Vesicles and mixed micelles in hypothyroid rat bile before and after thyroid hormone treatment: evidence for a vesicle transport system for biliary cholesterol secretion

Jeffrey P. Andreini,*† William F. Prigge,* Chenglie Ma, and Roger L. Gebhard[1,*†]**

Department of Medicine,* Minneapolis Veterans Affairs Medical Center; Department of Medicine,† University of Minnesota; and Department of Biochemistry,** University of Minnesota, Minneapolis, MN 55417

Abstract Hypothyroid rats show reduced secretion of biliary lipids, especially cholesterol. Secretion of biliary cholesterol is markedly augmented to levels above euthryoid beginning 12–24 h after administration of thyroid hormone. In the current studies, bile from hypothyroid and triiodothyronine-treated chronic bile-fistula rats was analyzed for vesicles and mixed micelles by metrizamide gradient ultracentrifugation. For euthyroid and hypothyroid animals, less than 12% of biliary cholesterol was in a vesicle gradient fraction. After treatment with triiodothyronine, biliary cholesterol increased markedly, and 50% of total cholesterol, 60% of excess cholesterol secreted, appeared in the vesicle fraction. Triiodothyronine stimulation of vesicle secretion resulted in cholesterol-rich vesicles (cholesterol:phospholipid ratio rose from less than 0.1 to 0.56), but no change in the distinct fatty acid composition of vesicle phospholipids. The microtubule inhibitor colchicine, given 12 h after triiodothyronine, prevented subsequent increase in cholesterol secretion in the form of vesicles. These studies, in a model that allows rapid changes in biliary lipid secretion, support the hypothesis that an important component of cholesterol and phospholipid secretion into bile involves microtubules and may involve a vesicle pathway.—**Andreini, J. P., W. F. Prigge, C. Ma, and R. L. Gebhard.** Vesicles and mixed micelles in hypothyroid rat bile before and after thyroid hormone treatment: evidence for a vesicle transport system for biliary cholesterol secretion. *J. Lipid Res.* 1994. **35:** 1405–1412.

Supplementary key words bile lipids • bile secretion • cholesterol

Biliary cholesterol can be shown to exist in both the vesicle and mixed micelle forms. In vesicles, cholesterol is complexed with phospholipid to form 700–1200 Å diameter particles of density <1.06 g/ml (1–4). In mixed micelles, approximately 50 Å in diameter and d > 1.06 g/ml (2–4), cholesterol is solubilized by bile acids and phospholipids.

It has previously been accepted that micellar secretion is the predominant form of biliary cholesterol and phospholipid secretion, with bile acids acting in a detergent fashion to dissolve canalicular membrane lipids (5, 6). During the last decade, however, characterization of cholesterol:phospholipid vesicles has led to a theory that the predominant secretory form may be as preformed vesicles utilizing a specific vesicular pathway (2, 3, 7–10). The two mechanisms are not necessarily exclusive, and evidence continues to suggest a primary bile acid effect at the canalicular level (11). It is not currently clear whether vesicles and mixed micelles arise from distinct secretory processes or whether they form in bile from physicochemical changes occurring after secretion of cholesterol:phospholipid vesicles in conjunction with bile acid secretion (9).

To a large extent, detection of vesicles or micelles in bile relates to the bile salt concentration and to the saturation of bile with cholesterol (9, 10). It has been demonstrated that saturated human bile may contain 93% of cholesterol in the form of vesicles and only 7% in the form of micelles (7). Rat bile, however, is normally secreted in a highly unsaturated state (12). Ulloa, Garrido, and Nervi (3) have shown that 50% or less of rat biliary cholesterol may appear in a vesicle fraction. Cohen, Angelica, and Carey (10) also found up to 70% bile vesicles, compared to mixed micelles, immediately after secretion from hepatocytes in the rat. The proportion of vesicles in bile decreased markedly as a function of both time and bile salt concentration. The particle form of cholesterol has clinical relevance to gallstone formation, as cholesterol

[1]To whom correspondence should be addressed at: Gastroenterology Section (111D), VA Medical Center, One Veterans Drive, Minneapolis, MN 55417.

may selectively crystallize from vesicles (2).

Thyroid hormone has been shown to have a myriad of effects on cholesterol metabolism. These effects include reduction in the levels of circulating lipoprotein cholesterol (12–14), augmentation of LDL receptor activity (15–17), increased synthesis of cholesterol and bile acids (12, 15, 18), and decreased absorption of cholesterol (19). This laboratory has recently reported that thyroid hormone status significantly affects biliary cholesterol secretion in the rat, and that this action might account for some of the reported effects of thyroid hormone on cholesterol metabolism (20, 21). Hypothyroid rats were shown to have low biliary cholesterol secretion, while thyroid hormone replacement markedly increased cholesterol secretion. Bile cholesterol secretion was out of proportion to bile acids and phospholipids, and was not dependent upon blood lipoproteins.

In the current study, vesicle secretion was evaluated during thyroid hormone-stimulated bile cholesterol secretion in the hypothyroid rat. The study aim was to determine whether vesicle or mixed micelle secretion predominates in this model of rapidly augmented secretion. Vesicle composition and the role of microtubules in bile lipid secretion were also evaluated.

METHODS AND MATERIALS

Male Sprague-Dawley rats weighing 120–140 g were made hypothyroid by at least 3 weeks of methimazole treatment (0.025% in drinking water), while maintained in a light-reversed room as described previously (12, 20). At time of surgery, hypothyroid rats and euthyroid controls weighed approximately 200 g and received silastic jugular vein catheters and PE-50 bile duct catheters while under metaphane anesthesia as described by Duane, Gilberstadt, and Wiegand (22). Catheters were exteriorized to the back and run through a spring harness, allowing animals to move freely in metabolic cages. Animals received standard Purina rat chow (St. Louis, MO) and either methimazole in water for hypothyroid rats or plain drinking water for euthyroid rats. After surgery, a solution of NaCl (77 meq/l), $NaHCO_3$ (30 meq/l), and KCl (8 meq/l) was infused into jugular catheters at a rate of 7.1 ml/24 h, while bile was collected by gravity syphonage.

After at least 48 h for stabilization and to allow maximal induction of bile acid synthesis, bile was collected on ice at 12-h increments continuously for 60–72 h. Hypothyroid animals were given triiodothyronine (T_3) dissolved in 0.05 N NaOH as a single intraperitoneal dose of 0.2 mg/100 g body weight after 12 h of baseline bile collection. The T_3 dose in this model is pharmacologic and saturates T_3 receptors for 48 h (23). Euthyroid controls received similar volumes of 0.05 N NaOH. Some hypothyroid animals also received colchicine (Sigma) at a dose of 0.05 mg/100 g body weight as an intravenous bolus 24 h after the intraperitoneal injection of T_3. This dose of colchicine has been shown to reduce hepatocyte microtubule volume density to near zero (24).

In some acute studies, a bile fistula was created and bile was immediately collected from hypothyroid, euthyroid, and T_3-treated animals for 2 h at room temperature under metaphane anesthesia.

A 2-ml aliquot of whole bile from each collection period was promptly ultracentrifuged to separate vesicles and mixed micelles. Using a modified method of Amigo, Covarrubias, and Nervi (7), metrizamide (15% w/v) was added to whole bile prior to ultracentrifugation in a vertical rotor (TLV-100, Beckman TL-100) at 338,000 *g* for 3 h at 4°C. Tubes were punctured with a needle at specific increments, and aliquots were aspirated with a syringe and weighed. The density of fractions was measured using a hexane–2,4 dichlorobenzene continuous gradient at 4°C, by a modified method of Beaufay et al. (25). Volumes were calculated from the sample weight.

Measurements of cholesterol by gas–liquid chromatography (GLC) (26), of phosphatidylcholine by enzymatic assay (27), and of bile salts by the methods of Talalay (28) were made in whole bile or gradient fractions, as previously described (12, 20). In some experiments, fatty acid composition of phospholipids in bile, serum, and hepatic microsomes was determined by GLC after transesterification (29), using a modification of a previously described method (30). Samples were extracted with nine volumes of isopropyl alcohol at 60°C for 5 min and protein was removed by centrifugation. Phospholipids were adsorbed on 200 mg alumina (Sigma) added directly to 13 × 100 mm Teflon-capped tubes. After shaking, centrifugation, and removal of supernate, the alumina was washed with 2 ml isopropyl alcohol. Residual alcohol was vacuum-removed and samples were directly transesterified.

Dynamic light scattering was done using standard components, with an argon-ion laser operating at 488 nm and a power output of 75 mW (31). Scattered light was collected by a photon counting photomultiplier. Photon-generated electron pulses were processed using a 64-channel Langley-Ford 1092 correlator. After accumulation of a correlation function, data were retrieved and second-order cumulant fit was used to calculate hydrodynamic radius. The homodyne photocurrent autocorrelation function measured with this instrument has been previously reported (32).

Statistical methods utilized Student's paired *t*-test for comparisons between time points among the same animals, and unpaired *t*-test for comparisons between groups.

RESULTS

Adequate separation of vesicles and mixed micelles by ultracentrifugation has previously been demonstrated in rat bile by Ulloa et al. (3) and by Amigo et al. (7) using this "rapid" method in human bile. **Figure 1** shows the ultracentrifugal separation of bile lipids from hypothyroid rats before T3 (basal, Fig. 1A) and 36–48 h after T3 (Fig. 1B). For this initial data, each 2 ml of bile was fractioned into ten 0.2-ml fractions having densities from 1.037 g/ml in the top fraction to 1.160 g/ml in the bottom fraction. The top 0.6 ml (top three fractions, d < 1.05 g/ml) contained a low bile salt concentration and much higher fractions of cholesterol and phospholipid 48 h after T_3 (Fig. 1B). This fraction has been shown to contain vesicles by prior studies (3, 7), and was defined as vesicles for

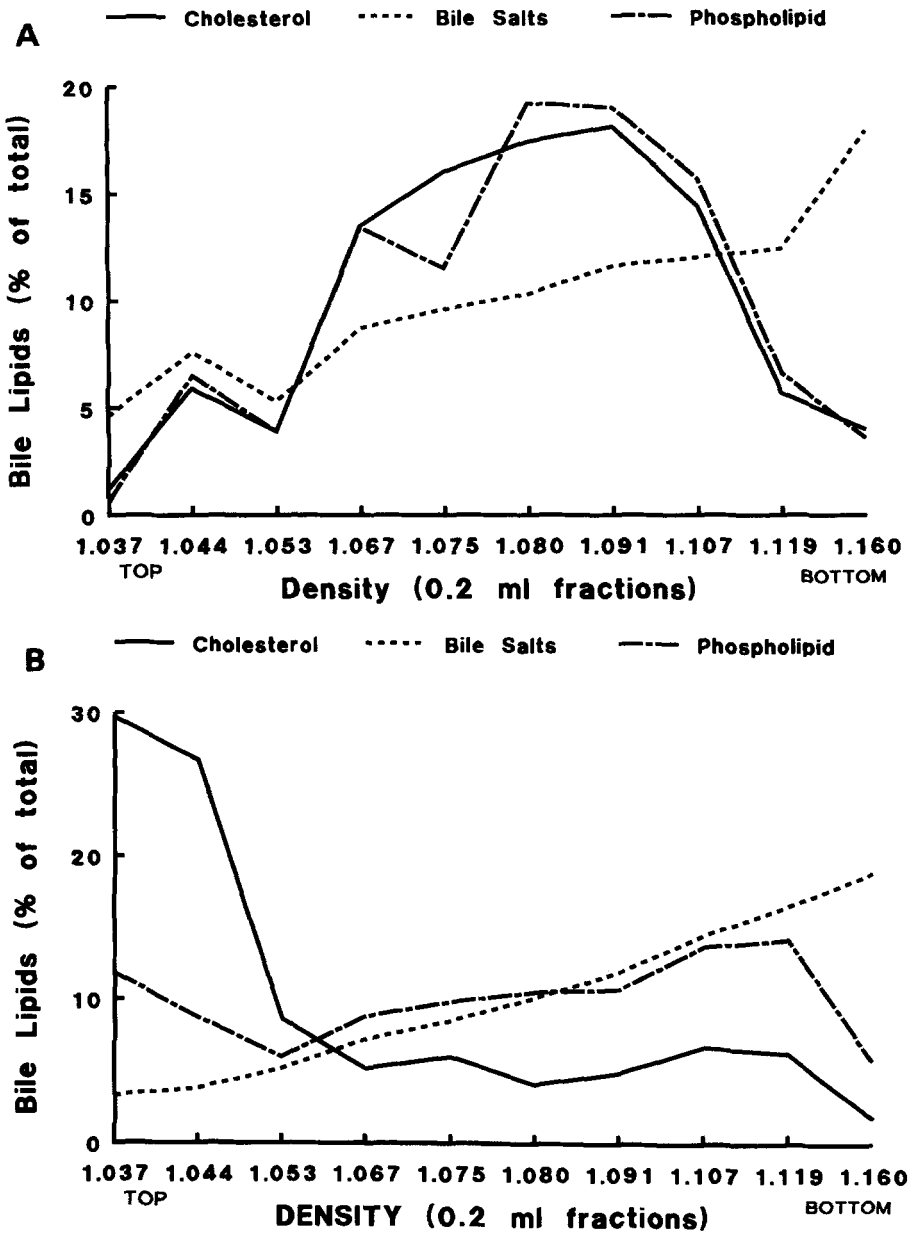

Fig. 1. Distribution of bile lipids in metrizamide gradients in hypothyroid and T_3-treated rats. Bile was collected for 12 h from chronic bile-fistula rats. After ultracentrifugation of 2 ml of bile with added metrizamide, bile lipids were measured in each 0.2-ml fraction from the top (lowest density) of the gradient to the bottom (most dense). Lipid distribution is reported as percent of total for each lipid. Fig. 1A shows bile cholesterol and phospholipids distributed in more dense mixed micelle fractions of bile from the hypothyroid rat. Fig. 1B shows more cholesterol distributed in lighter vesicle fractions of bile obtained between 36 and 48 h after T_3. Data are means of two animals having bile fractionated in 0.2-ml increments.

the current studies. Quasielastic light scattering (QELS) of this top 0.6-ml fraction 48 h after T_3 confirmed the presence of large particles of mean diameter of 980 Å. In the basal hypothyroid rat, the next 1.0-ml fraction was enriched in cholesterol, phospholipid, and bile salts (Fig. 1A). It is considered to represent predominantly mixed micelles, as shown by others (3, 7). QELS of this fraction showed a mean particle diameter of 88 Å. Whole uncentrifuged bile from hypothyroid rats prior to T_3 treatment showed particles having a mean diameter of 130 Å.

As previously reported (21), hypothyroid bile fistula rats given T_3 increased their total bile cholesterol secretion by 8-fold, bile phospholipid by 2.5-fold, and bile salts by 1.5-fold. **Figure 2** shows bile cholesterol secretion in the top 0.6-ml fraction ("vesicles") and in mixed micelles (whole bile cholesterol minus vesicle cholesterol) from euthyroid rats, and from hypothyroid rats before (basal) and after T_3. Euthyroid and basal hypothyroid bile-fistula rats showed no more than 12% of cholesterol in vesicles under these conditions. After T_3, total bile cholesterol increased well above euthyroid levels, and cholesterol in vesicles increased to 50% of total bile cholesterol. Vesicle cholesterol increased by 40-fold, while cholesterol found in more dense (mixed micelle) fractions increased by only 4-fold. Thus, the increase in whole bile cholesterol after T_3 appeared to a much greater extent in vesicles rather than mixed micelles.

To evaluate whether the apparent changes in vesicle cholesterol seen in chronic bile fistula animals might be an artifact of the 12-h collection on ice, bile was acutely collected from hypothyroid and T_3-treated animals for only 2 h at room temperature. Under these circumstances, 5 ± 1% (mean ± SE) of biliary cholesterol from hypothyroid animals appeared in the vesicle fraction, while 42 ± 4% of biliary cholesterol was in vesicles 48 h after T_3. In addition, the apparent vesicle and mixed micelle composition of bile was identical for samples centrifuged immediately after 12 h collection, and after storage of the same sample on ice for an additional 24 h.

Figure 3 shows the cholesterol:phospholipid lipid ratio in bile vesicle and mixed micelle fractions. Bile from euthyroid rats showed a constant cholesterol:phospholipid ratio of 0.1 over time. In hypothyroid rats, coincident with the increase in vesicle secretion after T_3, the ratio of cholesterol to phospholipid in vesicles rose from 0.08 to a value of 0.56.

As expected, **Table 1** shows that fatty acid composition of bile phospholipids was markedly different from composition of phospholipids in serum or liver (homogenate or microsomes), being enriched in 16:0 and depleted of 18:0, 20:4, and 22:6 fatty acids. Fatty acid compositions of bile vesicles and micelles after T_3 were comparable to each other and markedly different from profiles of serum and liver. Prior to T_3, micelle and vesicle phospholipid fatty

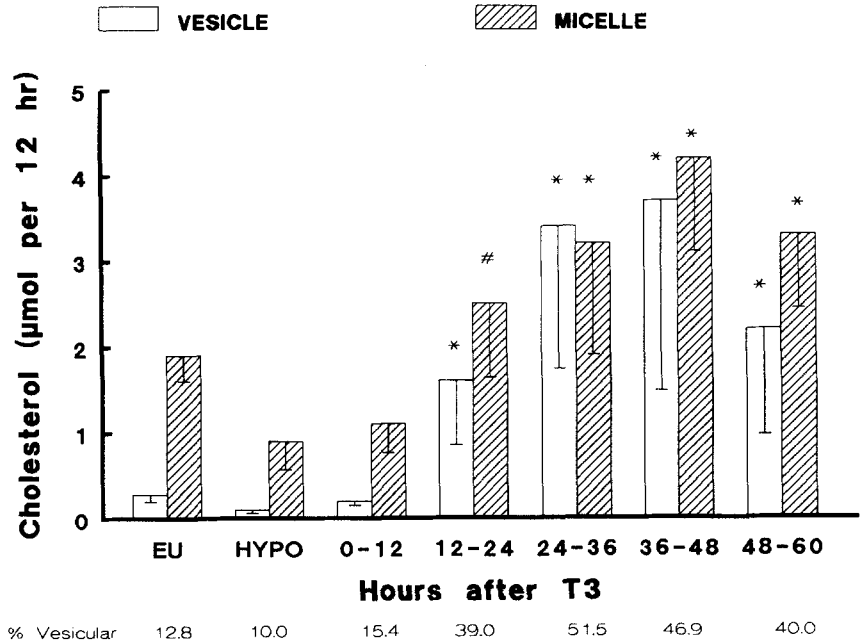

Fig. 2. The mass of cholesterol present in the top 0.6-ml fraction (vesicles) of the metrizamide gradient and in the remaining bile (mixed micelles) of 12-h collections of bile from untreated euthyroid rats (EU) and from T_3-treated hypothyroid rats (HYPO). Euthyroid vesicle and mixed micelle secretion remained constant over time. Bile cholesterol secretion was low in hypothyroid rats, but increased markedly after T_3, particularly vesicle cholesterol. Values are means ± SD for n = 7 animals. Mean percent of total bile cholesterol present in vesicles is noted below the graph. *, Increased from euthyroid and basal hypothyroid cholesterol output ($P < 0.05$); #, Increased from basal hypothyroid cholesterol output ($P < 0.05$).

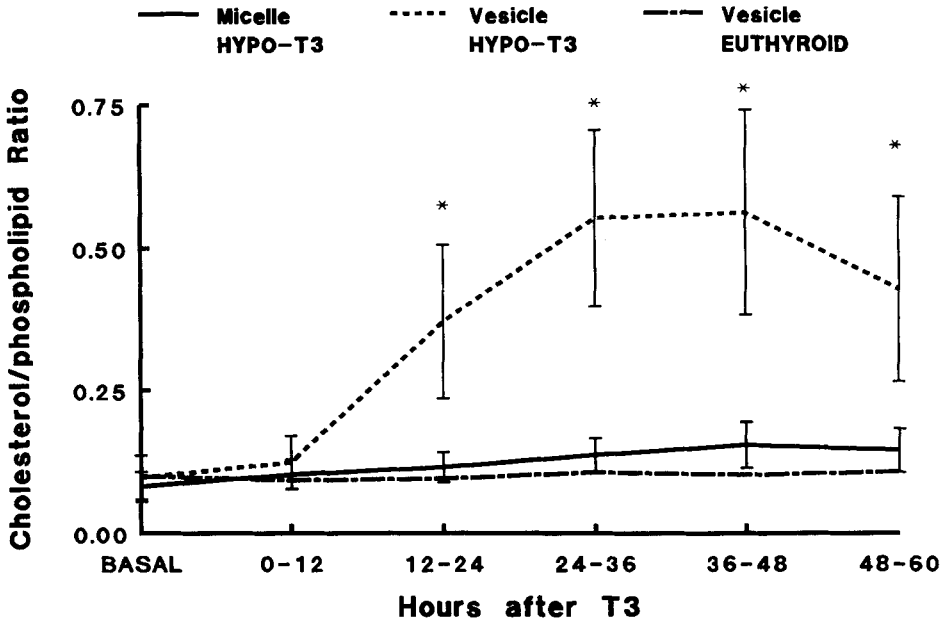

Fig. 3. The ratio of cholesterol to phospholipid in gradient fractions of bile from untreated euthyroid animals over time, and from hypothyroid rats before (basal) and at 12-h intervals after T_3. A constant ratio was seen in the euthyroid vesicle fraction and in hypothyroid mixed micelles, while the ratio in vesicles increased markedly after T_3 was given to hypothyroid rats. Values are mean ± SD for n = 7 animals. *, Increased from basal or euthyroid ratios ($P < 0.05$).

acid compositions were similar, possibly owing to micellar contamination of the small amount of vesicular phospholipid. After T_3, however, vesicles contained significantly less 16:0 and more unsaturated fatty acids (18:2, 20:4, 22:6) than did micelles. T_3 administration resulted in an increased percent of 16:0 and reduced percent of 18:2 and 22:6 in phospholipid of bile micelles.

Collections of bile from rats given the microtubule inhibitor colchicine 24 h after T_3 demonstrated significant blunting of subsequent total bile and vesicle cholesterol output. Total bile cholesterol in rats not given colchicine rose to 6.8 ± 2.6 and 7.9 ± 3.1 μmol/12 h by 36 and 48 h after T_3, respectively, while secretion decreased to 3.3 ± 0.5 and 3.7 ± 0.8 μmol/12 h in rats given colchicine ($P <$ 0.05). The effect of colchicine on the percent of cholesterol in vesicles is shown in **Figure 4.** In these studies, colchicine produced no significant effects on bile volume or on bile salt secretion, equating to bile acid synthesis in this model.

TABLE 1. Fatty acid composition of phospholipids from serum, liver microsomes, and bile vesicles and mixed micelles of hypothyroid rats after supplementation with T_3

	Percentage of Fatty Acid Species				
Source	16:0	18:0	18:2	20:4	22:6
Serum	25.9	20.9	19.9	11.6	6.3
Liver	22.0	24.8	13.9	18.7	10.2
Bile vesicles	39.0[a]	6.5	26.5[a]	8.3[b]	1.6[a]
Bile micelles	49.4	7.7	19.7	6.0	0.5

Mean of n = 3 animals.
[a]Different from micelles post-T_3 $P < 0.01$.
[b]Different from micelles post-T_3 $P < 0.05$.

DISCUSSION

Bile salts are actively extracted from portal blood, transported through hepatocytes, and secreted into bile by special transport protein processes (33). Bile flow is highly dependent upon rate of bile salt secretion, and biliary cholesterol and phospholipid secretion are also strongly linked to bile salt secretion under most conditions (34, 35). Controversy exists regarding the specific mechanism(s) for bile cholesterol and phospholipid secretion. Passive dissolution of canalicular membrane lipids by bile salts may occur (34), as well as a mechanism whereby cholesterol:phospholipid vesicles form in the Golgi and are transported to the canaliculus for direct secretion or for membrane repair (9). Microtubules may be involved in the vesicle transport process (36). After entry into the canaliculus, cholesterol and phospholipid may undergo physicochemical transition between vesicles and mixed micelles. The equilibrium between forms is time- and lipid concentration-dependent (10).

In chronic bile-fistula euthyroid (control) and basal (pre-T_3) hypothyroid rats, no more than 12% of total bile

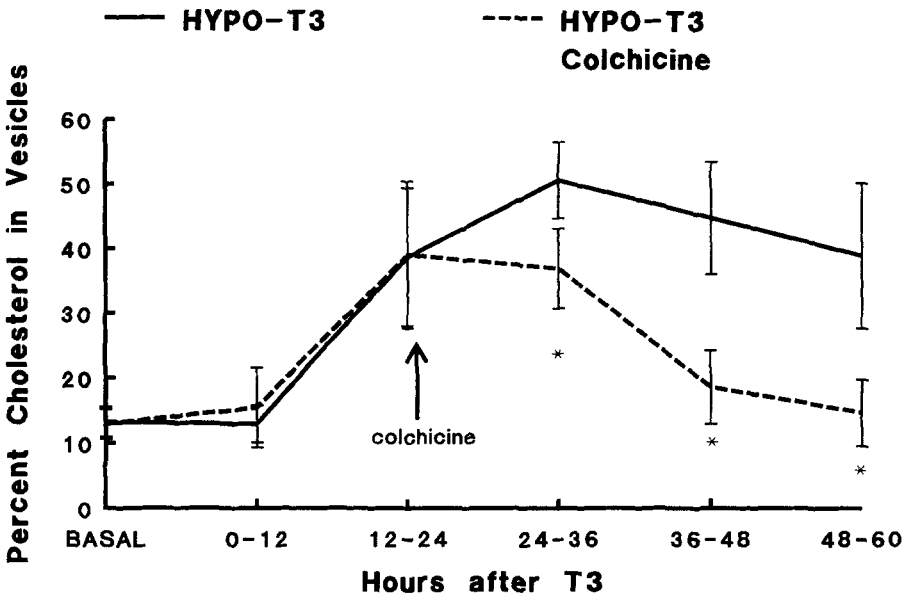

Fig. 4. The effect of colchicine on the percentage of cholesterol found in the vesicle fraction of secreted bile that has been stimulated by T_3. Colchicine is given intravenously 24 h after T_3 treatment of hypothyroid rats, in comparison to secretion without colchicine. Values are mean ± SD for n = 5–7 animals. *, Different from vesicle fraction without colchicine ($P < 0.05$).

cholesterol was found in the "vesicle" fraction. Bile collected from acute bile-fistula euthyroid rats and basal hypothyroid rats also showed only 10% of total cholesterol present in the vesicular layer. These results differ from those of Ulloa (3), who used methods of gel filtration and standard ultracentrifugation to determine that 50% of total bile cholesterol in euthyroid rats was in vesicles. The reason for this discrepancy is not clear, but transformation of vesicle lipid into mixed micelles over time in the very low cholesterol saturation of rat bile may be a factor.

This and previous studies show that T_3 has a prompt, marked effect on biliary lipid secretion, with cholesterol secretion augmented more than phospholipid or bile salt secretion (12, 21). In the present experiments, 8-fold augmentation of bile cholesterol secretion (4-fold above euthyroid levels) occurred predominantly in the form of cholesterol:phospholipid vesicles. Vesicle cholesterol increased 40-fold, so that vesicles held 50% of total bile cholesterol and 60% of the T_3-stimulated cholesterol. A measurable increase in vesicles appeared in bile 12 h after T_3, when bile saturation index was still quite low (12). Furthermore, the marked increase in vesicle lipids occurred with a minimal change in bile salt molar concentration. Cholesterol in mixed micelles only increased by 4-fold after T_3. This increase may have represented direct stimulation of micelle secretion or the action of bile salts on secreted bile vesicles.

The vesicle fraction in hypothyroid and euthyroid bile had a slightly higher ratio of cholesterol to phospholipid than did mixed micelles. T_3 markedly increased this ratio by stimulating secretion of cholesterol-rich vesicles, with only a modest increase in bile salt secretion. The observation that the fatty acid composition of vesicle phospholipids did not change after T_3 suggests that the origin of phospholipid for vesicle secretion was unaffected by T_3. The contrast in fatty acid composition of phospholipids in bile versus liver (and serum) also suggests that bile phospholipid originates from a subpool of hepatic phospholipid, rather than originating simply from bile salt detergent action on canalicular membranes.

Administration of the microtubule inhibitor colchicine to hypothyroid rats 24 h after T_3 prevented further augmentation of cholesterol output, primarily by decreasing output of cholesterol in vesicles by 80%. As bile volume, bile acid synthesis, and bile salt secretion were not affected by this dose of colchicine, its effect did not appear to be merely a toxic or cholestatic action. The observation suggests that T_3-induced vesicle secretion involves microtubules.

The implications of these observations for T_3 action on bile lipid secretion in humans is unclear. Basal biliary cholesterol secretion is much lower in rats than in humans, and the dose of T_3 administered to hypothyroid rats in this study is much higher than replacement doses given to humans. However, Honore (37) has reported an increased incidence of gallstones in treated hypothyroid patients, and increased bile saturation index during d-thyroxine therapy has been reported (38). In contrast, Angelin, Einarsson, and Leijd (39) found no increase in biliary cholesterol saturation following thyroid replace-

ment in hypothyroid subjects.

In summary, thyroid hormone administration to hypothyroid rats in this study promptly resulted in markedly increased secretion of cholesterol-rich vesicles in bile, by a process which appeared to involve microtubules. This model, which allows observations during rapid changes in biliary lipid secretion, supports the hypothesis that biliary secretion of cholesterol and phospholipids may involve a specific vesicle secretory pathway. This action of T_3 on biliary lipid secretion may be fundamental for other thyroid hormone effects on cholesterol metabolism.

Research was sponsored by the Department of Veterans Affairs Research Program.

Manuscript received 19 October 1993 and in revised form 9 February 1994.

REFERENCES

1. Somjen, G. J., and T. Gilat. 1983. A non-micellar model of cholesterol transport in human bile. *FEBS Lett.* **156:** 265–268.
2. Lee, S. P., H. Z. Park, H. Madani, and E. W. Kaler. 1987. Partial characterization of a nonmicellar system of cholesterol solubilization in human bile. *Am. J. Physiol.* **252:** G374–G383.
3. Ulloa, N., J. Garrido, and F. Nervi. 1987. Ultracentrifugal isolation of vesicular carriers of biliary cholesterol in native human and rat bile. *Hepatology.* **7:** 235–244.
4. Pattinson, N. R. 1985. Solubilization of cholesterol in human bile. *FEBS Lett.* **181:** 339–342.
5. Small, D. M., M. Bourges, and D. G. Dervichian. 1966. Ternary and quaternary aqueous systems containing bile salt, lecithin, and cholesterol. *Nature.* **211:** 816–818.
6. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile: relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
7. Amigo, L., C. Covarrubias, and F. Nervi. 1990. Rapid isolation of vesicular and micellar carriers of biliary lipid by ultracentrifugation. *J. Lipid Res.* **31:** 341–347.
8. Sömjen, G. J., and T. Gilat. 1985. Contribution of vesicular and micellar carriers to cholesterol transport in human bile. *J. Lipid Res.* **26:** 699–704.
9. Cohen, D. E., and M. C. Carey. 1990. Physical chemistry of biliary lipids during bile formation. *Hepatology.* **12:** 143S–147S.
10. Cohen, D. E., M. Angelico, and M. C. Carey. 1989. Quasielastic light scattering evidence for vesicular secretion of biliary lipids. *Am. J. Physiol.* **257:** G1–G8.
11. Verkade, H. J., R. Havinga, A. Gerding, R. J. Vonk, and F. Kuipers. 1993. Mechanism of bile acid-induced biliary lipid secretion in the rat: effect of conjugated bilirubin. *Am. J. Physiol.* **264:** G492–G469.
12. Day, R., R. L. Gebhard, H. L. Schwartz, K. A. Strait, W. C. Duane, B. G. Stone, and J. H. Oppenheimer. 1989. Time course of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and messenger ribonucleic acid, biliary lipid secretion, and hepatic cholesterol content in methimazole-treated hypothyroid and hypophysectomized rats after triiodothyronine administration: possible linkage of cholesterol synthesis to biliary secretion. *Endocrinology.* **125:** 459–468.
13. Epstein, A. A., and H. Lande. 1922. Studies on blood lipids. I. The relation of cholesterol and protein deficiency to basal metabolism. *Arch. Int. Med.* **30:** 563–677.
14. Peters, J. P., and E. B. Man. 1943. The interrelations of serum lipids in patients with thyroid disease. *J. Clin. Invest.* **22:** 715–720.
15. Ness, G. C., L. C. Pendleton, Y. C. Li, and Y. L. Chiang. 1990. Effect of thyroid hormone on hepatic cholesterol 7α-hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthase and apolipoprotein A-I mRNA levels in hypophysectomized rats. *Biochem. Biophys. Res. Commun.* **172:** 1150–1156.
16. Chait, A., E. Bierman, and J. Albers. 1979. Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultured human skin fibroblasts. *J. Clin. Endocrinol. Metab.* **48:** 887–889.
17. Walton, K., P. Scott, P. Dykes, and J. Davies. 1965. The significance of alterations in the serum lipids in thyroid dysfunction. II. Alterations of the metabolism and turnover of ^{131}I-low-density lipoproteins in hypothyroidism and thyrotoxicosis. *Clin. Sci.* **29:** 217–238.
18. Sample, C. E., L. C. Pendleton, and G. C. Ness. 1987. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels by L-triiodothyronine. *Biochemistry.* **26:** 727–731.
19. Mathe, D., and F. Chevallier. 1976. Effects of the thyroid state on cholesterol metabolism in the rat. *Biochim. Biophys. Acta.* **441:** 155–164.
20. Gebhard, R. L., B. G. Stone, J. P. Andreini, W. C. Duane, C. D. Evans, and W. F. Prigge. 1992. Thyroid hormone differentially augments biliary sterol secretion in the rat. I. The isolated-perfused liver model. *J. Lipid Res.* **33:** 1459–1466.
21. Gebhard, R. L., and W. F. Prigge. 1992. Thyroid hormone differentially augments biliary sterol secretion in the rat. II. The chronic bile fistula model. *J. Lipid Res.* **33:** 1467–1473.
22. Duane, W. C., M. L. Gilberstadt, and D. M. Wiegand. 1979. Diurnal rhythms of bile acid production in the rat. *Am. J. Physiol.* **236:** R175–R179.
23. Oppenheimer, J. H., P. Coulombe, and H. L. Schwartz. 1978. Nonlinear (amplified) relationship between nuclear occupancy by triiodothyronine and the appearance rate of hepatic glycerophosphate dehydrogenase and malic enzyme in the rat. *J. Clin. Invest.* **61:** 987–997.
24. Reaven, E. P., and G. M. Reaven. 1980. Evidence that microtubules play a permissive role in hepatocyte very low density lipoprotein secretion. *J. Cell Biol.* **84:** 28–39.
25. Beaufay, H., D. S. Bendall, P. Baudhuin, R. Wattiaux, and C. De Duve. 1959. Tissue fractionation studies: analysis of mitochondrial fractions from rat liver by density gradient centrifugation. *Biochem. J.* **73:** 628–637.
26. Duane, W. C. 1978. Simulation of the defect of bile acid metabolism associated with cholesterol cholelithiasis by sorbitol ingestion in man. *J. Lab. Clin. Med.* **91:** 969–978.
27. Gurantz, D., M. F. Laker, and A. F. Hofmann. 1981. The enzymatic measurement of choline-containing phospholipids in bile. *J. Lipid Res.* **22:** 373–376.
28. Talalay, P. 1960. Enzymatic analysis of steroid hormones. *Methods Biochem. Anal.* **8:** 119–143.
29. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:** 114–120.
30. Archer, S. L., G. J. Johnson, R. L. Gebhard, W. L. Castleman, A. S. Levine, J. Y. Westcott, N. F. Voelkel, D. P. Nelson, and E. K. Weir. 1989. Effect of dietary fish oil on lung

lipid profile and hypoxic pulmonary hypertension. *J. Appl. Physiol.* **66:** 1662–73.
31. Bloomfield, V. A., and T. K. Lim. 1978. Quasi-elastic laser light scattering. *Methods Enzymol.* **48:** 415–494.
32. Wei, G. J., and V. A. Bloomfield. 1979. Determination of polymer size distribution by combination of quasi-elastic light scattering and band transport: evaluation of the effect of diffusion. *Biophys. Chem.* **9:** 97–103.
33. Hofmann, A. F. 1990. Bile acid secretion, bile flow and biliary lipid secretion in humans. *Hepatology.* **12:** 17S–25S.
34. Wheeler, H. O., and K. K. King. 1972. Biliary excretion of lecithin and cholesterol in the dog. *J. Clin. Invest.* **51:** 1337–1350.
35. Wagner, C. I., B. W. Trotman, and R. D. Soloway. 1976. Kinetic analysis of biliary lipid secretion in man and dog. *J. Clin. Invest.* **57:** 473–477.
36. Crawford, J. M., C. A. Berken, and J. L. Gollan. 1988. Role of the hepatocyte microtubular system in the excretion of bile salts and biliary lipid: implications for intracellular vesicular transport. *J. Lipid Res.* **29:** 144–156.
37. Honore, L. H. 1981. A significant association between symptomatic cholesterol cholelithiasis and treated hypothyroidism in women. *J. Med.* **12:** 199–203.
38. Begemann, F., H. J. Roose, and E. Kempf. 1977. Drugs and gallstones. *Lancet.* **2:** 402.
39. Angelin, B., K. Einarsson, and B. Leijd. 1983. Bile acid metabolism in hypothyroid subjects: response to substitution therapy. *Eur. J. Clin. Invest.* **13:** 99–106.