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Dietary Taurine Reduces Hepatic Secretion of Cholesteryl Ester and Enhances Fatty Acid Oxidation in Rats Fed a High-Cholesterol Diet

Nobuhiro FUKUDA[1], Ayako YOSHITAMA[1], Satomi SUGITA[1], Michiko FUJITA[1] and Shigeru MURAKAMI[2]

[1]*Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, Gakuen Kibanadai, Miyazaki, Miyazaki 889–2192, Japan*
[2]*Medical Research Laboratories, Taisho Pharmaceutical Co. Ltd., 1–403 Yoshino-cho, Kitaku, Saitama 331–9530, Japan*

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Summary We investigated the fate of exogenous fatty acid in connection with decreased hepatic accumulation and secretion of cholesteryl esters in rats fed diets containing taurine. Providing taurine as 5% of the diet for 14 d significantly decreased concentrations of cholesterol, especially cholesteryl esters in both serum and liver. Ketone body production and incorporation of exogenous [1-^{14}C]oleate into ketone bodies in liver perfusate were consistently higher during a 4-h perfusion period in taurine-fed rats than in control rats. The elevation was accompanied by increased activity of liver mitochondrial carnitine palmitoyltransferase, a rate-limiting enzyme for fatty acid oxidation. Dietary taurine significantly reduced hepatic secretion of cholesteryl ester and decreased incorporation of exogenous oleic acid substrate into this lipid molecule. Further, the extent of reduction in hepatic secretion of cholesteryl ester was closely related to its diminished accumulation in the liver. The conversion pattern of exogenous [1-^{14}C]oleic acid substrate suggested a decreased esterification-to-oxidation ratio in the taurine group compared with the control. These results suggest that taurine-induced reduction in hepatic accumulation of cholesteryl ester was associated with reduced hepatic secretion of this lipid molecule, and was inversely related to enhanced ketone body production and fatty acid oxidation.

Key Words taurine, cholesterol ester secretion, fatty acid oxidation, rat liver

Taurine, a sulfur-containing amino acid present in high concentrations in animal tissues, is available from dietary sources and also can be synthesized in vivo from other amino acids. Dietary taurine exhibits various physiologic and pharmacological actions, including hypocholesterolemic and antiatherogenic effects, in experimental animals, especially rats, mice, and Japanese (LAP) quail fed high cholesterol diets (*1–6*). These beneficial effects of taurine have recently been reproduced in humans (*7*, *8*). Although these effects have attracted attention, the underlying mechanisms are not clearly understood. With regard to the hypocholesterolemic action of dietary taurine, we previously reported that in rats fed high-cholesterol diets, the lowering of serum cholesterol by taurine was partly attributable to a reduced secretion rate of cholesteryl ester by the liver, and that reductions in hepatic secretion rate and in hepatic accumulation of cholesteryl ester were inversely related to increased ketone body production, an index of fatty acid oxidation (*9*). This pattern of changes suggested that dietary taurine influences the metabolic fate of fatty acids, specifically whether they undergo esterification or oxidation. However, in that study, we did not assess the relative significance of these two pathways in the metabolism of exogenous fatty acids.

E-mail: nfukuda@miyazaki-u.ac.jp

We therefore undertook a more detailed examination of fatty acid metabolism using an exogenous [1-^{14}C]oleic acid, and found for the first time that the reduced hepatic synthesis and secretion of cholesteryl ester resulting from dietary taurine intake stimulates oxidation of exogenous fatty acid at the expense of esterification.

MATERIALS AND METHODS

Materials. Taurine (2-aminoethanesulfonic acid) was provided by Taisho Pharmaceutical (Saitama, Japan), while [1-^{14}C]oleic acid was purchased from Amersham Life Science (Buckinghamshire, England). Oleic acid and palmitoyl-CoA were obtained from Sigma Chemicals (St. Louis, MO). Bovine serum albumin (fraction V) was purchased from Boehringer Mannheim (Mannheim, Germany). Other chemicals used were of analytical grade.

Animals and diets. Male Wistar rats weighing 100 to 110 g (5 wk old) were purchased from Kyudo (Kumamoto, Japan) and housed in individual stainless-steel mesh cages in a temperature- and light-controlled room (22 to 24°C; lights on, 07:00 to 19:00) for 5 d for acclimatization. During this period, the rats were fed commercial powdered stock chow (CLEA Japan, Inc., Tokyo, Japan). After the acclimation, the animals were then divided into two groups with equal body weights: a tau-

Table 1. Effects of dietary taurine on body weight, food intake, liver weight, serum and liver lipid concentrations, and activity of liver mitochondrial carnitine palmitoyltransferase.

	Control	Taurine
Experiment 1[1]		
Body weight (g)		
Initial	146±4	145±7
Final	261±9	258±7
Food intake (g/d)	18.5±0.8	18.5±0.6
Liver weight (g/100 g body weight)	6.5±0.1	6.3±0.1
Serum lipids (μmol/dL)		
Cholesteryl ester	935±102	568±48.4*
Free cholesterol	256±62	121±17.0*
Triglyceride	389±60	301±65
Phospholipids	299±28	225±13
Liver lipids (μmol/g liver)		
Cholesteryl ester	80.8±4.4	66.3±6.4*
Free cholesterol	7.1±0.4	6.1±0.3
Triglyceride	26.3±1.0	24.9±1.5
Phospholipids	29.5±0.6	34.8±0.7
Mitochondrial carnitine palmitoyltransferase activity (nmol CoA/min/mg protein)	8.2±0.5	9.8±0.6*
Experiment 2[2]		
Body weight (g)		
Initial	166±4	167±2
Final	270±6	258±4*
Food intake (g/d)	18.6±0.7	18.3±0.9
Liver weight (g/100 g body weight)	6.7±0.7	5.5±0.2*
Lipid content of postperfused liver (mmol/g liver)		
Cholesteryl ester	62.4±2.61	48.9±1.5*
Free cholesterol	6.3±0.5	6.0±0.3
Triglyceride	37.9±3.8	30.7±1.9
Phospholipids	28.5±0.4	30.7±1.9

[1] Data are the mean±SE of 7 rats.
[2] Data are the mean±SE of 6 rats.
* Significantly different from control group at $p<0.05$.

rine-free control group and a taurine-fed group. The control group was fed a diet prepared as follows (by weight percent): casein, 20.0; lard, 9.0; soybean oil, 1.0; mineral mixture (AIN[76]), 3.5; vitamin mixture (AIN[76]), 1.0; cellulose, 4.0; choline chloride, 0.15; cholesterol, 0.5; sodium cholate, 0.15 and sucrose, 60.7. Relative high amounts of carbohydrate in the form of sucrose or fructose stimulate lipogenesis and therefore have been shown to be associated with an increased circulating concentration of serum triglyceride; we supplemented the sucrose as a carbohydrate source to the diets (*10*). The taurine group was fed the same diet except for supplementation with 5% taurine at the expense of sucrose, as described previously (*9*). Two separate experiments were performed as described later. The animals had free access to the diets and deionized water for 14 d.

Assay for liver mitochondrial carnitine palmitoyltransferase. In the first experiment, the rats (7 rats in each group) were sacrificed by decapitation and the liver was excised and weighed. A portion of liver was rapidly homogenized in 9 volumes of chilled 0.25 M sucrose solution. The homogenate was centrifuged at 1,000 ×*g* for 10 min to sediment the nuclei and cell debris, and the supernatant was then centrifuged at 12,500 ×*g* for 20 min to sediment the mitochondrial fraction (*11*), which was resuspended in a solution containing 0.3 M mannitol, 10 mM HEPES, and 0.1 mM EGTA, and stored at −45°C until analysis. Carnitine palmitoyltransferase activity in the mitochondrial fraction was assayed in 58 mM Tris-HCl (pH 8.0) containing 1.25 mM EDTA, 0.25 mM DTNB, 37.5 mM palmitoyl-CoA, and 1.25 mM L-carnitine according to the method of Markwell et al. (*12*). The activity obtained in the absence of L-carnitine was used as a negative control. Mitochondrial protein was analyzed by the method of Lowry et al. (*13*). Blood serum was collected by centrifugation, and stored at −45°C until analysis.

Liver perfusion. In the second experiment, at approximately 9:00 am on the 15th day, rats from the control and taurine groups (6 rats in each group) were anesthetized with pentobarbital (i.p., 5 mg/100 g body weight) and their livers were surgically isolated and perfused shortly thereafter (beginning between 9:00 and 9:30 am) with 120 mL of recirculating Krebs-Henseleit buffer (pH 7.4) containing 25 mM glucose, 1.5% (w/v)

bovine serum albumin, and 25% (v/v) washed bovine erythrocytes, at a rate of 20 mL/min at 37°C. The rate of synthesis and secretion of triglyceride and cholesterol is influenced by the quality and quantity of exogenous fatty acids added in the perfusion medium; Kohout et al. (*14*) and Goh and Heimberg (*15*) and found that oleic acid substrate is the best substrate for the synthesis and secretion of triglyceride and cholesterol by the isolated rat liver perfused with equimolar quantities of palmitic, oleic, or linoleic acid, although the rate of hepatic uptake of free fatty acids was similar for all fatty acid substrates. In the present study, we therefore employed oleic acid as an exogenous fatty acid substrate. At the beginning of recirculation, 5 mL of 20 mM potassium [1-^{14}C]oleate (100 μmol; specific activity, 0.25 MBq/mmol) was added as an exogenous fatty acid, and the same solution was continuously infused at a rate of 4.5 mL/h (90 μmol) by a mechanical infusion pump. At 1-h intervals, 20 mL of perfusate was taken for analyses of ketones and lipids. An equal volume of fresh perfusion medium was added after each sampling to maintain the total volume of perfusion medium at 120 mL. The liver perfusions of the control and taurine groups were performed at the same time, and continued for a total of 4 h, as described in detail previously (*9*, *16–18*).

Chemical analyses. All assays of the perfusate were carried out after removing erythrocytes by centrifugation. Acetoacetate and β-hydroxybutyrate were measured enzymatically in a deproteinized sample of liver perfusate (*9*, *16–18*). Lipids in serum and liver (experiment 1), and those in perfusate and postperfusion liver (experiment 2) were isolated and purified according to the method described by Folch et al. (*19*). Total- and free-cholesterol, triglyceride, and phospholipid in the lipid extract were measured as described elsewhere (*9*, *16–18*). Cholesteryl ester was calculated as the difference between total and free cholesterol. Radioactivity derived from [1-^{14}C]oleate in ketone bodies was measured as described previously (*16*, *17*), while activities in lipid fractions were measured after separation by thin-layer chromatography on silica gel 60G using a solvent mixture of n-hexane, diethyl ether, and acetic acid (80/20/1, v/v/v) (*16*, *17*). Bands corresponding to cholesteryl ester, triglyceride, free fatty acid, diglyceride, and phospholipids were detected by iodine vapor and scraped into vials. Radioactivity was measured in a liquid scintillation counter after the addition of a toluene-based scintillation cocktail.

Statistical analyses. Data are expressed as the mean±SE. The statistical significance of differences between means was evaluated by Student's *t*-test with significance defined as $p<0.05$ (*20*).

RESULTS

Concentrations of lipids in serum and liver

Food intake and growth were comparable between the groups in both experiments, as shown in Table 1. Relative liver weight tended to be lower in the taurine groups than in the control groups. Dietary taurine caused 39% and 53% reductions in the concentration of serum cholesteryl ester and free cholesterol, respectively (experiment 1). No significant effects of taurine feeding on the concentration of other serum lipid components such as triglyceride and phospholipid were noted. A significant reduction was seen in the hepatic concentration of cholesteryl ester (18% and 22% reductions in experiments 1 and 2, respectively), but not in other lipid components in either experiment, although in experiment 2 lipid components were measured at the end of perfusion.

Table 2. Ketogenesis and incorporation of [1-^{14}C]oleic acid substrate into ketone bodies by isolated perfused livers (Experiment 2).[1]

Time (h)	Control	Taurine
	(mmol/whole liver)	
Ketogenesis		
1	158±30	216±8
2	233±44	307±16
3	320±59	418±28
4	407±69	506±37
Incorporation of [1-^{14}C]oleic acid into ketone bodies		
1	5.5±0.9	8.6±1.3
2	7.0±1.4	12.1±1.9*
3	9.8±2.1	17.8±2.2*
4	13.0±2.7	22.0±2.8*

[1] Data are the mean±SE of 6 rats.
* Significantly different from control group at $p<0.05$.

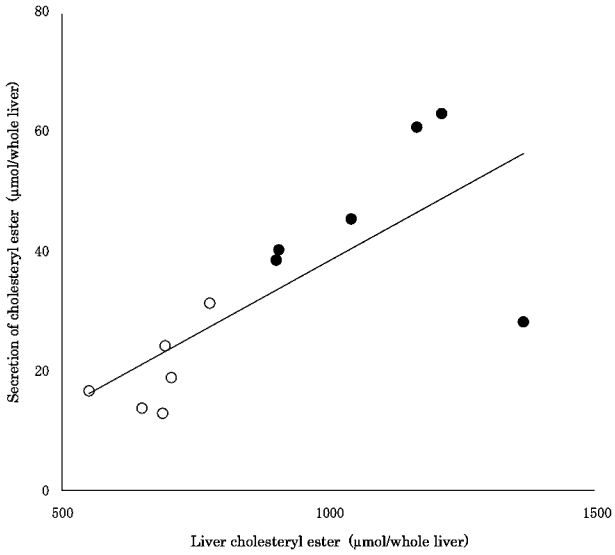

Fig. 1. Relationship of cholesterol ester content in liver to secreted cholesterol ester in liver perfusate in rats fed with taurine (●) and without taurine (○). Data are the mean±SE of 6 rats.

Hepatic fatty acid oxidative enzyme activity

The activity of liver mitochondrial carnitine palmitoyltransferase, the rate-limiting enzyme for fatty acid oxidation, was significantly higher in the taurine groups than in the control groups (Table 1).

Table 3. Effect of taurine on net secretion of cholesteryl ester and free cholesterol by the perfused liver and the incorporation of [1-^{14}C]oleic acid substrate into cholesteryl ester in perfusate (Experiment 2).[1]

Time (h)	Control (μmol/whole liver)	Taurine (μmol/whole liver)
Cholesteryl ester secretion		
1	17.0±2.1	8.1±1.2*
2	28.6±3.4	14.5±0.9*
3	34.5±4.8	16.2±1.9*
4	40.3±5.4	17.2±1.0*
Free cholesterol secretion		
1	5.2±1.0	3.5±0.6
2	8.4±1.3	5.0±0.8
3	12.2±2.0	6.6±0.6
4	14.2±2.5	8.9±1.1
Incorporation of [1-^{14}C]oleic acid		
1	2.9±0.8	2.3±0.7
2	5.9±1.0	4.6±0.7
3	7.5±1.1	4.8±0.5*
4	8.5±1.5	5.0±0.6*

[1] Data are the mean±SE of 6 rats.
* Significantly different from control group at $p<0.05$.

Table 4. Conversions of infused oleic acid substrate by the perfused liver (Experiment 2).[1]

	Control (% of total uptake)	Taurine (% of total uptake)
Products of esterification		
Perfusate lipids		
Cholesteryl ester	2.0±0.3	1.1±0.2*
Triglyceride	16.8±3.4	9.3±1.2
Partial glycerides	0.2±0.0	0.2±0.0
Phospholipid	0.4±0.0	0.3±0.1
Liver lipids		
Cholesteryl ester	7.51±0.3	5.8±0.4*
Triglyceride	31.6±1.8	29.4±2.6
Free fatty acid	1.5±0.3	1.4±0.2
Partial glycerides	1.6±0.1	1.1±0.2
Phospholipid	9.1±0.1	9.3±1.1
Products of oxidation		
Ketone body	3.1±0.6	5.2±0.7*
Undetermined[2]	26.2±2.9	36.8±3.1*

[1] Data are the mean±SE of 6 rats.
[2] These values represent the total radioactivities not present in lipids and ketone bodies. This is primarily CO_2 but also contains some other water soluble intermediates of oxidation such as acetyl-CoA and krebs cycle intermediates.
* Significantly different from control group at $p<0.05$.

Hepatic ketone body production and lipid secretion

Hepatic uptakes of exogenous [1-^{14}C]oleic acid, as described previously (*16*, *17*), were similar in the control and taurine groups after each hour of perfusion (control group, 175.9±2.7, 266.4±2.1, 352.9±3.3, and 426.3±6.0 μmol/whole liver vs. taurine group, 171.3±2.2, 263.3±2.6, 350.6±2.3, and 423.1±4.7 μmol/liver). This result indicates that direct comparisons can be made concerning utilization of exogenous oleic acid substrate by the livers in each group (i.e., oxidation vs. esterification pathways).

Ketone body production and incorporation of [1-^{14}C]oleic acid into ketone bodies are shown in Table 2. Dietary taurine tended to stimulate ketone body production to amount to 1.2-times the production in controls, which was consistent with previous observations (*9*). Incorporation of exogenous [1-^{14}C]oleic acid substrate into perfusate ketone bodies likewise significantly increased in the taurine group by 1.7-times. On the other hand, dietary taurine caused a 57% reduction in the hepatic secretion rate of cholesteryl ester at 4 h, compared with controls (Table 3). Incorporation of infused exogenous [1-^{14}C]oleic acid substrate into secretory cholesteryl ester was also significantly repressed by 45%. Secretion of free cholesterol was reduced by 40% in the taurine group compared with the control group (6.6±0.8, 9.9±1.4, 14.4±2.1, and 16.2±3.3 for the control group vs. 4.3±0.5, 5.5±1.2, 6.8±1.0, and 9.8±1.5 for the taurine group; the differences at 2 and 3 h were statistically significant).

In this study, the decreased concentration of hepatic cholesteryl ester appeared to relate to reduced secretion of this lipid molecule by the liver, so we analyzed the relationship between amounts of cholesteryl ester accumulated in the liver and those secreted by the liver using linear regression analysis. A very close positive correlation was evident between these two cholesteryl ester pools ($r=0.737$, $p<0.01$, Fig. 1).

Cumulative secretion of triglyceride and incorporation of [1-^{14}C]oleate into triglyceride in the perfusate were consistently lower in taurine-fed rats than in control rats at all time points during perfusion, although the differences were not statistically significant due to large variation (data not shown).

Fate of exogenous [1-^{14}C]oleic acid substrate

The fate of exogenous [1-^{14}C]oleic acid is summarized in Table 4. Some undetermined products were derived from exogenous [1-^{14}C]oleic acid, mainly CO_2 and various water-soluble intermediates such as acetyl-CoA and Krebs cycle intermediates (*16*, *17*). In control livers, 71% of the [1-^{14}C]oleic acid taken up by the liver was incorporated into perfusate and liver lipids, especially triglyceride, and to a lesser extent phospholipid and cholesteryl ester. The other 29% of the labeled compound was converted to oxidation products, including undetermined fractions and ketone bodies. This indicates that the free fatty acid supplied exogenously during the perfusion period was metabolized in the liver via either the pathway of esterification or that of oxidation. On the other hand, dietary taurine significantly decreased conversion of oleic acid into perfusate and hepatic cholesteryl esters, and increased conversion into ketone bodies and undetermined fractions. On the whole, then, dietary taurine altered the metabolism of exogenous oleic acid in favor of oxidation pathways at

the expense of esterification, especially of the cholesteryl ester fraction.

DISCUSSION

The addition of taurine to a cholesterol-enriched diet, but not to cholesterol-free diets, has been reported to reduce serum and liver cholesterol concentrations, particularly those of cholesteryl ester, in rats (*21*). We have previously reported that addition of taurine to a diet enriched with cholesterol significantly ameliorated dietary cholesterol-dependent increases in serum and hepatic concentrations of cholesteryl ester of in rats (*9*). The present study again showed that this sulfur-containing amino acid reduces serum and hepatic concentrations of cholesteryl ester (Table 1). These observations indicate that dietary taurine can improve hypercholesterolemia and development of fatty liver induced by high-cholesterol diets in rats.

With regard to hepatic metabolism of fatty acid derived from endogenous and exogenous fatty acid substrates, it has been previously reported that there is a reciprocal response in the pathways of esterification and oxidation of these fatty acids, in the livers of rats under various physiological and nutritional conditions (*9*, *14–18*, *22*, *23*). In these conditions, we examined whether dietary taurine-dependent reduction in the concentration of serum and liver cholesteryl ester is related to an altered hepatic metabolism of fatty acid in the liver. The mechanisms by which dietary taurine lowers serum cholesterol are not completely understood. Gandhi and Mulky reported that oral administration of taurine to rats with Triton-induced hyperlipidemia leads to a significant reduction in the serum concentration of total cholesterol (*24*), suggesting that hepatic secretion of total cholesterol is decreased by taurine administration. We previously reported that dietary taurine reduces the rate of secretion of cholesterol, especially cholesteryl ester, by the rat liver (*9*). The present studies showed similar results (Table 3). These observations therefore suggest that the hypocholesterolemic effect of dietary taurine results partly from a decreased rate of hepatic secretion of cholesteryl ester, since the influx of cholesterol entering the blood stream from the liver in the form of lipoprotein-cholesterol is a critical determinant of serum cholesterol concentration (*22*).

The present study clearly showed that the reduced hepatic concentration of cholesteryl ester induced by the feeding of taurine is highly correlated with a reduced hepatic secretion rate of this lipid molecule (Fig. 1). Fungwe et al. reported that increasing the amount of cholesterol in the diet caused a dose-dependent accumulation of cholesteryl ester in the liver, while the hepatic secretion rate of cholesteryl ester was proportional to cholesteryl ester accumulation in the liver (*23*). A possible mechanism for the lowering of hepatic cholesterol including cholesteryl ester by dietary taurine in the presence of high cholesterol intake involves increased conversion of cholesterol to bile acids, which are secreted in the bile (*4*). Thus, these observations suggest that taurine-induced suppression of hepatic cholesteryl ester caused by dietary cholesterol is critical for the hepatic secretion rate of cholesteryl ester in the rat.

The fatty acid composition of serum cholesteryl ester has long been recognized to resemble that of cholesteryl ester in the rat liver (*25*). This implies that a portion of serum cholesteryl ester content is derived from direct secretion by the liver after being synthesized from fatty acyl-CoA and free cholesterol by acyl CoA:cholesterol acyltransferase (ACAT). Consistent with these observations, the present study showed that exogenous oleic acid infused during hepatic perfusion was incorporated into hepatic cholesteryl ester and secreted into perfusate in the form of lipoprotein-cholesteryl ester (Table 3). On the other hand, dietary taurine clearly slowed the hepatic secretion rate of cholesteryl ester, accompanying a concomitant reduction in incorporation of exogenous oleic acid into this lipid molecule (Tables 3 and 4). This finding is consistent with the observation that taurine represses ACAT activity in the liver (*4*, *5*). The reduction of these two cholesterol parameters is probably due to a decrease in a putative hepatic metabolic pool of cholesterol through increased conversion of cholesterol to bile acids by dietary taurine as described above.

On the other hand, we previously suggested that taurine-dependent decreases in the hepatic accumulation and secretion rate of cholesteryl ester in the rat are inversely related to stimulation of hepatic ketogenesis (*9*). This reciprocal response was confirmed in the present studies. We first measured liver mitochondrial carnitine palmitoyltransferase, a rate-limiting enzyme of fatty acid transport across the mitochondrial membrane preceding β-oxidation, and found that the feeding of taurine resulted in significantly elevated enzyme activity. This result suggests that taurine enhances an influx of fatty acids across the mitochondrial membrane, which accounts for its stimulatory effects upon ketone body production, consistent with previous experiments (*9*), but a tendency toward elevation of ketone body production was observed in the present study (Table 2). Since this observation was further supported by augmentation of radioactivity from exogenous [1-^{14}C]oleic acid substrate in perfusate ketone bodies and undetermined fractions, as shown in Table 4, we concluded that dietary taurine can enhance ketone body production and thus fatty acid oxidation, especially from exogenous fatty acid substrate. Considered together with the finding concerning ACAT, these observations therefore suggest that the decrease in incorporation of exogenous oleic acid into cholesteryl ester in rats fed taurine could result in enhanced diversion of fatty acids to the oxidation pathway, resulting in a higher formation of ketone bodies and some other undetermined fractions. Consequently, the altered balance between fatty acid oxidation and esterification to cholesteryl ester may be secondary to changes in the cholesterol metabolism caused by dietary taurine.

The results of present and previous studies (*9*) sug-

gest that dietary taurine has hypocholesterolemic activity via reduced hepatic secretion of cholesteryl ester, and enhanced oxidation of exogenous fatty acids may contribute to reduced cholesteryl ester synthesis and secretion by the liver.

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