p. at 1200 or 2400 h, and rats were killed 1 h later. Hepatic lipid extracts were saponified and fatty acids were separated by thin-layer chromatography. Data are expressed as µmol fatty acid/g liver per h. Each bar represents the mean ± SEM for six animals; *different from rats fed control diet and pulsed at the same time ($P < 0.05$); #different from rats fed the same diet and pulsed during the light period ($P < 0.05$).

Effects of duration of dietary treatment on hepatic lipogenesis

The effects of feeding cholesterol for 3, 7, or 21 days on synthesis of fatty acid are shown in **Table 2.** Incorporation of newly synthesized ^3H-labeled fatty acids into hepatic triglyceride and cholesteryl ester fatty acids and into total saponifiable lipids over a 24-h period was elevated with cholesterol in the diet. The duration of feeding had little effect on net hepatic synthesis of fatty acid in either the cholesterol-fed or control groups. In these experiments, incorporation into phospholipid fatty acids was not altered by dietary cholesterol. As expected, net synthesis of hepatic cholesterol was diminished by cholesterol feeding; this decrease in utilization of labeled substrate was not sufficient to account for the increase in net fatty acid synthesis.

Effects of increased dietary fat content on cholesterol-induced lipogenesis

To determine whether the observed stimulation of lipogenesis by dietary cholesterol could be altered by increasing the fat intake, rats were fed their respective diets for 7 days and exposed to ^3H_2O for 24 h. The effect of high fat (20% corn oil) and cholesterol feeding on fatty acid synthesis is shown in **Table 3.** Relative to rats on the 5% corn oil diet (Table 2), the rate of incorporation of newly synthesized fatty acid into the hepatic saponifiable fatty acid fraction was reduced. However, incorporation of newly synthesized fatty acids into both cholesteryl ester and triglyceride was still elevated by dietary cholesterol.

Effects of dietary fatty acids on cholesterol synthesis

In addition to evaluating the effects of cholesterol on fatty acid synthesis, in the presence of either low or high fat in the diet, we also measured the effects of fat content on cholesterol biosynthesis. As expected, dietary cholesterol reduced total hepatic cholesterol synthesis and decreased the amount of newly synthesized cholesterol in plasma (**Fig. 3**). In the cholesterol-fed animals significant amounts of newly synthesized cholesterol (more than 50% of total hepatic cholesterol) were esterified. Of more importance, Fig. 3 also illustrates that dietary fat stimulated total hepatic cholesterol synthesis, not only in animals fed the 0.0% cholesterol diet but also in those fed 0.5% cholesterol, albeit at a lower level.

TABLE 2. Effects of dietary cholesterol on fatty acid and cholesterol synthesis in rats fed a diet containing 5% corn oil

Treatment	Synthesis Rate: 0.0% Cholesterol in Diet (n)	Synthesis Rate: 0.5% Cholesterol in Diet (n)
3-Day feeding period	(7)	(8)
Plasma total FA (µmol/ml)	2.15 ± 0.33	2.98 ± 0.48
Liver (µmol/g)		
Total FA	27.10 ± 2.72	35.31 ± 2.72[a]
PLFA	16.69 ± 1.08	16.83 ± 1.05
TGFA	4.14 ± 0.59	8.60 ± 0.71[a]
CEFA	0.25 ± 0.03	2.76 ± 0.19[a]
Total cholesterol	0.67 ± 0.05	0.26 ± 0.02[a]
7-Day feeding period	(6)	(6)
Plasma total FA (µmol/ml)	1.32 ± 0.15	2.82 ± 0.40[a]
Liver (µmol/g)		
Total FA	17.10 ± 0.97	33.66 ± 6.07[a]
PLFA	13.53 ± 1.40	18.53 ± 2.42
TGFA	3.33 ± 0.82	10.34 ± 1.39[a]
CEFA	0.16 ± 0.03	3.04 ± 0.38[a]
Total cholesterol	0.46 ± 0.03	0.16 ± 0.02[a]
21-Day feeding period	(6)	(6)
Plasma total FA (µmol/ml)	2.23 ± 0.25	2.97 ± 0.32
Liver (µmol/g)		
Total FA	23.12 ± 2.18	33.52 ± 2.71[a]
PLFA	17.60 ± 0.87	17.70 ± 1.48
TGFA	4.47 ± 0.71	12.32 ± 2.20[a]
CEFA	0.21 ± 0.02	2.94 ± 0.48[a]
Total cholesterol	0.64 ± 0.06	0.18 ± 0.01[a]

Data are means ± SEM and indicate µmol fatty acid or cholesterol synthesized per g liver or per ml plasma. Figures in parentheses indicate number of animals in each group. All animals received the diets containing 5% (w/w) corn oil for the indicated period. Experiments were carried out for 24 h.

[a] $P < 0.05$, compared to 0.0% cholesterol.

TABLE 3. Effects of dietary cholesterol on fatty acid and cholesterol synthesis in rats fed a diet containing 20% corn oil

	Synthesis Rate	
	0.0% Cholesterol (8)	0.5% Cholesterol (8)
Plasma total FA	*μmol/ml*	
	0.60 ± 0.09	0.63 ± 0.15
Liver	*μmol/g*	
Total FA	11.60 ± 1.45	14.90 ± 2.37
PLFA	8.21 ± 1.09	8.53 ± 1.56
TGFA	3.17 ± 0.34	5.75 ± 1.10[a]
CEFA	0.21 ± 0.05	0.62 ± 0.13[a]
Total cholesterol	0.86 ± 0.21	0.32 ± 0.04[a]

Data are means ± SEM. Figures in parentheses indicate number of animals in each group. All animals received the diet containing 20% (w/w) corn oil for 7 days. Experiments were carried out for 24 h.
[a] $P < 0.05$, compared to 0.0% cholesterol.

DISCUSSION

At the diurnal peak of fatty acid synthesis, dietary cholesterol augmented incorporation of ^3H_2O into fatty acids of hepatic phospholipid, triglyceride, and cholesteryl ester. Incorporation of newly synthesized fatty acid into phospholipid and cholesteryl ester at the diurnal nadir was also augmented by dietary cholesterol, although the increase in incorporation into triglyceride and into total saponifiable fatty acid achieved only borderline statistical significance ($P < 0.06$). These data are consistent with those of Davis, Malone-McNeal, and Moses (10), who found that adding 2% cholesterol to a diet containing 20% olive oil increased incorporation of ^3H_2O into triglyceride by isolated cultured hepatocytes.

The normal diurnal rhythm in hepatic fatty acid synthesis in the rat (highest in the dark period) has been attributed to a change in the phosphorylation state of acetyl-CoA carboxylase, without a diurnal change in the amount of the enzyme protein (11). Parallel increases in both the light and dark periods might be expected if cholesterol augments expression of acetyl-CoA carboxylase without altering the diurnal changes in phosphorylation state. Alternatively, a selective increase in lipogenesis in the dark period suggests an effect of dietary cholesterol on phosphorylation of the enzyme or some diurnally sensitive change in the concentration of an allosteric regulator.

We cannot discount the possibility that diminished oxidation (5) or preferential esterification (4, 5) of the newly synthesized fatty acid contributes to the observed increase in net lipogenesis. In our previous experiments with isolated perfused rat livers (5), 46% of the radioactivity from exogenous [1-^{14}C]oleic acid taken up by livers from control animals was recovered in products of beta-oxidation; in livers from cholesterol-fed animals, however, this dropped to 27% and was accompanied by a corresponding 37% increase in radioactivity recovered as esterified fatty acid. In those experiments, total hepatic uptake of the fatty acid was the same in both groups. If oxidation of newly synthesized fatty acid were reduced, or the efficiency of esterification increased, in the same proportion as observed for exogenous oleic acid, it would still be insufficient to account for the approximately 2-fold increase in net synthesis of fatty acid that was observed. The results suggest that an increase in the rate of fatty acid synthesis, as well as changes in the disposition of the newly synthesized fatty acid, is produced by cholesterol feeding.

Dietary cholesterol increased the incorporation of exogenous [1-^{14}C]oleic acid into neutral lipids, but not into

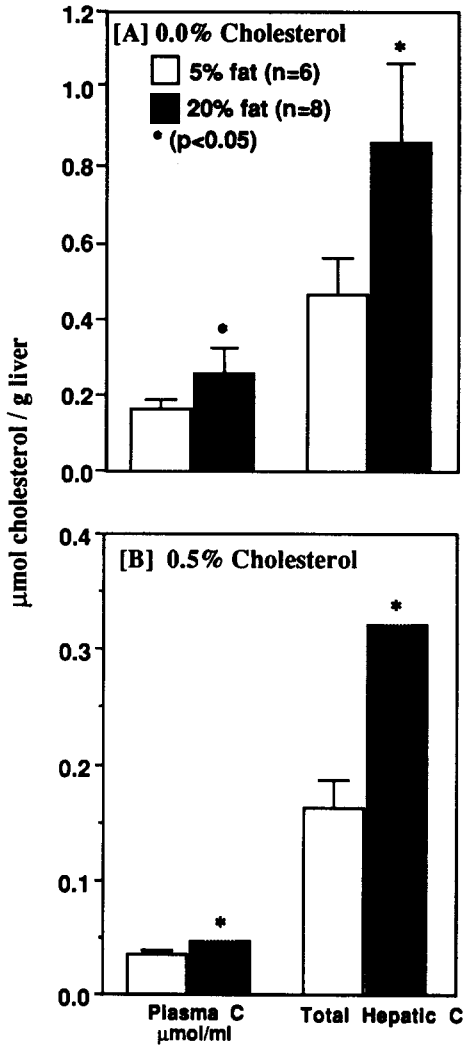

Fig. 3. Effects of dietary fat on biosynthesis of total plasma or hepatic cholesterol recovered from plasma or liver at the end of 24 h in rats fed either a cholesterol-free diet containing 5% or 20% corn oil (panel A) or the same diets supplemented with 0.5% cholesterol (panel B) for 1 week. Cholesterol was recovered from total lipid extracts by thin-layer chromatography after saponification. Data are expressed as μmol cholesterol synthesized/g liver per 24 h.

phospholipid, both in vivo and in the perfused liver in vitro (5). It may be that saturation of phospholipid synthesis occurs at lower concentrations of fatty acid precursor than does synthesis of triglyceride or cholesteryl esters (12). In addition, changes in the rate of de novo fatty acid synthesis would have a relatively small impact on the specific activity of the fatty acid pool available for esterification when exogenous free fatty acid at high concentration is the source of radioactive label. In the previous experiments, perfused livers from rats killed during the light period took up 9 μmol of oleic acid/g liver per h; under the present experimental conditions, rates of endogenous fatty acid synthesis were 1 to 2 μmol/ g per h. In contrast, when ^3H_2O is used to label newly synthesized fatty acids under the same conditions, the specific activity of the precursor pool should increase with increases in the rate of lipogenesis and result in corresponding increases in the amount of label incorporated into all products of esterification, whether or not the absolute rate of these reactions is altered. This was observed. Incorporation of the ^3H-labeled fatty acyl moieties into phospholipid and triglyceride was increased to approximately the same extent (1.78- and 2.23-fold for phospholipid and triglyceride, respectively, in the dark period). However, this does not exclude a contribution of increased fatty acid synthesis to increased absolute rate of triglyceride synthesis. If phospholipid synthesis or turnover is saturated at the prevailing intracellular fatty acid concentration, increased utilization of newly synthesized fatty acid would occur at the expense of fatty acid from other sources. If triglyceride synthesis is not saturated under the same conditions, more newly synthesized fatty acid can be incorporated into this pool without decreasing the incorporation of unlabeled fatty acid.

Fatty acyl-CoA is an allosteric inhibitor of acetyl-CoA carboxylase (reviewed in ref. 13). A primary action of dietary cholesterol on the efficiency of fatty acid esterification or oxidation might affect rates of fatty acid synthesis by changing the concentration of fatty acyl-CoA in the cytoplasm. If the direct effect of cholesterol is to promote esterification of fatty acids, and diminished oxidation is a consequence of less fatty acid being available for uptake by mitochondria, higher rates of fatty acid synthesis would be anticipated. One would expect the opposite if inhibition of oxidation were the primary effect, and the increased esterification reflected increased availability of fatty acyl-CoA. Resolution of this question awaits experiments in which oxidation is blocked with suitable inhibitors.

Regardless of the mechanism, newly synthesized fatty acid is more rapidly incorporated into hepatic triglyceride in cholesterol-fed rats than in controls. This may contribute to the hepatic triglyceride accumulation and hypertriglyceridemia that accompanies cholesterol feeding in the rat (3).

The results of a 24-h pulse of ^3H_2O (Tables 2 and 3) are, in general, consistent with those of 1-h pulses during the light and dark periods. The net synthesis of saponifiable fatty acid within the liver was augmented by cholesterol feeding. Incorporation of newly synthesized fatty acid into neutral lipid fractions was increased. Taken together with evidence that these fractions are secreted more rapidly by livers from cholesterol-fed animals (Table 2 and ref. 3), these results are consistent with an effect of dietary cholesterol to enhance either the rate of hepatic fatty acid synthesis, or fractional incorporation of newly synthesized fatty acids into triglyceride, or both. However, in contrast to the results of 1-h pulses, there was no increase in incorporation of newly synthesized fatty acid into hepatic phospholipid in livers from cholesterol-fed animals. The reason for this difference is not clear at this time.

Three days of cholesterol feeding seemed to be sufficient to promote increased incorporation of newly synthesized fatty acid into hepatic neutral lipids. There was little change in the overall rate of synthesis and incorporation of fatty acids into hepatic neutral lipids between 3 and 21 days, despite increased hepatic content of triglyceride and cholesteryl ester. Craig et al. (14) failed to observe an increase in the activity of acetyl-CoA carboxylase or fatty acid synthase after feeding rats for 2 days with a diet supplemented with 2% cholesterol. This might indicate that 2 days is not sufficient to bring about an increase in the activity of these enzymes. However, in that study acetyl-CoA carboxylase activity was measured under conditions that would not preserve the endogenous phosphorylation state of the enzyme (13). Changes in phosphorylation brought about by dietary cholesterol would therefore have been masked; it is also possible that the locus of the cholesterol effect on fatty acid synthesis resides elsewhere.

As expected, increasing the fat content of the diet to 20% reduced the rate of fatty acid synthesis within the liver (Table 3). Nevertheless, the effect of cholesterol to promote incorporation of newly synthesized fatty acid into triglyceride was still observed.

Exogenous oleic acid stimulates cholesterol synthesis in perfused rat liver (15, 16) and in rat hepatocytes in culture (17). This is accompanied by increase in HMG-CoA reductase activity (18–20) and in the activity of cytosolic acetoacetyl-CoA thiolase and HMG-CoA synthase (21). Moreover, HMG-CoA reductase activity increased in proportion to the concentration of infused oleate (22) and, in intact fed rats, is correlated with secretion of the VLDL (15). Inhibition of fatty acid oxidation at the level of carnitine palmitoyl transferase has also been reported to increase HMG-CoA reductase activity in rat liver (19, 23). This effect is supported by data in this study, since increasing the dietary fat content from 5% to 20% in-

creased hepatic synthesis of cholesterol in animals on both the cholesterol-free diet and the diet supplemented with 0.5% cholesterol.

Net synthesis of triglyceride can draw upon free fatty acid taken up from the circulation and fatty acid synthesized de novo. Under physiological conditions, the rate of free fatty acid uptake exceeds endogenous synthesis. Nevertheless, changes in the rate of fatty acid synthesis can contribute to altered rates of triglyceride formation. Fukuda and Ontko (24) reported that, in rat livers perfused without exogenous fatty acid, newly synthesized fatty acid was incorporated into triglyceride at a rate of 0.62 μmol/g per h; fatty acid supplied to the perfusion medium at a rate of 90 μmol/h was incorporated into triglyceride at 2.18 μmol/g per h. In the present study we found that, during the light period, newly synthesized fatty acid was incorporated into hepatic triglyceride in rats fed the control diet at a rate of 0.27 μmol/g per h. While we do not have data for incorporation of exogenous free fatty acid under the same conditions, in perfused livers isolated from similarly treated animals and perfused with [1-^{14}C]oleic acid at 0.5 mM (infused at a rate of 140 μmol/h), this rate was 2.72 μmol/g per h (5). Thus, doubling the rate of incorporation of newly synthesized fatty acid might be expected to add about 10% to the overall rate of triglyceride formation within the liver.

The rate of endogenous fatty acid synthesis is one determinant of the rate of triglyceride synthesis in perfused liver (9). Inhibition of de novo fatty acid synthesis by TOFA produced an 84% reduction in triglyceride secretion by rat liver perfused in the absence of exogenous fatty acids (24), despite the fact that only 20% of the fatty acid in secreted triglyceride is derived from this source (the balance coming from preformed lipid). TOFA produced a 40% reduction in the rate of VLDL triglyceride secretion in intact hamsters (25). Interpretation of these results is complicated by the fact that the effects of TOFA on lipid metabolism are not limited to inhibition of acetyl-CoA carboxylase (24).

In a study of the effects of nutritional state on VLDL secretion by rat hepatocytes cultured in the absence of exogenous fatty acid, Gibbons and Burnham (26) found that fatty acids synthesized during the pulse period contributed 20% or less to the secreted triglyceride; the bulk of secreted triglyceride came from preformed stores. However, these stores result from past synthesis of triglyceride from fatty acid produced within the liver and taken up from the circulation. Our previous findings (5) and the results of the present investigation show that both processes are enhanced in rats fed cholesterol and both may contribute to the hypertriglyceridemic response to dietary cholesterol. The mechanisms underlying these effects will be the subject of future investigation.

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