

# Thyroid hormone differentially augments biliary sterol secretion in the rat. I. The isolated-perfused liver model

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**Abstract** Thyroid hormone lowers serum cholesterol and alters sterol metabolic processes. This laboratory has previously reported increased biliary lipid secretion as an early effect of triiodothyronine ( $T_3$ ) in the rat. To evaluate whether the bile lipid action of  $T_3$  is a primary or secondary effect, the isolated-perfused rat liver model was used. Red blood cells in lipid-free buffer were used to perfuse livers of euthyroid and methimazole-hypothyroid rats, as well as hypothyroid rats given  $T_3$  at intervals before perfusion. Bile flow was maintained by taurocholate perfusion. Hypothyroid rats had elevated pre-perfusion serum cholesterol compared to euthyroid ( $107 \pm 4$  vs.  $65 \pm 2$  mg/dl) and decreased biliary cholesterol ( $0.016 \pm 0.001$  vs.  $0.031 \pm 0.004$   $\mu$ mol/g liver/h) secretion. Serum cholesterol decreased to euthyroid levels by 18 h after  $T_3$ , an effect that was prevented by bile duct ligation. Bile cholesterol secretion doubled by 18 h, and reached levels twice euthyroid by 42 h, while phospholipid secretion doubled to levels just above euthyroid. The fourfold increase in biliary cholesterol secretion occurred with lipid-free perfusion and unchanging bile acid uptake or output. It occurred without a fall in hepatic lipoprotein cholesterol secretion. Blockade of cholesterol synthesis with lovastatin failed to alter  $T_3$ -augmented bile cholesterol secretion. **■** We conclude that  $T_3$  induces biliary cholesterol secretion concomitantly with the fall in serum cholesterol. This augmented biliary secretion did not appear to depend upon lipoprotein uptake, increased bile acid transport, or cholesterol synthesis. It did not occur at the expense of hepatic lipoprotein secretion. Facilitated biliary lipid secretion may be a primary effect of  $T_3$ .—Gebhard, R. L., B. G. Stone, J. P. Andreini, W. C. Duane, C. D. Evans, and W. Prigge. Thyroid hormone differentially augments biliary sterol secretion in the rat. I. The isolated-perfused liver model. *J. Lipid Res.* 1992. 33: 1459–1466.

**Supplementary key words** cholesterol • biliary lipids • HMG-CoA reductase

Thyroid hormone status has long been known to influence serum cholesterol levels (1, 2). Lipoprotein levels are increased in hypothyroidism, while thyroid hormone administration results in reduced levels of low density lipoproteins (LDL), intermediate density lipoproteins (IDL), and cholesteryl ester-rich forms of high density lipoproteins (HDL) (3–6). Consistent with the reduction

in lipoprotein cholesterol by thyroid hormone, LDL receptor activity in cultured cells has been shown to be induced by triiodothyronine ( $T_3$ ) in vitro (7), and rat liver LDL receptor mRNA has been shown to increase after  $T_3$  in vivo (8). Correlative findings for receptor-mediated LDL clearance have been reported in humans (9). Thyroid hormone actions on rat apolipoprotein metabolism have also been reported. These include hepatic apolipoprotein A-I metabolism and effects on hepatic processing of apolipoprotein B (5, 6, 8, 10–12).

Thyroid hormone has further been shown to have widespread effects on other aspects of cholesterol metabolism. In accord with the pattern of lipoprotein cholesterol changes, cholesterol absorption is reported to be increased in hypothyroidism and decreased after  $T_3$  (13, 14). These effects are perhaps caused by changes in gut motility. In contradistinction to what might be expected with the changes in serum cholesterol levels and LDL receptor activity, hepatic cholesterol synthesis is decreased in the low thyroid state and increased by  $T_3$  (15–17). Activity and mRNA levels for the rate-determining enzyme of hepatic cholesterol synthesis, HMG-CoA reductase, are low in hypothyroid states and increase markedly after  $T_3$  (8, 15–17). The increase in these parameters occurs 30 h and more after  $T_3$  (8, 17). This contrasts with the rapid and more direct nuclear  $T_3$  effects on other mRNA transcription (8, 18). One such rapid response to  $T_3$  is reported to be an increase in mRNA levels for hepatic  $7\alpha$ -hydroxylase, the rate-limiting enzyme for bile acid synthesis, and bile acid synthesis itself (8, 13). Thus, augmented synthesis of

Abbreviations:  $T_3$ , triiodothyronine; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; BW, body weight.

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bile acids from cholesterol may contribute to the decrease in serum cholesterol levels after thyroid hormone administration.

This laboratory previously reported a marked reduction in biliary lipid secretion in the hypothyroid rat (17). Administration of  $T_3$  in vivo resulted in rapid and substantial facilitation of biliary cholesterol and phospholipid secretion with only a modest increase in bile acid secretion (17). In these studies, there also appeared to be a temporal disparity between the biliary cholesterol and bile acid secretion rates. A direct action of thyroid hormone on biliary cholesterol and phospholipid secretion was postulated, with uncoupling from the usual linkage to bile acid secretory rate. The current studies were undertaken in order to determine whether changes in bile acid secretion rate are required for the thyroid hormone effect on biliary cholesterol and phospholipid secretion. Furthermore, the source of augmented biliary sterol secretion after  $T_3$  was not determined in the previous study. Bile sterols might increase as a result of induced lipoprotein uptake, at the expense of hepatic lipoprotein secretion, or as a consequence of increased synthesis. Thus, the second goal of these studies was to determine whether facilitated hepatic lipoprotein uptake or diminished hepatic lipoprotein production are essential to  $T_3$  induction of biliary lipid secretion. To address these issues, we have used the isolated-perfused rat liver model. This system allows the measurement of biliary cholesterol and phospholipid secretion while maintaining bile acid secretion constant. As livers are perfused with lipid-free medium, the model eliminates lipoprotein cholesterol uptake as a source for biliary secretion. Finally, the model allows measurement of simultaneous hepatic lipoprotein secretion into hepatic venous blood (perfusate).

## METHODS AND MATERIALS

Male Sprague-Dawley rats (Biolab Corporation, St. Paul, MN) initially weighing 120–140 g were maintained in a light-reversed room, with mid-dark point at 1200. Animals were fed standard laboratory rat chow (Purina Mills, St. Louis, MO) and were made hypothyroid with 0.025% methimazole (Aldrich, Milwaukee, WI) in drinking water for at least 3 weeks. Concurrent control euthyroid rats were fed and housed in the same manner but did not receive methimazole. Some hypothyroid animals received a single intraperitoneal dose of 200  $\mu\text{g}$   $T_3$ /100 g body weight, a dose calculated to saturate nuclear  $T_3$  receptors and provide more than 90% receptor occupancy for at least 54 h. Donor livers for perfusion were taken at time 1000–1200 from euthyroid and hypothyroid animals as well as  $T_3$ -treated hypothyroid rats 6, 18, 24, and 42 h after  $T_3$  replacement.

## Liver perfusion studies

At time of study, animals were anesthetized with pentobarbital. Blood was obtained for serum cholesterol determination, and the bile duct was cannulated with PE-10 tubing for bile collection. A recirculating isolated liver perfusion was established by the methods of Mortimer (19), as previously described (20). The perfusate consisted of 21–25% washed fresh sheep or dog red blood cells suspended in Earle's balanced salt solution (GIBCO, Grand Island, NY) containing 3 g/dl of lipid-free bovine serum albumin (Sigma, St. Louis, MO). Perfusate was oxygenated with 95%  $\text{O}_2$ /5%  $\text{CO}_2$  using a silastic lung, while liver and perfusate temperatures were strictly maintained at 36–37°C. Perfusate pH was titrated to 7.4 with saturated  $\text{NaHCO}_3$  and circulated at a rate of 49.5 ml/min per kg body weight for 2–2.5 h. Bile acid secretion was maintained constant during perfusion by continuous delivery of sodium taurocholate (Sigma, St. Louis, MO) into the portal vein cannula at a physiologic rate of 120  $\mu\text{mol/kg}$  body weight per h. The sodium taurocholate was initially dissolved at 25 mM concentration in Earle's with albumin but without red blood cells to avoid hemolysis, and then injected into the circulating perfusate at appropriate rate.

Bile was collected from the isolated-perfused livers at half-hour intervals for measurement of volume and bile lipids. At the end of perfusion, perfusate was centrifuged at low speed for 15 min to remove RBCs. The cell-free phase was adjusted with KBr to a density of 1.006 g/ml and VLDL obtained by centrifugation at 186,000  $g$  for 16 h. The infranate was then adjusted to a density of 1.21 g/ml, and an HDL fraction (containing LDL and IDL) was isolated by further gradient centrifugation (21). Livers were weighed at the end of perfusion and all values were expressed per gram liver weight to correct for differences in animal size.

## Bile obstruction studies

Euthyroid, hypothyroid, and  $T_3$ -treated hypothyroid animals were also studied for the effect of bile duct ligation on blood cholesterol levels and biliary lipid secretion. Under metofane anesthesia, common bile ducts of experimental animals were ligated three times with 4-0 silk ligatures, and the duct was severed between ligatures to prevent reestablishment of bile flow. Four days after bile duct obstruction, animals were again anesthetized and bile was collected from the dilated duct, proximal to the obstruction, for 0.5–1 h to measure bile lipids. Animals were then bled for measurement of serum cholesterol, and liver was removed for weight and measurement of cholesterol and microsomal HMG-CoA reductase activity. Some hypothyroid animals were given 200  $\mu\text{g}$   $T_3$ /100 g BW IP 2 days after bile duct ligation, so that measurements were made 48 h after the  $T_3$ .

TABLE 1. Basal characteristics of isolated-perfused liver animals

	Age-Matched Euthyroid (n = 6)	Weight-Matched Euthyroid (n = 7)	Hypothyroid (n = 6)
Body weight (g)	308 ± 10 <sup>a</sup>	233 ± 4 <sup>b</sup>	227 ± 23 <sup>b</sup>
Liver weight (g)	13.1 ± 0.5 <sup>a</sup>	10.4 ± 0.4 <sup>b</sup>	8.8 ± 0.8 <sup>b</sup>
Liver cholesterol (mg/g protein)	11.5 ± 0.3 <sup>a</sup>	12.1 ± 0.3 <sup>a</sup>	10.0 ± 0.2 <sup>b</sup>
Bile flow (μl/g liver/h)	88 ± 3 <sup>a</sup>	88 ± 8 <sup>a</sup>	61 ± 3 <sup>b</sup>
Bile lipids (μmol/g liver/h)			
Cholesterol	0.031 ± 0.004 <sup>a</sup>	0.067 ± 0.008 <sup>b</sup>	0.016 ± 0.001 <sup>c</sup>
Phosphatidylcholine	0.26 ± 0.02 <sup>a</sup>	0.26 ± 0.01 <sup>a</sup>	0.17 ± 0.02 <sup>b</sup>
Bile acids	2.59 ± 0.14 <sup>a</sup>	2.10 ± 0.13 <sup>b</sup>	2.66 ± 0.13 <sup>a</sup>

Liver weight and liver cholesterol at the end of perfusion, bile flow and lipid secretion during perfusion of euthyroid and hypothyroid rats, and body weight obtained just prior to perfusion. Values are mean ± SE. Values with the same superscript are not significantly different ( $P > 0.05$ ); most others are  $P < 0.01$ . Weight-matched euthyroid rats are younger than hypothyroid or age-matched euthyroids and show higher bile cholesterol secretion than older, larger euthyroid rats. Both control groups are shown for comparison. Bile cholesterol concentration in hypothyroid rats (0.26 μmol/ml) was lower than in age-matched (0.35 μmol/ml) or weight-matched (0.76 μmol/ml) controls.

### Chemical analyses

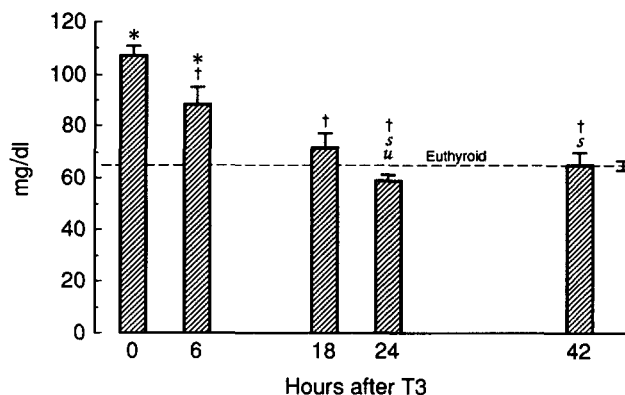
Free cholesterol was extracted with hexane from bile that had been diluted with 2–3 volumes of methanol. Total cholesterol was extracted from serum, perfusate lipoprotein fractions, and liver homogenates using the method of Abell et al. (22). Cholesterol was measured as the trimethylsilyl ether using a Hewlett-Packard 5830A gas chromatograph (Palo Alto, CA) (23). Total bile acid concentration in bile was measured by the procedure of Talalay (24). Phosphatidylcholine concentration in bile (essentially the only bile phospholipid in the rat) was measured by an enzymatic choline assay (25) using the PL kit-K from Nippon-Shoji Kaisha, Ltd. (Osaka, Japan). Protein was measured by the method of Lowry et al. (26). Hepatic microsomes were prepared from liver homogenates and microsomal HMG-CoA reductase was assayed as we have previously described (27). Radio-labeled [<sup>14</sup>C]HMG-CoA and [<sup>3</sup>H]mevalonate were purchased from New England Nuclear (Boston, MA). Lovastatin (formerly mevinnolin) was a generous gift from Merck Laboratories. All results are expressed as mean ± standard error. Data were analyzed for significance by Student's unpaired *t*-testing.

### RESULTS

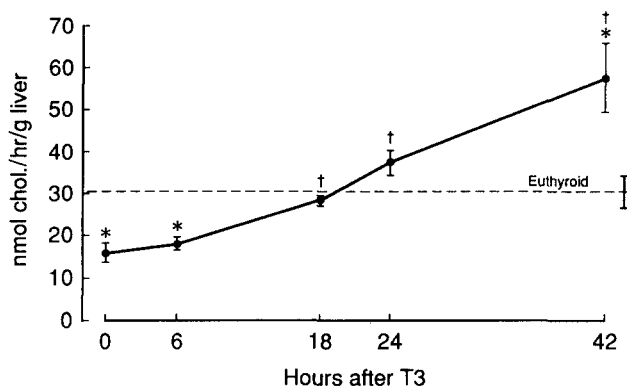
Shown in **Table 1** are body weights immediately prior to perfusion, and liver cholesterol values at the end of perfusion for hypothyroid animals (Hypo) compared to age-matched and body weight-matched euthyroid control animals (Eu). Also shown in the table are bile flow and biliary lipid secretion rates, expressed as μmol/g liver per h for bile acids, cholesterol, and phosphatidylcholine during the time of perfusion. Because sodium taurocho-

late was infused at a physiologic rate of 120 μmol/kg BW per h to maintain bile acid secretion, its secretory rate was constant at 88–110 μmol/kg BW per h (1.7–2.6 μmol/g liver per h) for all study groups. Biliary cholesterol and phospholipid secretion was lower in hypothyroid animals compared to euthyroid controls, despite the fact that bile acid secretion rate was artificially maintained at euthyroid levels. Biliary cholesterol concentration was significantly reduced in hypothyroid rats (mean 0.26 μmol/ml) compared to age-matched (0.35 μmol/ml) or weight-matched (0.76 μmol/ml) animals. Bile flow was also decreased, making the biliary cholesterol secretion rate substantially less than controls. Bile cholesterol secretion, but not phospholipid, was greater in young, weight-matched euthyroid animals compared to older ones ( $P < 0.05$ ).

**Fig. 1** shows that pre-perfusion serum cholesterol levels were higher for hypothyroid animals than for euthyroid



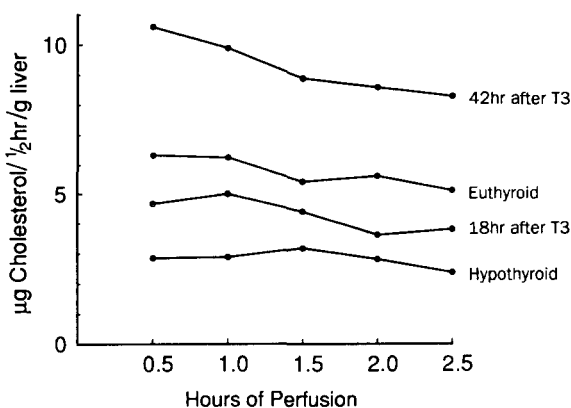
**Fig. 1.** Pre-perfusion serum cholesterol levels. Blood samples were obtained from hypothyroid and single-dose T<sub>3</sub>-treated hypothyroid rats immediately prior to liver perfusion. Euthyroid value is shown for comparison. Values are mean ± SE for n = 6–7 rats; \*,  $P < 0.01$  vs. euthyroid; †,  $P < 0.02$  vs. hypothyroid; ‡,  $P < 0.02$  vs. 6 h; §,  $P < 0.02$  vs. 18 h.



**Fig. 2.** Bile cholesterol secretion into bile during liver perfusion. Bile was obtained during perfusion of hypothyroid and single-dose T<sub>3</sub>-treated hypothyroid livers. Euthyroid secretion (age-matched) is shown for comparison. Hypothyroid bile flow increased by 50% after T<sub>3</sub>, reaching euthyroid levels of  $93 \pm 6 \mu\text{g/g liver/h}$  at 42 h after T<sub>3</sub>. Values are mean  $\pm$  SE,  $n = 6-7$  perfusions; \*,  $P < 0.02$  vs. euthyroid; †,  $P < 0.01$  vs. hypothyroid.

age-matched controls. However, when hypothyroid animals received T<sub>3</sub> prior to study, their serum cholesterol fell to euthyroid levels by 18 h after administration.

As shown in **Fig. 2**, biliary cholesterol secretion rate reached euthyroid levels by 18 h after in vivo T<sub>3</sub> treatment and was well above euthyroid levels by 42 h after T<sub>3</sub>. As bile flow increased only modestly (by 50% at 42 h), most of the augmented secretion was due to increased concentration. As expected, essentially all biliary cholesterol was in the free form, even during maximal secretion at 42 h. The differences in cholesterol secretion rates were maintained throughout the 2.5 h of lipoprotein-free perfusion, with output during the last half hour being 80% of initial output for all T<sub>3</sub> treatment groups (**Fig. 3**). This con-

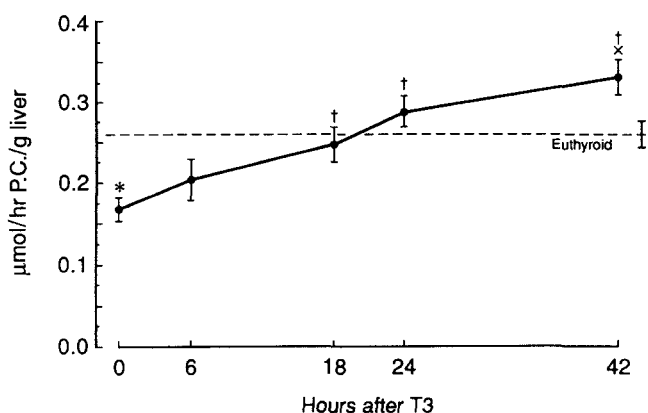


**Fig. 3.** Bile cholesterol secretion in half-hour increments during perfusion. Values are means for  $n = 6-7$  perfusions. Secretory rate falls off modestly during lipid-free perfusion for euthyroid, hypothyroid, and T<sub>3</sub>-treated hypothyroid preparations, probably because liver viability deteriorates. However, relative levels of secretion persist in the absence of lipid uptake.

sistency suggests that continuing lipoprotein cholesterol uptake was not essential for the T<sub>3</sub> augmentation of biliary cholesterol secretion.

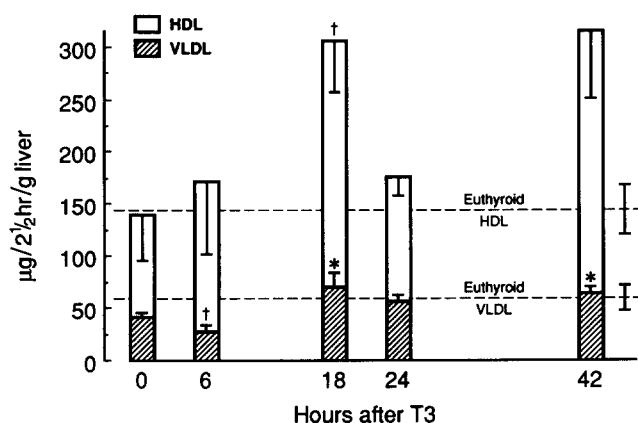
To further test whether lipoprotein cholesterol uptake was required for the facilitated biliary cholesterol secretion after T<sub>3</sub>, additional measurements were made in a group of animals studied 42 h after T<sub>3</sub>. Total cholesterol was measured in liver homogenate and hepatic microsomes from a small lobe of liver obtained prior to perfusion and from the entire liver at completion of 2.5 h of lipid-free perfusion. Microsomal cholesterol fell significantly ( $P < 0.05$ ) during perfusion from  $23.9 \pm 1.9 \mu\text{g/mg protein}$  to  $20.7 \pm 1.6 \mu\text{g/mg protein}$ . Total cholesterol in liver homogenate fell from  $2.35 \pm 0.08 \text{ mg/g wet weight}$  pre-perfusion to  $2.11 \pm 0.09 \text{ mg/g wet weight}$  ( $P < 0.01$ ) at the end of perfusion. Total liver cholesterol was calculated to fall by  $1324 \pm 148 \mu\text{g}$ , while the total amount of cholesterol secreted into bile was  $353 \pm 121 \mu\text{g}$  over the same period. Liver also secreted lipoprotein cholesterol. Thus, the increase in biliary cholesterol output seen after T<sub>3</sub> occurred at the expense of total liver cholesterol, again suggesting that lipoprotein cholesterol was not essential.

Biliary phosphatidylcholine secretion during perfusion is shown in **Fig. 4**. Phospholipid secretion by hypothyroid livers was also low, and increased to euthyroid levels by 18 h after T<sub>3</sub>. Although the phosphatidylcholine secretion by hypothyroid livers was significantly below euthyroid levels prior to T<sub>3</sub> and significantly above euthyroid levels 42 h after T<sub>3</sub>, the twofold increase in secretion could be in large measure accounted for by increased bile flow and was less marked than the fourfold increase seen for cholesterol secretion. In consequence, the ratio of cholesterol to phospholipid in bile doubled after T<sub>3</sub>.



**Fig. 4.** Bile phosphatidylcholine secretion into bile during liver perfusion. Bile was obtained during perfusion of hypothyroid and single-dose T<sub>3</sub>-treated hypothyroid livers. Age-matched euthyroid secretion is shown for comparison. As bile flow increased by 50% at 42 h after T<sub>3</sub>, much of the phospholipid response was related to flow. Values are mean  $\pm$  SE,  $n = 6-7$  perfusions; \*,  $P < 0.01$  vs. euthyroid; †,  $P < 0.05$  vs. hypothyroid; x,  $P < 0.02$  vs. hypothyroid.





**Fig. 5.** Lipoprotein cholesterol secretion into perfusate during 2.5 h liver perfusion. Lipoproteins (VLDL,  $d < 1.006$  g/ml; HDL, density between 1.006 and 1.21 g/ml) were isolated from final perfusate of hypothyroid and  $T_3$ -treated hypothyroid rat livers. Total, HDL, and VLDL cholesterol values are mean  $\pm$  SE,  $n = 6-7$  perfusions. Euthyroid values are shown for comparison; \*,  $P < 0.05$  vs. hypothyroid; †,  $P < 0.05$  vs. euthyroid.

**Fig. 5** shows the appearance of VLDL or HDL cholesterol into perfusate after 2 h of liver perfusion. There was no difference in hepatic secretion of VLDL or HDL cholesterol by hypothyroid livers compared to euthyroid livers. After  $T_3$ , secretion of lipoprotein cholesterol did not decrease in the face of increasing biliary cholesterol secretion. In fact, there was a tendency toward increased secretion of the higher density lipoprotein.

In order to evaluate the role of new cholesterol synthesis in the  $T_3$ -mediated induction of biliary cholesterol secretion, 1.2 mg of lovastatin dissolved in 0.12 ml ethanol was added to the recirculating perfusate at the initiation of liver perfusion. Lovastatin is a competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis. This dose has previously been shown to inhibit cholesterol synthesis and produce a decrease in measured activity of HMG-CoA reductase (28). After

2.5 h of perfusion, the perfusate was still able to inhibit hepatic microsomal HMG-CoA reductase activity by 80–90% in a standard assay. As shown in **Table 2**, this perfusion with lovastatin, with or without lovastatin pre-feeding, did not significantly affect lipoprotein cholesterol secretion by hypothyroid, 42 h  $T_3$ -treated hypothyroid, or young euthyroid livers, when compared to results in **Fig. 5**. More importantly, lovastatin inhibition of cholesterol synthesis did not block the  $T_3$ -mediated increase in biliary cholesterol or phospholipid secretion, as compared to **Table 1** or **Figs. 2** and **4**.

Finally, rats with surgically obstructed bile ducts were studied in order to evaluate whether the reduction in serum cholesterol after  $T_3$  is a result of increased hepatic uptake resulting from increased secretion of sterol into bile, or might occur independently of the facilitated biliary sterol secretion. Data are shown in **Table 3**. All animals lost weight after bile duct ligation. Duct obstruction is a complicated metabolic condition known to result in an elevated level of serum cholesterol and rate of cholesterol synthesis (hepatic HMG-CoA reductase activity), as well as altered bile acid synthesis (29, 30). In spite of the complexity of the model, several observations were made. Serum cholesterol levels were elevated in obstructed euthyroid rats, but they were elevated to a much greater degree in the hypothyroid rats. Treatment with  $T_3$  had no effect on serum cholesterol of ligated hypothyroid animals. Bile cholesterol concentration was significantly elevated in  $T_3$ -treated hypothyroid rats, compared to euthyroid. As expected, HMG-CoA reductase activity was high in all obstructed animals. Reductase activity 48 h after  $T_3$  administration to hypothyroid rats was higher than that of any other animal group.

## DISCUSSION

As previously shown *in vivo*, a marked reduction is found for bile flow and biliary lipid secretion by isolated-

**TABLE 2.** Lovastatin perfusion studies

	Bile Cholesterol	Bile Phosphatidylcholine	Cholesterol in Perfusate	
			VLDL	HDL
	$\mu\text{mol/g liver/h}$		$\mu\text{g/g liver/2.5 h}$	
Euthyroid (weight-matched; $231 \pm 6$ g)	$0.052 \pm 0.006^a$	$0.33 \pm 0.02^a$	$91 \pm 10^c$	$144 \pm 15^a$
Hypothyroid	$0.017 \pm 0.002^b$	$0.15 \pm 0.02^b$	$69 \pm 6^c$	$206 \pm 23^{a,b}$
Hypothyroid (42 h after $T_3$ )	$0.047 \pm 0.002^a$	$0.43 \pm 0.03^a$	$51 \pm 11^b$	$219 \pm 11^b$
Hypothyroid (42 h after $T_3$ , pre-fed lovastatin*)	$0.139 \pm 0.028^c$	$0.43 \pm 0.3^a$	$45 \pm 11^b$	$263 \pm 45^b$

Liver perfusions ( $n = 3-8$  rats/group) with lovastatin (10  $\mu\text{g/ml}$ ) added to all perfusates. At the end of perfusion, perfusate with this dose of lovastatin was able to inhibit 80–90% of HMG-CoA reductase activity when added to a microsomal assay. Values are mean  $\pm$  SE. Values with the same superscript are not significantly different ( $P > 0.05$ ). Bile cholesterol and phospholipid secretion with lovastatin is not significantly reduced from values without lovastatin shown in **Figs. 2** and **3**. Lipoprotein secretion with lovastatin is also not significantly reduced from values without lovastatin in **Fig. 5**. Euthyroid liver perfused with lovastatin is not significantly reduced from euthyroid without lovastatin (**Table 1**).

\*Animals were given  $T_3$  IP and were pre-fed 2% lovastatin in chow for 2 days prior to perfusion with lovastatin also in perfusate.

TABLE 3. Bile duct obstruction studies

	Euthyroid (n = 6)	Hypothyroid (n = 8)	Hypothyroid + T <sub>3</sub> (48 h) (n = 8)
Weight, Day 4 (g)	195 ± 22 <sup>a</sup>	157 ± 3 <sup>a</sup>	160 ± 5 <sup>a</sup>
Serum cholesterol (mg/dl)	161 ± 8 <sup>a</sup>	673 ± 78 <sup>a</sup>	655 ± 69 <sup>b</sup>
Bile lipid composition (μmol/ml)			
Cholesterol	0.209 ± 0.035 <sup>a</sup>	0.354 ± 0.092 <sup>a,b</sup>	0.542 ± 0.081 <sup>b</sup>
Phosphatidylcholine	1.68 ± 0.22 <sup>a</sup>	2.89 ± 0.29 <sup>b</sup>	1.99 ± 0.29 <sup>a,b</sup>
Bile acids	32.9 ± 2.0 <sup>a</sup>	39.2 ± 9.9 <sup>a</sup>	27.0 ± 3.5 <sup>a</sup>
Hepatic HMG-CoA reductase (pmol/mg/min)	548 ± 50 <sup>a</sup>	584 ± 120 <sup>a</sup>	1241 ± 178 <sup>b</sup>

Euthyroid and hypothyroid rats had their bile ducts totally obstructed for 4 days. Two days prior to study, hypothyroid rats in column 3 received an IP dose of T<sub>3</sub>. Values are mean ± SE. Values with the same superscript are not significantly different ( $P > 0.05$ ).

perfused livers of hypothyroid rats. Triiodothyronine injection results in an increase in bile flow and biliary secretion of cholesterol and phosphatidylcholine from the perfused liver prepared at least as early as 18 h after treatment. This increase occurs in the same time-frame as the fall in animals' serum cholesterol.

The direct relationship between thyroid status and biliary secretion of cholesterol and phospholipid did not appear to depend upon bile acid secretion, as this was consistent for all animal groups during the period of liver perfusion. Under most circumstances, there is reported to be a strong linkage of cholesterol/phospholipid secretion to bile acid secretion (31–33). Effects were studied after a single high dose of T<sub>3</sub>. However, augmented bile cholesterol secretion also occurs after a dose one-tenth that used here, as reported in a companion article (34). Thyroid hormone appears to stimulate a cholesterol and phospholipid secretory mechanism, perhaps in the form of vesicles, that is not directly related to the rate of bile acid transport. This unlinking was previously observed during in vivo experiments, where the saturation index of bile from T<sub>3</sub>-treated hypothyroid rats increased from normal values of 0.4 to values above full saturation (17). While increased bile acid synthesis and secretion are factors in sterol removal after T<sub>3</sub> (8, 13), current data demonstrate that they are not essential for the facilitated biliary cholesterol secretion.

Studies in the hypothyroid rat model have previously raised the question whether T<sub>3</sub> treatment primarily facilitates hepatic lipoprotein uptake with the resultant secretion of excess cholesterol into bile, or whether a primary T<sub>3</sub> action on secretion of hepatic cholesterol might stimulate lipoprotein uptake (17). The two events occurred within the same time-frame. Other investigations have suggested a primary action of T<sub>3</sub> on LDL receptor function (7–9). However, the current studies demonstrate that the increase in biliary cholesterol and phospholipid secretion after T<sub>3</sub> treatment of hypothyroid animals was not dependent upon uptake of circulating lipoproteins by the liver, at least during the period of lipid-free liver perfu-

sion. It appears that facilitated biliary cholesterol secretion continued throughout the time of perfusion, at the expense of total liver cholesterol and cholesterol in the microsomal compartment. Furthermore, although the bile duct obstruction model results in very complex changes in sterol metabolism, prevention of biliary sterol secretion by duct ligation was associated with failure of the usual reduction in blood cholesterol following thyroid hormone. Again, this suggests that the reduction in blood cholesterol after T<sub>3</sub> is a consequence of enhanced biliary sterol secretion. However, the possibility that T<sub>3</sub> acts on both biliary cholesterol secretion and lipoprotein uptake or LDL receptors cannot be ruled out by these studies, since lipoprotein uptake and receptors were not measured.

The reduced biliary cholesterol secretion seen in hypothyroid rats and the supranormal secretion seen after T<sub>3</sub> also did not appear to occur in conjunction with altered hepatic lipoprotein secretion. Lipoprotein cholesterol in the perfusate at termination of perfusion was identical, on a per gram liver basis, for hypothyroid and euthyroid rats. After T<sub>3</sub> injection, lipoprotein secretion during perfusion actually tended to increase during the time of increased biliary cholesterol secretion. Thus, T<sub>3</sub>-facilitated biliary cholesterol secretion in this model does not occur at the expense of hepatic lipoprotein cholesterol secretion.

Thyroid hormone is also known to stimulate cholesterol synthesis, albeit at a somewhat later time point than other sterol metabolic effects of T<sub>3</sub> (8, 15–17). It has been speculated that increased hepatic HMG-CoA reductase activity, seen after T<sub>3</sub>, might be a consequence of the facilitated biliary sterol secretion rather than a cause. This suggestion was based on the time relationships of the two events (17). In the current studies, the competitive inhibitor of HMG-CoA reductase enzyme, lovastatin, was added to perfusate during the perfusion period. The drug, at a similar dose, has previously been shown to inhibit cholesterol synthesis and measured activity of microsomal HMG-CoA reductase in the rat (28), and the perfusate containing lovastatin inhibited HMG-CoA reductase activity in a microsomal bioassay. No effect on biliary

cholesterol and phosphatidylcholine secretion during perfusion was seen with lovastatin, suggesting that cholesterol synthesis is not the primary driving force for T<sub>3</sub>-augmented secretion.

We conclude that thyroid hormone has an early action to increase bile cholesterol and phospholipid secretion in the hypothyroid rat. In the isolated-perfused rat liver model, this increase does not appear to be dependent upon the level of bile acid secretion, does not depend upon ongoing hepatic lipoprotein uptake or increased cholesterol synthesis, and does not occur at the expense of hepatic lipoprotein secretion into blood. The decrease in serum cholesterol observed after T<sub>3</sub> administration is probably due to increased lipoprotein clearance, perhaps as a consequence of hepatic cholesterol uptake stimulated by augmented biliary lipid secretion. A direct T<sub>3</sub> effect on lipoprotein uptake is also possible. The effect of thyroid hormone on biliary cholesterol secretion appears to be a primary one, perhaps mediated by enhanced cholesterol-phospholipid vesicle transport. ■

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