Thyroid hormone differentially augments biliary sterol secretion in the rat. II. The chronic bile fistula model

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Abstract To further define thyroid hormone effects on bile acid synthesis and biliary lipid secretion, studies were done in chronic bile fistula rats. Euthyroid and methimazole-hypothyroid rats, with and without triiodothyronine (T_3) injection, had total bile diversion for timed bile collections. With interrupted enterohepatic circulation, cholesterol absorption is negligible and bile acid secretion equals bile acid synthesis rate. Hypothyroid rats had diminished levels of bile acid synthesis and biliary secretion of cholesterol and phospholipid. Single dose T_3 injection produced a 13-fold increase in bile cholesterol secretion and a 3-fold increase in phospholipid secretion, both initiated 12 h after T_3. Bile acid synthesis increased by 50%, but the increase did not begin until 24 h after T_3. Neither hypothyroidism nor T_3 treatment abolished diurnal rhythms of bile acid synthesis and biliary lipid secretion. Inhibition of cholesterol synthesis with lovastatin resulted in a persistent 33% decrease in bile acid synthesis in euthyroid and hypothyroid rats, while bile cholesterol secretion only transiently decreased. Inhibition of cholesterol synthesis did not alter T_3-induced bile cholesterol secretion, with a 10-fold increase seen. However, bile acid synthesis was not stimulated by T_3 in the presence of lovastatin. We conclude that facilitated bile acid synthesis and biliary cholesterol secretion are early effects of T_3 and may account for the hypocholesterolemia of T_3. Cholesterol synthesis does not appear to be required for the T_3-induced bile cholesterol secretion.—**Gebhard, R. L., and W. F. Prigge.** Thyroid hormone differentially augments biliary sterol secretion in the rat. II. The chronic bile fistula model. *J. Lipid Res.* 1992. **33:** 1467–1473.

Supplementary key words bile acid synthesis • cholesterol • biliary lipids

Thyroid hormone has been reported to have a myriad of effects on cholesterol metabolism. Important observations after thyroid hormone administration in animals and humans include the following: reduction of circulating lipoprotein cholesterol levels (1–3), augmentation of LDL receptor activity (4–6), increase in cholesterol and bile acid synthesis (1, 4, 7), and decrease in the absorption of cholesterol (8). We have recently observed an early augmentation of bile flow and biliary lipid secretion, particularly bile cholesterol, after replacing thyroid hormone in hypothyroid rats (1, 9). Which of these changes in cholesterol metabolism are primary actions of thyroid hormone and which are indirect consequences is unclear. However, two of the earliest effects appear to be decreased blood cholesterol levels and increased biliary cholesterol secretion (1, 9).

In a companion article (9), it is reported that the thyroid hormone-induced increases in biliary cholesterol and phospholipid secretion do not appear to depend upon hepatic lipoprotein uptake or cholesterol synthesis. Furthermore, they do not occur at the expense of hepatic lipoprotein cholesterol secretion into blood (9). Previous work has suggested that the cholesterol secreted into bile and that utilized for bile acid synthesis are derived from a common pool (10). Therefore, thyroid hormone might act on this common pool to augment biliary lipid secretion. To further assess the relationship between biliary lipid secretion and bile acid synthesis with respect to thyroid hormone status, rats with chronic bile fistulae were studied. The enterohepatic circulation is interrupted in the chronic bile fistula model, so that bile acid synthesis is maximal and the rate of bile acid secretion equals the rate of bile acid synthesis. The role of cholesterol synthesis in biliary lipid secretion was also studied by administration of an inhibitor of HMG-CoA reductase activity or by provision of mevalonate, the product of HMG-CoA reductase and a key precursor of cholesterol synthesis.

METHODS AND MATERIALS

Male Sprague-Dawley rats, with initial weights of 120–140 g, were made hypothyroid by at least 3 weeks of methimazole treatment (0.025% in drinking water).

Abbreviations: LDL, low density lipoprotein.

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Animals were maintained in a light-reversed room as described in a companion article (9). At time of surgery, hypothyroid rats and euthyroid controls (weighing between 180 and 220 g) were surgically prepared with silastic jugular vein catheters and PE-50 bile duct catheters under metofane anesthesia as previously described by Duane, Gilberstadt, and Wiegand (11). Catheters were exteriorized to the back and were run through a spring harness that allowed animals to move freely in metabolic cages. Animals had access to standard Purina rat chow (St. Louis, MO), and either 0.025% methimazole for hypothyroid rats or plain drinking water for euthyroid rats. A solution of NaCl (77 meq/l), $NaHCO_3$ (30 meq/l), and KCl (8 meq/l) was infused into the jugular catheter at a rate of 7.1 ml/24 h. Bile was continually drained by gravity syphonage.

After at least 2 days for stabilization and to allow maximum induction of bile acid synthesis, bile was collected using a timed fraction collector, in 90-min increments continuously for 5–7 days. Some hypothyroid animals were given triiodothyronine (T_3) as a single intraperitoneal dose of 200 μg/100 g body weight after 48 h of basal bile collection. T_3 was given at the mid-dark time point, and the collections were continued. This dose of T_3 was calculated to maintain occupancy of at least 90% of nuclear T_3 receptors for at least 54 h. Some animals also received a soluble form of the HMG-CoA reductase inhibitor lovastatin intravenously (mevinolinic acid 10 mg/kg per day) as a continuous infusion or lovastatin orally (0.3% by weight in chow) at the same time as T_3 or beginning 12 h after the T_3. Other animals received intravenous mevalonate, the product of HMG-CoA reductase enzyme, in the form of mevalonolactone at a dose of 374 mg/day.

Bile volume was measured by weighing tared tubes. Bile measurements included cholesterol by gas–liquid chromatography (12), phosphatidylcholine by enzymatic choline assay (13), and bile acids by the method of Talalay (14), as we have previously described (1, 9). Some data are reported on an hourly basis in order to demonstrate the time course of secretory changes. Most secretion data were combined into 12-h intervals to allow for ease of statistical comparisons. For some experiments, activity of hepatic microsomal HMG-CoA reductase, the rate determining enzyme of cholesterol synthesis, was measured as previously reported (15). Protein was measured by the method of Lowry et al. (16).

Chemicals, drugs, and reagents were obtained from commercial sources as described in a companion article (9). Lovastatin (formerly mevinolin) was a gift from Merck Sharp and Dohme Research Laboratories and was converted to mevinolinic acid by dissolving 4 mg in 0.1 ml absolute ethanol, adding 0.15 ml of 0.1 N NaOH, heating at 50°C for 2 h, neutralizing to pH 7.2 with HCl, and bringing to a final volume of 1 ml with distilled water (personal communication from Dr. Alfred W. Alberts, Merck Sharp and Dohme Research Laboratories). Mevalonolactone was obtained from Sigma (St. Louis, MO) and a stock solution was prepared by dissolving 10 g in water to a final volume of 20 ml, and storing at −20°C until used. Sufficient quantity of this stock was then added to the intravenous infusion solution to provide 374 mg/day. Values are reported as mean ± standard error. Data were tested for significance using Student's unpaired *t*-test.

RESULTS

As shown in **Table 1,** bile flow as well as secretion of biliary cholesterol and phosphatidylcholine were reduced in hypothyroid rats when compared to weight-matched euthyroid controls. Secretion of bile acids, which also represents bile acid synthesis rate in this model, was also reduced in comparison to the euthyroid animals. Also shown, treatment of hypothyroid rats with a single high dose of T_3 resulted in a substantial increase in bile flow, cholesterol secretion (reaching 13-fold by 48–60 h after T_3), and phospholipid secretion (reaching threefold). These increases became significant as early as the second 12-h period after T_3 injection. Bile acid synthesis also significantly increased by 50%, but not until the time period of 24–36 h after T_3.

Bile acid synthesis and secretion of bile lipids are

TABLE 1. Bile fistula secretion

	EU Basal	HYPO Basal	HYPO-T_3 12–24 h	HYPO-T_3 48–60 h
Volume (ml/12 h)	5.7 ± 0.2[a]	3.4 ± 0.1[b]	4.5 ± 0.4[c]	6.0 ± 0.4[a]
Cholesterol (μmol/12 h)	3.3 ± 0.3[a]	0.9 ± 0.2[b]	3.7 ± 0.7[a]	11.8 ± 4.5[c]
Bile acids (μmol/12 h)	83 ± 5[a]	44 ± 4[b]	62 ± 4[c]	69 ± 4[a,c]
Phosphatidylcholine (μmol/12 h)	30 ± 2[a]	8 ± 0.4[b]	15 ± 1[c]	25 ± 2[a]

Bile volume and biliary lipids secreted over 12-h intervals by bile fistula rats weighing 190–200 g. Data are given for bile collected from euthyroid rats (EU) and hypothyroid rats (HYPO) prior to T_3 and at intervals beginning 12 h and 48 h after T_3 administration. Values are mean ± SE for 5–6 rats per group. Values with the same superscripts are not significantly different ($P > 0.05$).

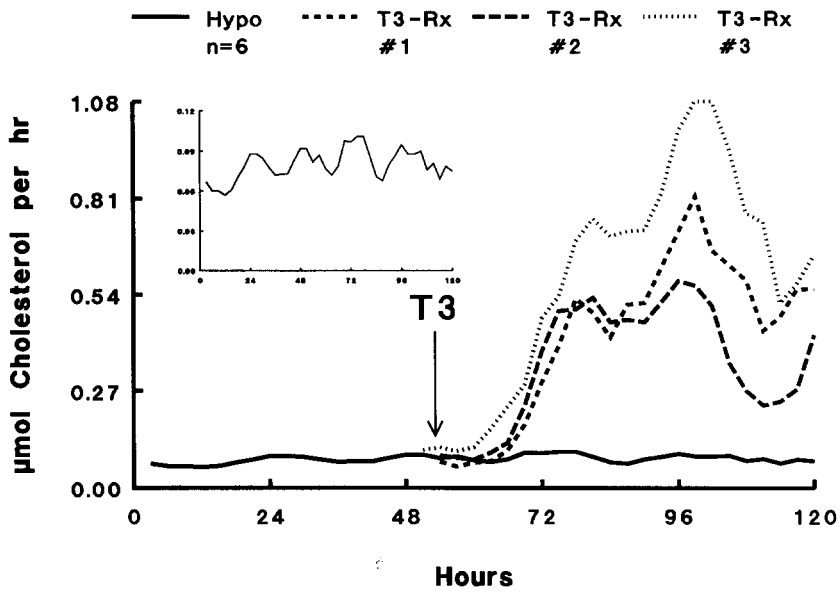

Fig. 1. Diurnal rhythm of biliary cholesterol secretion. Insert: Cholesterol secretion in bile fistula hypothyroid rats (mean values for n=6 rats). Peak activity occurs at the mid-point of the dark period, consistent with the time of highest bile acid synthesis and secretion. Main graph: Enlarged scale of cholesterol secretion by three representative hypothyroid rats after T_3. The diurnal rhythm persists at a higher secretory level.

known to have diurnal rhythms in the rat (11, 17). The diurnal rhythm for cholesterol secretion (**Fig. 1,** insert) and the rhythm for bile acid synthesis (not shown) were intact in hypothyroid rats. As in the previous reports, peak bile acid synthesis and cholesterol secretion occurred during the mid-dark point of the diurnal cycle, the time of feeding. These diurnal rhythms continued at higher levels after T_3 treatment, as shown with an expanded scale for cholesterol secretion by three individual rats in Fig. 1.

Although bile collections were obtained and measured at 90-min intervals, data were summed for 12-h increments in order to allow statistical comparisons of this large number of time points. Expressed as mean secretion per 12 h, biliary cholesterol secretion increased markedly to levels well above those of weight-matched euthyroid controls (**Fig. 2**). Phosphatidylcholine secretion increased

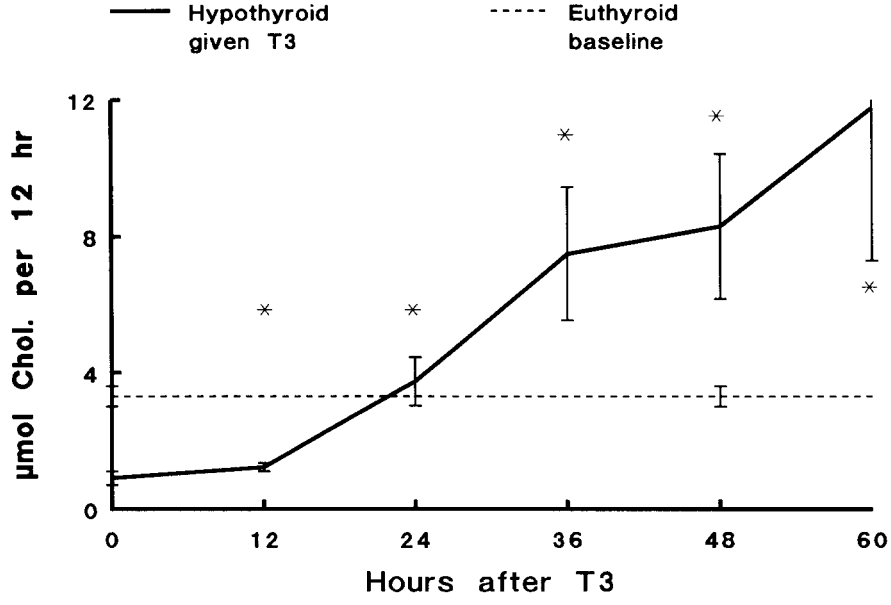

Fig. 2. Bile cholesterol (Chol) secretion. Secretion by hypothyroid bile fistula rats during the 12 h prior to T_3 (0 h) and during 12-h intervals after T_3. Time points shown indicate the end of collection. Values are mean ± SE. Euthyroid secretion is shown for comparison; *,$P < 0.05$ versus hypothyroid secretion; n=5–6 rats per time point.

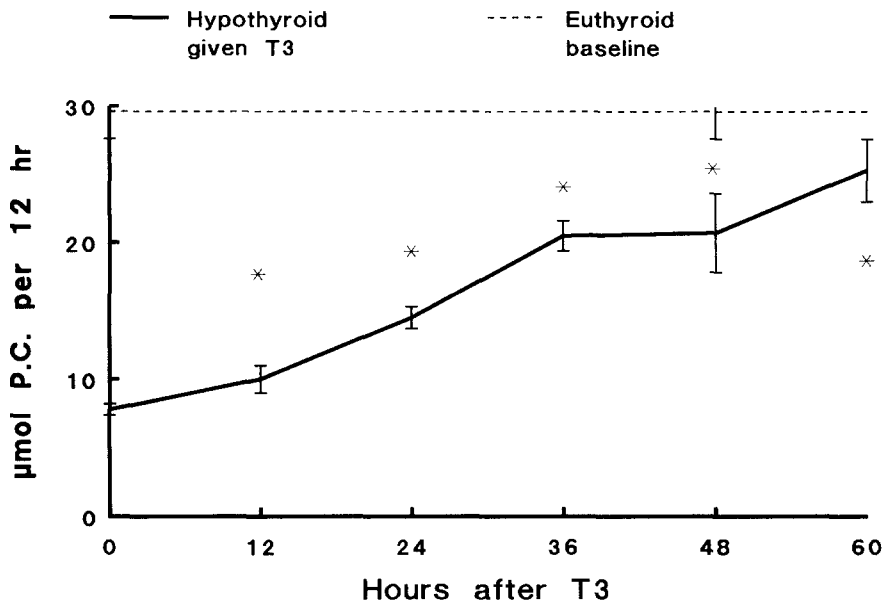

Fig. 3. Bile phosphatidylcholine (PC) secretion. Twelve hour secretion by hypothyroid bile fistula rats prior to and at intervals after T_3. Values are mean ± SE for n=5-6 animals. Euthyroid synthesis is shown for comparison; *, $P < 0.05$ versus hypothyroid secretion.

only to levels consistent with euthyroid values (**Fig. 3**). Bile acid secretion, and therefore synthesis, increased by only 50% and remained below the values of euthyroid controls (**Fig. 4**). Administration of a much lower single dose of T_3, 20 µg/100 g body weight or one-tenth of the study dose, still resulted in a significant doubling of cholesterol and phospholipid secretion, while bile acid synthesis rate only increased by 30%. Cholesterol secretion rose from a mean of 1.15 µmol/12 h to a rate of 2.31 µmol/12 h between 24 and 36 h after T_3, while phospholipid secretion increased from 10.1 to 18.4 µmol and bile acid synthesis from 51.7 to 62.9 µmol.

Lovastatin was administered either intravenously or orally to some hypothyroid bile fistula animals in order to inhibit HMG-CoA reductase activity and cholesterol synthesis. Bile acid synthesis decreased promptly and

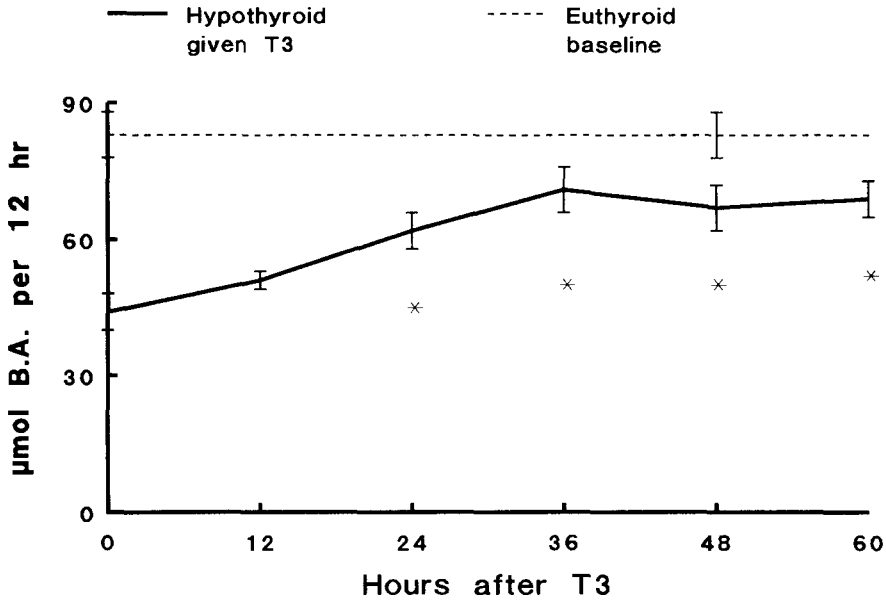

Fig. 4. Bile acid (BA) synthesis (secretion). Twelve hour synthesis by hypothyroid bile fistula rats prior to and at intervals after T_3. Values are mean ± SE for n=5-6 animals. Euthyroid synthesis is shown for comparison; *,$P < 0.05$ versus hypothyroid synthesis.

significantly to 66% of baseline and remained at this level (**Fig. 5a**). Cholesterol secretion also quickly and significantly decreased to 66% of baseline, but then recovered to baseline levels within an additional 12 h (Fig. 5b). The route of administration, intravenous or oral, made no difference in these secretory effects of lovastatin, and administration of lovastatin to euthyroid bile fistula rats had identical effects (data not shown). Administration of lovastatin concomitantly with T_3 also produced the initial decrease in bile lipid secretion. However, this was followed by the prompt appearance of T_3-augmented cholesterol secretion (10-fold, from 1.13 ± 0.12 μmol/12 h prior to T_3 to 11.0 ± 4.2 μmol/12 h by 36–48 h after T_3, n=4) and phospholipid secretion, while bile acid synthesis remained low (**Fig. 6**). Lovastatin was also administered in a delayed manner, beginning 12 h after the T_3 injection, in order to have maximal impact on the T_3 effects. In agreement with the data above, the T_3-augmented secretion of cholesterol still reached levels well above euthyroid rates, while bile acid synthesis fell by one-third and remained low.

Administration of intravenous mevalonolactone, which is promptly converted to cholesterol, was given to test whether cholesterol synthesis rate was limiting the increase in biliary cholesterol secretion triggered by thyroid hormone. This infusion did result in an expected decrease in measured HMG-CoA reductase activity from a mean of 3140 pmol/mg microsomal protein per min in T_3-treated fistula rats to a value of 1540 pmol/mg per min with T_3 and mevalonate provided, and a value of 350 pmol/mg per min in fistula rats with mevalonate only. However, provision of mevalonate by itself had no significant effect on basal or T_3-augmented biliary cholesterol or bile acid output from either hypothyroid or euthyroid bile fistula animals. When T_3 was administered to hypothyroid rats concurrently with mevalonate infusion, a fivefold increase in cholesterol secretion and 40% increase in bile acid synthesis were seen. This T_3-related rise was actually less than that seen with T_3 alone. Cholesterol secretion was constant at 1.1–1.3 μmol/12 h in hypothyroid rats and rose to 5.1 μmol/12 h after T_3 plus mevalonate.

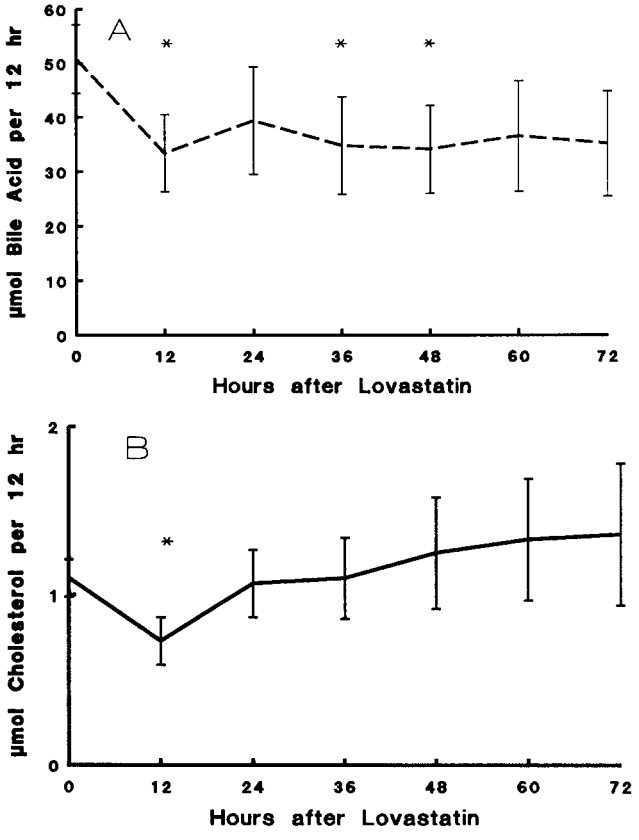

Fig. 5. Secretion of bile sterols by hypothyroid bile fistula rats given lovastatin either by continuous intravenous infusion at 10 mg/kg per day or orally by addition to the diet as 0.3% of chow weight. Route of administration made no difference in results, so animals were grouped for comparison. Fig. 5a shows bile acid synthesis (secretion) prior to (0 h) and at 12-h intervals during lovastatin for n=6 rats. Fig. 5b shows cholesterol secretion during the same intervals for these animals; *, $P < 0.05$ versus 0 h, prior to lovastatin.

DISCUSSION

In the hypothyroid bile fistula rat, synthesis of bile acids and secretion of bile volume and biliary lipids were reduced compared to euthyroid levels. In spite of the reduced secretion, a diurnal rhythm of bile acid synthesis and biliary lipid secretion was observed both in the absence and in the presence of intraperitoneal T_3. It appears, therefore, that thyroid hormone is not a critical determinant for these hepatic circadian rhythms.

Administration of thyroid hormone, in the form of T_3, to hypothyroid bile fistula rats resulted in an increase in the maximal rate of bile acid synthesis. This finding is consistent with our previous observation that bile acid secretory rate increased in vivo after T_3 treatment of hypothyroid or hypophysectomized rats (1), and the observation that 7α-hydroxylase mRNA levels increase under these conditions (4). In the current studies, a significant increase in bile acid synthesis was first demonstrated at 24–36 h after T_3. Ness et al. (4) have shown an increase in 7α-hydroxylase mRNA in liver of hypophysectomized rats as early as 6 h after T_3.

Biliary lipid secretion was also augmented following T_3 administration to hypothyroid bile fistula rats. Previous studies have shown that biliary secretion of cholesterol and phospholipid are closely linked to bile acid secretion, under most circumstances (18, 19). Cholesterol secretion in our studies increased more rapidly and to a much greater proportion than either bile acids or phosphatidylcholine. Each of the latter lipids increased towards the

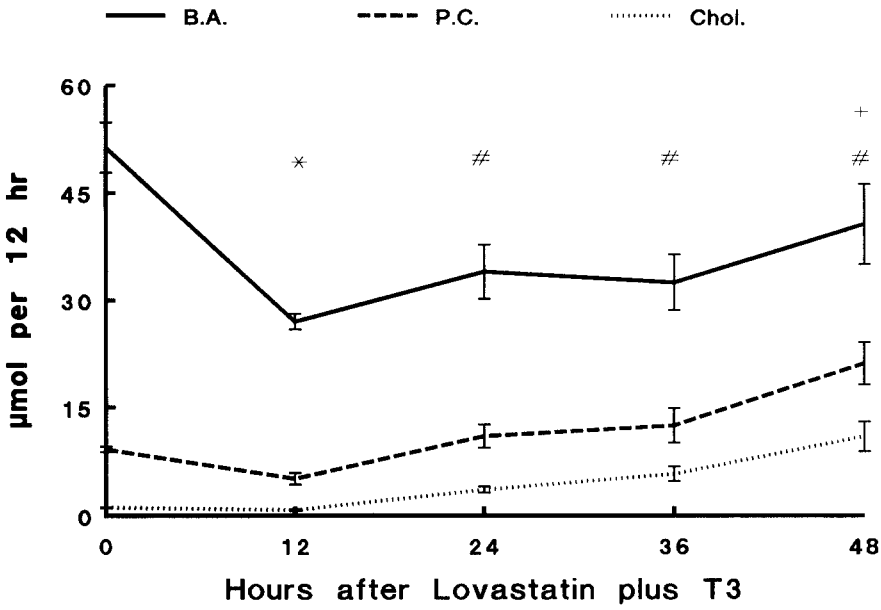

Fig. 6. Bile lipid secretion after T_3 plus lovastatin. Hypothyroid bile fistula rats (n=4) were given a single dose of T_3 and begun on continuous lovastatin, either orally or intravenously. Bile was collected prior to and at 12-h intervals after treatment to measure cholesterol (Chol) secretion, phosphatidylcholine (PC) secretion, and bile acid (BA) synthesis (secretion); *,$P < 0.05$ decrease for all lipids vs basal secretion; #, at least $P < 0.05$ increase for cholesterol vs. basal secretion; +,$P < 0.05$ increase for phosphatidylcholine secretion vs. basal secretion.

range of normal euthyroid secretion under these conditions, while cholesterol secretion increased by 13-fold to levels well above euthyroid. The striking increase in biliary cholesterol secretion was not dependent upon cholesterol absorption, since sterol absorption would be expected to be negligible in bile fistula animals. Administration of T_3 augmented biliary sterol secretion by 25 µmol of bile salts per 12 h and by 11 µmol of cholesterol per 12 h, so that one-third of new sterol secretion consisted of cholesterol. Thus, new lipid secretion had a markedly different ratio of cholesterol (and phospholipid) to bile salt. This T_3-mediated uncoupling of bile cholesterol and phospholipid from bile acid secretion has previously been observed in vivo (1) and in the isolated-perfused rat liver (9). One possible explanation for such bile lipid uncoupling is that thyroid hormone may facilitate secretion of cholesterol-phospholipid vesicles into bile.

The studies utilizing lovastatin as an inhibitor of cholesterogenesis suggest that T_3-stimulated biliary cholesterol secretion does not depend on cholesterol synthesis. In the rat, lovastatin is well known to rapidly induce the quantity of HMG-CoA reductase, resulting in the short-term recovery of cholesterol synthesis (20). It is possible that such recovery could have occurred in these experiments. However, a similar lack of requirement for cholesterol synthesis was also observed in very short-term isolated-perfused rat liver studies (9). Lovastatin did, however, inhibit the T_3-mediated rise in bile acid synthesis, demonstrating that bile acid synthesis and secretion after T_3 do appear to require ongoing cholesterol synthesis. Cholesterol synthesis has previously been suggested to be a regulating factor for bile acid synthesis in the rat (21). As mevalonate was not administered concurrently with lovastatin in these studies, however, we cannot rule out the possibility that lovastatin may have had a separate effect on bile acid synthesis or secretion. Nevertheless, the differences that we observed between cholesterol and bile acid secretion suggest that the thyroid hormone effect is not on a common bile acid and biliary cholesterol pool. Provision of mevalonate alone, in an attempt to prevent cholesterol synthesis rate from acting as a limiting factor for secretion, failed to alter either biliary cholesterol or bile acid secretion.

In conclusion, thyroid hormone appears to play an important role in biliary cholesterol and phospholipid secretion and in bile acid synthesis in the rat. During the acute transition from the hypothyroid to the hyperthyroid state, secretion of cholesterol and, to a lesser extent, phospholipid are uncoupled from their usual dependency upon bile acid secretion (18, 19). The increased biliary cholesterol secretion seen after T_3 administration occurs rapidly and prior to secretory consequences of the T_3-mediated action on bile acid synthesis.

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