Thyroxine and propylthiouracil supplements reduce the lithogenic index and cholesterol gallstones in hamsters

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Alteration of thyroid status affects cholesterol and bile acid metabolism. In the present study, dietary supplements of DL-thyroxine and propylthiouracil were used to modulate plasma thyroxine levels, plasma lipoproteins, bile acid composition, and gallstone formation in hamsters. Male Syrian hamsters (Lakeview strain) were fed a gallstone-inducing purified diet (5% butter, 0.4% cholesterol) or the same diet supplemented with 0.005% thyroxine or 0.05% propylthiouracil for 5 weeks. Thyroxine administration greatly increased plasma triiodothyroxine (total T_3) and thyroxine (total T_4), whereas propylthiouracil depressed both. Compared with the gallstone diet, plasma cholesterol and triglycerides were significantly elevated by thyroxine and depressed by propylthiouracil. On autopsy, three of 10 hamsters fed the gallstone diet had cholesterol gallstones. No cholesterol gallstones were found in hamsters treated with thyroxine, but 9 of 10 animals had pigment stones. By contrast, only one of 10 hamsters supplemented with propylthiouracil had cholesterol stones, whereas 9 of 10 were without any stones. The lithogenic index was significantly reduced with thyroxine (1.1 ± 0.4) compared with the gallstone diet (2.6 ± 0.7), while the lithogenic index in hamsters fed propylthiouracil was intermediate (1.8 ± 0.7). With the gallstone diet, the cheno pool was slightly larger than the cholate pool. Thyroxine resulted in three times more cholate than cheno (percent distribution), while propylthiouracil resulted in twice as much cheno as cholate. As a result the cholate to cheno ratio was tripled by thyroxine and reduced 40% by propylthiouracil compared with the gallstone diet. Thus, despite enhanced lipemia, thyroxine-treated hamsters were protected against cholesterol gallstones, possibly by their ability to maintain a predominantly cholate bile acid profile. On the other hand, propylthiouracil protected against gallstones of all types despite a high cheno profile and elevations in hydrophobicity index. These results indicate that cholesterol gallstone formation in hamsters does not depend solely on plasma hyperlipemia, the bile acid profile, or either the lithogenic index or hydrophobicity index. (J. Nutr. Biochem. 5:397–405, 1994.)

Keywords: thyroid status; lipoproteins; biliary lipids; bile acid profile; gallstones; hamsters

Introduction

The influence of thyroid hormone status on cholesterol and bile acid metabolism in humans is well recognized,[1,2] i.e., hypothyroidism is associated with hypercholesterolemia, whereas hyperthyroidism decreases plasma cholesterol.[3] Furthermore, elevated plasma triglyceride concentrations are frequently observed in hypothyroid patients, whereas depressed triglyceride concentrations are generally found with hyperthyroidism.[4] Evidence suggests that enhanced bile acid synthesis from hepatic cholesterol,[5] increased hepatic receptor-mediated low density lipoprotein (LDL) uptake,[6,7] and decreased cholesterol absorption[1,8] all contribute to the hypocholesterolemic effect of thyroid hormones in humans and rats.

Administration of thyroid hormones in rats stimulated bile acid synthesis, increasing chenodeoxycholate synthesis and decreasing the cholate to cheno ratio.[9] In contrast, propylthiouracil (PTU)-induced hypothyroidism did not alter the bile acid pool size of intact rats,[9] but PTU decreased the bile acid output and increased the cholate to cheno ratio slightly in bile fistula rats.[10] The altered rates of cholate and chenodeoxycholate synthesis observed after thyroxine

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administration in the rat were attributed to an inhibition of hepatic 12α-hydroxylase,[11] the key enzyme in cholate biosynthesis.

Typically, hamsters[12–17] and prairie dogs[18–20] fed dietary cholesterol develop combined hyperlipidemia, hepatic cholesterol accumulation, and an exaggerated chenodeoxycholate (cheno) profile during the process of developing cholesterol gallstones. Similar to the situation following thyroxine administration to rats,[11] the decline in cholate during cholesterol consumption in rats[21] and hamsters[22] is associated with depressed 12α-hydroxylase activity. Because thyroxine and dietary cholesterol both depress 12α-hydroxylation, one might expect that both would increase cholesterol gallstone formation in the hamster. However, previous studies feeding a sucrose-rich diet with 2% lard revealed mostly pigment gallstones in hamsters given thyroxine,[23–25] whereas methylthiouracil seemed to favor cholesterol gallstone formation in this model.[25]

Thus, to further evaluate thyroid hormone function on gallstone induction in the cholesterol-fed hamster, the present study manipulated plasma lipoproteins and bile acid composition with dietary supplements of DL-thyroxine and PTU. We reasoned that modulation of bile acid metabolism by thyroxine would alter the cholate to cheno ratio by depressing cholate to favor cheno and thereby enhance cholesterol gallstone formation, whereas PTU would have the opposite effect.

Methods and materials

Thirty male golden Syrian hamsters (Lakeview strain, Charles River Breeding Labs, Wilmington, MA USA), weighing 53 ± 4 g were randomly assigned to three groups (n = 10) and fed purified cholesterol-enriched diets with or without supplements of 0.005% DL-thyroxine or 0.05% propylthiouracil (both from Sigma Chemicals, St. Louis, MO USA) for 4 or 5 weeks. Hamsters were housed in groups of three to four animals per cage and kept in a temperature-controlled environment with a 12 hr light-dark cycle (lights on 18:00 hr). The basal composition of the purified diet was (g/kg dry weight): casein 200, cornstarch 335, glucose 200, cellulose 100, wheat bran 50, butter 50, Ausman-Hayes mineral mix 46, Hayes-Cathcart vitamin mix 12, choline chloride 3, and cholesterol 4. The composition of the mineral and vitamin mix was detailed previously.[26] Diets were fed as starch gels that were prepared by withholding 60 g/kg of cornstarch from the formulation and premixing it with 800 mL of simmering water to form a gel to which the remaining ingredients were added. Food and fresh water were provided daily ad libitum. Food consumption was recorded daily, and body weights were monitored on a weekly basis. All protocols and procedures were approved by the Brandeis University Animal Care and Use Committee.

Blood samples for plasma lipid and thyroid hormone analysis were collected at 2, 4, and 5 weeks under light anesthesia into an EDTA-wetted syringe by cardiac puncture, and plasma was separated immediately by centrifugation at 12,000g for 5 min. Prior to blood sampling, hamsters were fasted overnight (18 hr) individually in wire-bottomed cages. Total plasma cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were determined by enzymatic assays (Sigma kit #352 for cholesterol and #336 for triglycerides, Sigma Chemicals). HDL-cholesterol was assayed following Mg^{2+}-phosphotungstate precipitation of lipoproteins containing apo B and apo E using HDL-cholesterol Reagent Set (Boehringer Diagnostics, Indianapolis, IN USA) according to the procedure described by Weingard and Daggy.[27]

At 4 and 5 weeks, hamsters (five per diet group) were exsanguinated by cardiac puncture, and the liver, cecum, and perirenal adipose fat excised (right side only), blotted, and weighed. Portions of the livers were removed and frozen for hepatic cholesterol analysis. Gallbladder bile was aspirated for analysis of biliary lipid and bile acid profile prior to inspection for gallstones. The gallbladder was dissected from the liver, opened under a dissecting microscope, and examined along with the remaining gallbladder bile for gallstones under regular and polarized light microscopy as previously described.[28]

Additionally, in the 4-week plasma samples, lipoproteins were separated by density-gradient ultracentrifugation[29] in a Beckman L5-50 ultracentrifuge (Beckman Instruments, Palo Alto, CA USA) using an SW-41 rotor at 35,000 rpm and 15° C for 24 hr, with each tube stained with minimal Sudan Black to visualize lipoproteins.[30] Plasma from two hamsters with similar cholesterol and triglyceride concentrations from each group were pooled for lipoprotein isolation. Three lipoprotein fractions were isolated: very low density plus intermediate density lipoprotein (VLDL plus IDL, $d < 1.019$ g/dL), low density lipoprotein (LDL, $1.019 < d < 1.055$ g/dL), and high density lipoprotein (HDL, $1.055 < d < 1.21$ g/mL). Each lipoprotein fraction was dialyzed extensively against 0.15 M NaCl (pH 7.4) containing 100 mM butylated hydroxytoluene (BHT). Cholesterol, triglycerides, and phospholipids were determined using enzymatic assays (Sigma kit #352 for cholesterol and #336 for triglycerides, and Wako phospholipids B kit for phospholipids, Wako Chemicals, Richmond, VA USA). Protein concentration was determined according to the Lowry procedure.[31]

Plasma thyroid hormones (triiodothyronine [total T_3] and thyroxine [total T_4]) were determined at 2, 4, and 5 weeks using radioimmunoassay kits (Gammacoat [^{125}I] T_3 kit and GammaCoat [^{125}I] T_4 kit, Incstar Corporation, Stillwater, MN USA).

Hepatic cholesterol was extracted by grinding a 100-mg portion of liver with 5 g anhydrous sodium sulfate and extracting three times with 10 mL chloroform:methanol (2:1,vol/vol). The liquid phase was evaporated to dryness and redissolved in 1 mL chloroform. An aliquot of the chloroform phase was evaporated to dryness and redissolved in 2-propanol for cholesterol analysis. Total cholesterol was determined enzymatically after hydrolysis with alcoholic KOH for 1 hr as adapted from Tercyak,[32] using the Wako Free cholesterol C kit for cholesterol analysis.

Bile lipids were isolated using a modified Folch extraction.[33] An aliquot of 10 μL gallbladder bile was extracted with 3 mL chloroform:methanol (2:1, vol/vol) and 0.75 mL of 1.12% KCl solution. Biliary cholesterol and phospholipids were measured enzymatically using Wako Free cholesterol C kit and Wako Phospholipids B kit (Wako Chemicals, Richmond, VA USA). Bile acids were analyzed with an isocratic high pressure liquid chromatography (HPLC) procedure adapted from Rossi et al.[34] using an aliquot of the methanol/KCl layer from the Folch extract, evaporated under a stream of nitrogen and redissolved in the mobile phase. A standard containing 60 mmol/L of taurine and glycine conjugates of cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid was used to calculate the individual bile acid concentration. Total bile acid concentration was calculated as the sum of individual bile acids (taurine and glycine conjugates of cholate, cheno, deoxycholate, and lithocholate) determined by HPLC. Taurine and glycine conjugates of ursodeoxycholate were present only in trace amounts and therefore are not included in the calculation.

The lithogenic index (LI) was calculated according to published procedures,[35] based on the relative molar ratios of lipid components and total lipid concentration using a computerized version of cholesterol solubility.[36] The hydrophobicity index (HI) was calculated as previously described.[37]

Statistical differences were calculated using one-factorial analysis of variance (ANOVA) followed by Scheffe's F-test.

Results

Growth response

All hamsters were healthy throughout the dietary regimen and demonstrated normal weight gain. Ad libitum food consumption was 20% higher with thyroxine supplementation compared with the gallstone diet or the diet supplemented with propylthiouracil *(Table 1)*. Final body weights were similar between the three groups, although hamsters fed propylthiouracil tended to weigh less. Thyroxine supplementation increased the relative liver weight 1.4-times but resulted in a significantly smaller perirenal fat pad *(Table 1)*.

Plasma lipids and lipoprotein profile

Plasma cholesterol and triglyceride concentrations substantially increased with all three diets, but to a different extent *(Table 2)*. After 5 weeks, plasma cholesterol was significantly elevated (+30%) in hamsters fed thyroxine and depressed (−17%) in hamsters fed propylthiouracil compared with the gallstone diet. HDL-cholesterol at 2 and 4 weeks was significantly lower in hamsters fed propylthiouracil compared with thyroxine-fed animals, but after 5 weeks HDL-cholesterol was similar for all three diets. However, the TC to HDL-cholesterol ratio was always lowest in hamsters fed propylthiouracil and highest with thyroxine. Compared with the gallstone diet, plasma triglyceride concentrations after 5 weeks were more than doubled with thyroxine and reduced by more than 50% with propylthiouracil.

The distribution of cholesterol and triglycerides among lipoproteins revealed that both thyroxine and propylthiourasil reduced cholesterol carried by VLDL while increasing HDL-cholesterol *(Figure 1)*.

Plasma thyroid hormones

The effect of thyroxine and propylthiouracil administration was reflected in the plasma triiodothyronine (total T_3) and thyroxine (total T_4) concentrations, i.e., thyroxine greatly increased and propylthiouracil depressed both of these thyroid hormones *(Table 3)*. After 5 weeks of thyroxine, T_3 and T_4 concentrations were increased sixfold and sevenfold, respectively, compared with the gallstone diet. In contrast, propylthiouracil supplementation decreased T_4 by 53% compared with the gallstone diet, whereas T_3 concentration was not affected. When the relationship between plasma lipids (cholesterol and triglycerides) and thyroid hormone status (T_3 and T_4) was plotted, a direct correlation with the T_3 or T_4 concentration was observed *(Figure 2)*.

Hepatic cholesterol

Liver cholesterol (total, free, and esterified cholesterol) was substantially increased above normal concentrations (total cholesterol normally about 10 mmol/kg) in all groups, with no significant differences noted (4- and 5-week data combined). However, hamsters fed the diet with PTU had slightly higher hepatic cholesterol concentrations than hamsters fed the gallstone diet or the thyroxine supplement *(Table 2)*.

Biliary lipids

When biliary lipid composition was expressed as molar ratios, the mol % of bile acids, phospholipids, and cholesterol were not affected by thyroid status after 4 weeks *(Table 4)*. The LI exceeded 1.0 for all three diets and was highest (1.7 ± 0.3) for the gallstone diet. Biliary lipid composition, especially the mol % cholesterol, increased rapidly during the week 5 in all groups, and the LI increased 50% with both the gallstone diet and the PTU supplement. The slightly supersaturated LI was not altered between weeks 4 and 5 with thyroxine supplementation.

Bile acid profile

The bile acid profile was modified slightly after 4 weeks (data not shown), but differences were striking after 5 weeks.

Table 1 Body weight gain, liver, cecum, and perirenal fat weight in hamsters fed a gallstone diet or that diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil

	Gallstone diet	+ Thyroxine	+ Propylthiouracil
Food consumption, g wet weight/day*	19 ± 3[a]	24 ± 1[a,b]	20 ± 3[b]
Final body weight, g**			
4 wks	111 ± 5	106 ± 3	105 ± 9
5 wks	120 ± 4	119 ± 7	113 ± 4
Daily weight gain, g/day			
4 wks	2.2 ± 0.1[a,b]	1.8 ± 0.1[a]	1.9 ± 0.1[b]
5 wks	1.8 ± 0.1	1.9 ± 0.1[a]	1.7 ± 0.1[a]
Liver weight, % body wt			
4 wks	5.1 ± 0.3[a]	7.4 ± 0.6[a,b]	4.7 ± 0.3[b]
5 wks	5.0 ± 0.3[a]	7.2 ± 0.4[a,b]	4.8 ± 0.2[b]
Cecum weight, % body wt			
4 wks	1.3 ± 0.3	1.5 ± 0.2	1.2 ± 0.2
5 wks	1.2 ± 0.2	1.6 ± 0.6	1.2 ± 0.2
1/2 Perirenal fat, % body wt			
4 wks	1.1 ± 0.2[a]	0.6 ± 0.1[a]	0.8 ± 0.2
5 wks	1.0 ± 0.05[a]	0.7 ± 0.1[a]	0.9 ± 0.1

*Hamsters were fed ad libitum (25 g wet weight/day/hamster)

**Initial body weight, 53 ± 4 g. Values are mean ± SD with $n = 5$ per group.

[a,b,c]Values sharing a common superscript are significantly different ($P < 0.05$) using one-factorial ANOVA and Scheffe's F test.

Table 2 Plasma and hepatic lipids in hamsters fed a gallstone diet and the same diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil

	Gallstone diet	+ Thyroxine	+ Propylthiouracil
Plasma			
TC, mmol/L			
2 wks	9.6 ± 2.1[a,b]	13.5 ± 2.2[a,c]	7.4 ± 0.8[b,c]
4 wks	11.1 ± 1.8	13.5 ± 5.6	8.1 ± 0.5
5 wks	10.5 ± 2.4	13.9 ± 2.8[a]	8.8 ± 0.6[a]
HDL-C, mmol/L			
2 wks	4.4 ± 0.8	5.5 ± 1.2[a]	4.2 ± 0.9[a]
4 wks	5.5 ± 0.5	6.3 ± 0.9[a]	4.9 ± 0.5[a]
5 wks	5.3 ± 0.6	6.0 ± 0.7	5.6 ± 0.5
HDL-C % of TC			
2 wks	47 ± 11	42 ± 10[a]	57 ± 10[a]
4 wks	50 ± 8	56 ± 14	61 ± 6
5 wks	52 ± 11	44 ± 10[a]	63 ± 2[a]
TG, mmol/L			
2 wks	2.3 ± 1.1[a]	6.9 ± 3.2[a,b]	1.1 ± 0.2[b]
4 wks	5.2 ± 2.9	13.4 ± 10.2[a]	2.0 ± 0.5[a]
5 wks	4.5 ± 2.3[a]	10.0 ± 4.8[a,b]	2.1 ± 0.3[b]
Liver		mmol/kg	
Total cholesterol	168 ± 29	159 ± 27	187 ± 33
Free cholesterol	27 ± 8	28 ± 5	29 ± 10
Esterified cholesterol*	142 ± 21	131 ± 23	157 ± 29

Values are mean ± SD.
*Calculated as the difference between total and free cholesterol.
[a,b,c]Values sharing a common superscript are significantly different $P < 0.05$ using one-factorial ANOVA and Scheffe's F test.

Thyroxine supplementation significantly lowered the concentration (mM) of chenodeoxycholate, especially taurocheno, compared with the gallstone diet *(Table 5)*. Furthermore, thyroxine resulted in almost three times more cholate than cheno, while propylthiouracil hamsters had twice as much cheno as cholate. As a result, the cholate to cheno ratio was tripled by thyroxine and 40% reduced by propylthiouracil compared with the gallstone diet. The conjugation pattern was also affected, with thyroxine resulting in a two and one half times higher glycine to taurine ratio than with the gallstone diet. In contrast, propylthiouracil did not alter the conjugation pattern. The HI, a measure of the hydrophobic-hydrophilic balance of bile acids, was significantly decreased with thyroxine compared with the other two diets *(Table 5)*.

Gallstone incidence

After 4 weeks one of five hamsters fed the gallstone diet had cholesterol gallstones, whereas two of five developed cholesterol stones after 5 weeks, but no pigment stones were observed in this group. No cholesterol stones or crystals were observed in hamsters treated with thyroxine, but nine of 10 animals had pigment stones (4- and 5-week data combined). By contrast, nine of 10 hamsters supplemented with propylthiouracil had no stones, and only one hamster developed cholesterol stones at 4 weeks *(Table 4)*.

Discussion

The present study examined the impact of thyroid hormone status (after treatment with thyroxine or propylthiouracil) on lipid and bile acid metabolism and its relationship to gallstone development in hamsters. Although it was apparent from the fluctuation in data between weeks 4 and 5 that a steady state had not yet been achieved, distinct differences in plasma lipoproteins, biliary lipids, and bile acid composition were observed in response to changes in thyroid hormone status. However, the results of these experiments on thyroid function in cholesterol-fed hamsters contrast with findings in rats (intact as well as bile fistula rats) and human investigations.

Thyroid status and plasma cholesterol

Although the cholesterol-lowering effect of thyroid hormone is well established in humans and animal models like the rat,[1,3] thyroxine exacerbated the cholesterolemia in our cholesterol-fed hamsters, confirming the trend noted by others.[2,3] Also, despite the fact that PTU treatment induced hypothyroidism, a modest decrease occurred in plasma cholesterol, in contrast to frequent reports of elevated plasma cholesterol in human hypothyroidism.[1] Even more striking was the parallel response in plasma triglycerides. In the hyperthyroid hamsters the triglyceride concentration increased more than 100% compared with the gallstone-inducing diet, whereas PTU caused a 50% decrease.

Even though mechanisms underlying the opposing effects of thyroxine and PTU were not revealed, one can speculate on their atypical effects in the cholesterol-fed hamster. For instance, the hypocholesterolemic effect of thyroxine in rats is associated with enhanced bile acid synthesis, upregulation of hepatic LDL receptors, and reduced hepatic VLDL secretion.[5,7] Paradoxically, thyroid hormone in rats also enhances hepatic HMG-CoA reductase activity and increases cholesterol synthesis.[5,38] Apparently, in the rat, stimulation of bile acid synthesis (removing cholesterol) by thyroxine more than compensates for the increase in cholesterol synthesis,

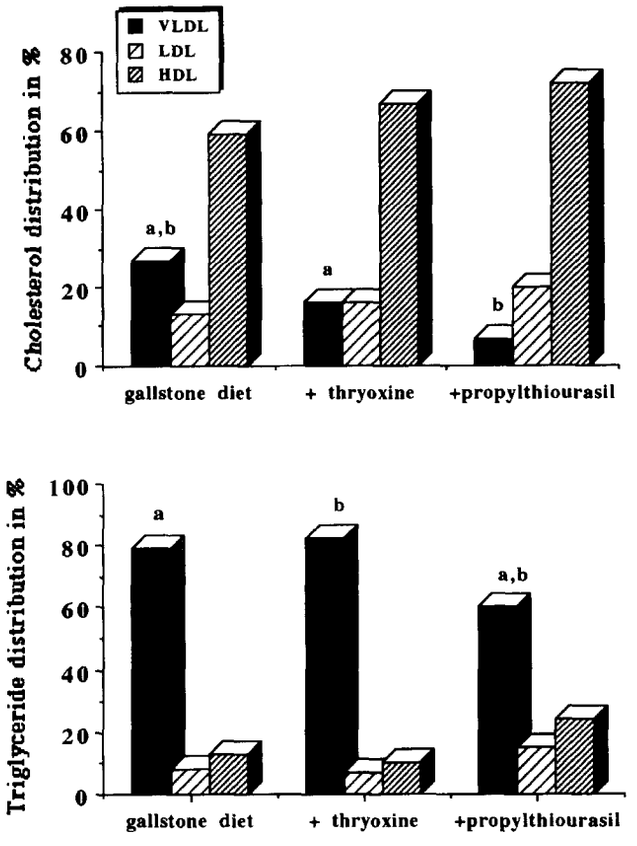

Figure 1 The percent distribution of cholesterol and triglycerides in the lipoprotein fraction. VLDL, LDL, and HDL are shown for the gallstone-inducing diet or that diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil. Values sharing a common superscript are significantly different ($P < 0.05$).

i.e., stimulation of bile acid synthesis (indicated by a rapid increase in mRNA for hepatic cholesterol 7α-hydroxylase) would appear to be greater than the induction of hepatic cholesterol synthesis (indicated by a lesser increase in HMG-CoA reductase).[5,38] In essence, the hypocholesterolemic effect of thyroxine in rats can be attributed to enhanced bile acid synthesis and increased biliary cholesterol secretion.[5,39,40] This does not seem to be the case for humans[2] and probably not for hamsters.

Hyperthyroidism in humans does not enhance conversion of cholesterol into bile acids,[1,2,41] but rather it appears to inhibit cholesterol-7α-hydroxylase.[2] Therefore, unlike rats, the cholesterol-lowering effect of thyroid hormone in humans cannot be explained by augmented bile acid synthesis. Furthermore, thyroxine treatment in humans had no effect on fecal bile acid excretion and only slightly increased neutral steroid excretion.[1] Thus, a detailed explanation for the cholesterol-lowering effect of thyroid hormone awaits further investigation. LDL-receptor activity, intestinal cholesterol absorption, as well as hepatic cholesterol synthesis and biliary cholesterol conversion to bile acids may all be differentially modulated by thyroxine in different species. Cholesterol catabolism, i.e., conversion of cholesterol into bile acids, seems to be exceptional in the rat (high cholesterol 7α-hydroxylase activity), whereas hamsters and humans demonstrate rather limited bile acid synthesis, even under normal physiological conditions. The reason for this species variation is not obvious. However, the hypercholesterolemic action of thyroxine in our hamster model might be explained partly by their poor capacity for bile acid synthesis, their tendency to consume a more cholesterol-rich diet and the exaggerated sensitivity of male hamsters to dietary cholesterol.[42–44] In addition, the potential for thyroxine to enhance LDL receptor uptake in hamsters seems unlikely because the relatively limited LDL receptor activity of hamsters was probably extremely down-regulated by the dietary cholesterol load. In part, the latter reflects the inability of male hamsters to compensate for a cholesterol balance because of their already marginal hepatic cholesterol synthesis.[45] Thus, the atypical effect of thyroxine administration in our hamster gallstone model probably relates to the substantial load of dietary cholesterol. This suggests that the initial balance between HMG-Co A reductase activity, 7α-hydroxylase activity, and LDL receptor activity collectively determines the host's response to thyroxine.

Cholesterol absorption in humans and rats usually is increased by hypothyroidism, while thyroid hormone treatment reduces cholesterol absorption.[1,8] Both situations are accompanied by changes in plasma lipoproteins. In the present study, hepatic cholesterol accumulation was increased, but differences between the three diets were not observed, suggesting that cholesterol absorption was not affected by altered thyroid function in hamsters. Interestingly, despite the reduced thyroid function, hamsters treated with PTU

Table 3 Plasma concentration of triiodothyronine (total T_3) and thyroxine (total T_4) in hamsters fed a gallstone diet and the same diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil

	Gallstone diet	+ Thyroxine	+ Propylthiouracil
Total T_3		nmol/L	
2 weeks	1.9 ± 0.3[a]	11.8 ± 2.6[a,b]	1.1 ± 0.3[b]
4 weeks	2.1 ± 0.2[a]	13.7 ± 4.6[a,b]	2.4 ± 1.1[b]
5 weeks	2.1 ± 0.6[a]	12.9 ± 1.8[a,b]	2.3 ± 0.6[b]
Total T_4		nmol/L	
2 weeks	42.5 ± 8.4[a]	266.2 ± 101.5[a,b]	4.6 ± 11.1[b]
4 weeks	46.2 ± 15.3[a]	309.5 ± 34.3[a,b]	9.1 ± 14.1[b]
5 weeks	54.1 ± 9.3[a,b]	355.3 ± 22.6[a,c]	25.1 ± 8.5[b,c]

Values are mean ± SD.
[a,b,c]Values sharing a common superscript are significantly different ($P < 0.05$) using one-factorial ANOVA and Scheffe's F test.

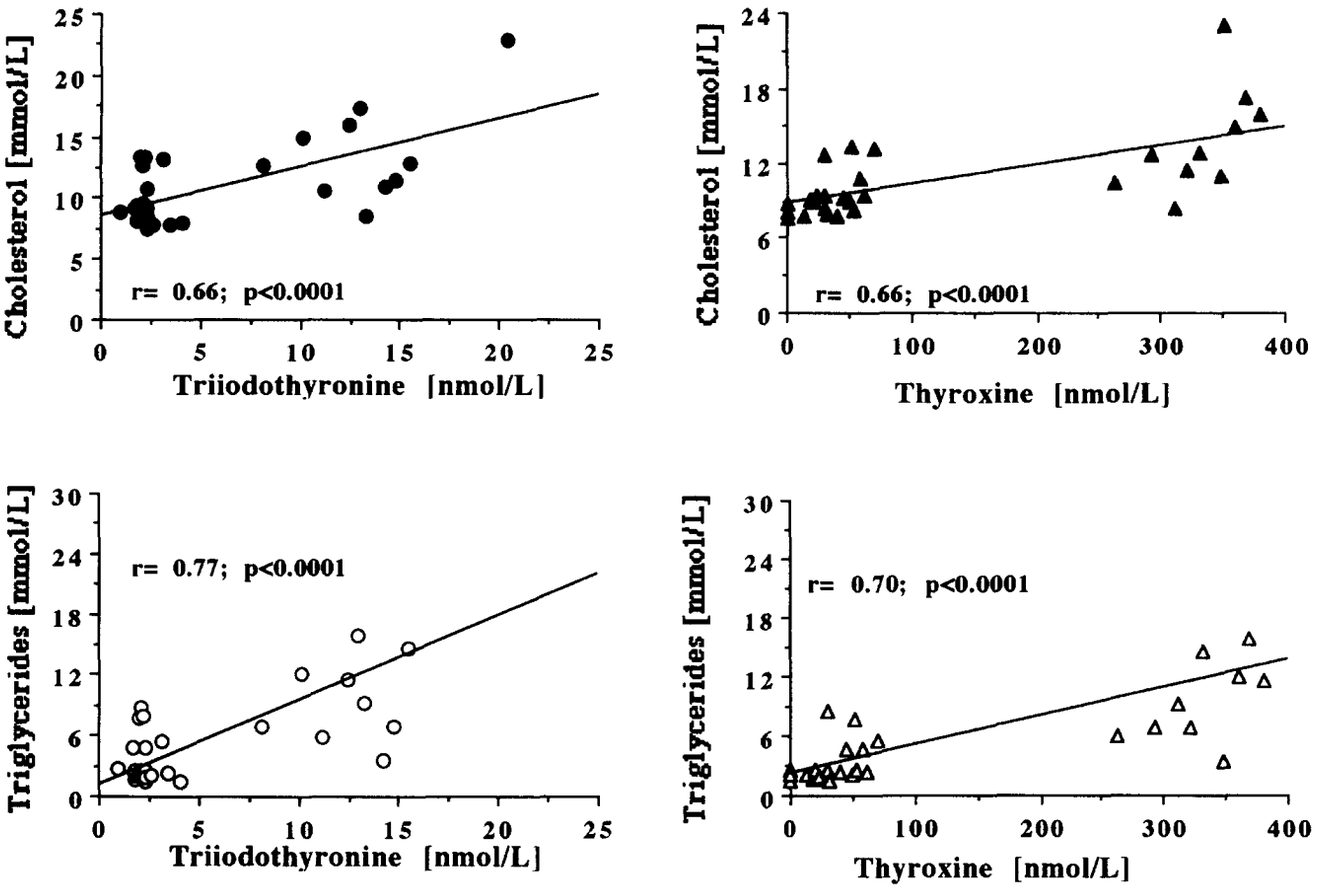

Figure 2 Correlations are depicted between plasma concentrations of thyroxine and triiodothyronine and plasma cholesterol and triglycerides from individual hamsters fed the gallstone-inducing diet or that diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil.

did not decrease their food (caloric) intake compared with hamsters fed the gallstone diet.

Thyroid status and lithogenic index

Biliary cholesterol supersaturation, expressed by a lithogenic index exceeding 1.0 or 100%,[35] generally results from either hypersecretion of biliary cholesterol or hyposecretion of bile acids. All three diets led to an LI that exceeded full saturation. However, thyroxine supplementation caused only a mild supersaturation (LI of 1.1), whereas PTU treatment clearly elevated the LI (LI of 1.8). In the hypothyroid rat, biliary lipid secretion is markedly reduced. Treatment of hypothyroid rats with thyroid hormone resulted in a striking increase in biliary cholesterol and phospholipid secretion and only a modest increase in bile acid secretion.[39,46] The enhanced biliary cholesterol secretion was accentuated by stimulated bile flow, leading to supersaturated bile. In fact, the LI increased from 0.4 to levels above full saturation.[46] Hypothyroid humans frequently have supersaturated bile,[1,41] most likely caused by increased cholesterol synthesis and enhanced biliary cholesterol secretion, which presumably enhances susceptibility to cholesterol gallstones.

Thyroid status and bile acid profile

The bile acid profile was also affected by thyroid hormone status with striking differences occurring in the cholate to cheno and glycine to taurine ratios. Thyroxine treatment reversed the predominance of chenodeoxycholic acid typically encountered in hamsters fed the gallstone-inducing diet.[16,17] In fact, thyroxine supplementation raised the cholate to cheno ratio to a level higher than that normally observed in hamsters fed a cholesterol-free diet.[17,47] Concurrently, the HI was decreased to a level found only in hamsters supplemented with cholestyramine.[17] As a result, the elevation in cholate together with the decline in the LI appeared to preclude development of cholesterol gallstones in thyroxine-treated hamsters, despite the accentuation of hyperlipemia in this group. The predominance of cholate seems to exert a protective influence against cholesterol gallstone induction, at least for hamsters. However, thyroxine supplementation resulted in an increased formation of pigment stones, which corroborates earlier studies in thyroxine-treated hamsters.[23–25]

The increase in the cholate to cheno ratio to almost 3:1 following thyroxine is in contrast to reports in rats in which the normal cholate to cheno ratio decreased from 3:1 to 1:3,

Table 4 Biliary lipids in gallbladder bile from hamsters fed a gallstone diet or the same diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil

	Gallstone diet	+ Thyroxine	+ Propylthiouracil
Hamsters at 4 weeks			
		mM	
Bile acids	99.5 ± 33.2	92.3 ± 18.6	113.6 ± 28.2
Phospholipids	14.8 ± 2.9	16.9 ± 4.6	20.5 ± 3.8
Cholesterol	9.2 ± 3.2	6.4 ± 2.4	8.6 ± 3.1
		mol%	
Bile acids	79.9 ± 3.5	80.0 ± 2.4	79.4 ± 2.5
Phospholipids	12.4 ± 2.1	14.6 ± 2.3	14.5 ± 1.0
Cholesterol	7.6 ± 1.8	5.4 ± 1.0	6.1 ± 2.0
Total lipids, g/dL	6.4 ± 1.9	6.1 ± 1.3	7.5 ± 1.7
Lithogenic index	1.7 ± 0.3[a]	1.1 ± 0.2[a]	1.2 ± 0.4
		Gallstone incidence	
Cholesterol stones/crystals	1/5	0/5	1/5
Pigment stones	0/5	4/5	0/5
No stones	4/5	1/5	4/5
Hamsters at 5 weeks			
		mM	
Bile acids	67.4 ± 14.8	68.3 ± 25.6	87.5 ± 34.7
Phospholipids	9.8 ± 3.4[a]	20.9 ± 4.9[a]	18.7 ± 6.6
Cholesterol	8.6 ± 1.3	6.4 ± 1.9	11.1 ± 4.7
		mol %	
Bile acids	78.4 ± 3.2	70.4 ± 6.2	74.0 ± 4.2
Phospholipids	11.2 ± 2.2[a]	22.5 ± 4.6[a,b]	16.1 ± 1.2[b]
Cholesterol	10.3 ± 2.4	7.1 ± 3.1	9.9 ± 3.5
Total lipids, g/dL	4.4 ± 0.9	5.2 ± 1.5	6.2 ± 2.3
Lithogenic index	2.6 ± 0.7[a]	1.1 ± 0.4[a]	1.8 ± 0.7
		Gallstone incidence	
Cholesterol stones/crystals	2/5	0/5	0/5
Pigment stones	0/5	5/5	0/5
No stones	3/5	0/5	5/5

Values are mean ± SD.
[a,b,c]Values sharing a common superscript are significantly different ($P < 0.05$) using one-factorial ANOVA and Scheffe's F test.

Table 5 Bile acid profile in gallbladder bile of hamsters fed a gallstone diet or the same diet supplemented with 0.005% DL-thyroxine or 0.05% propylthiouracil for 5 weeks

	Gallstone diet	+ Thyroxine	+ Propylthiouracil
		mM	
Taurocholate	23.7 ± 8.9	22.8 ± 8.4	19.7 ± 9.8
Glycocholate	12.8 ± 6.5	25.6 ± 12.6	10.9 ± 6.9
Taurochenodeoxycholate	34.6 ± 13.8[a]	11.5 ± 3.6[a,b]	45.0 ± 15.4[b]
Glycochenodeoxycholate	7.8 ± 7.0	5.1 ± 2.4	10.1 ± 7.5
Taurodeoxycholate	4.5 ± 3.3	2.7 ± 2.1	3.1 ± 3.2
Glycodeoxycholate	1.8 ± 2.5	0.5 ± 1.1	2.1 ± 1.8
		% Distribution	
Taurocholate	28.0 ± 4.5	34.2 ± 7.8[a]	20.3 ± 4.0[a]
Glycocholate	16.2 ± 3.3[a]	36.4 ± 8.1[a,b]	12.5 ± 4.1[b]
Total cholate	44.2 ± 1.9[a,b]	70.6 ± 2.3[a,c]	32.8 ± 5.2[b,c]
Taurochenodeoxycholate	42.0 ± 2.4[a]	17.8 ± 6.2[a,b]	48.7 ± 10.5[b]
Glycochenodeoxycholate	8.2 ± 3.8	7.2 ± 2.7	13.7 ± 8.8
Total cheno	50.2 ± 1.7[a,b]	25.0 ± 4.7[a,c]	62.3 ± 6.8[b,c]
Taurodeoxycholate	5.0 ± 0.8	3.6 ± 2.5	3.0 ± 2.3
Glycodeoxycholate	0.5 ± 1.0	0.8 ± 1.8	2.0 ± 1.3
Taurine conjugates	75.0 ± 6.7	55.6 ± 11.1	72.0 ± 12.0
Glycine conjugates	25.0 ± 6.7	44.4 ± 11.1	28.0 ± 12.3
		Ratios	
Cholate: cheno	0.88 ± 0.07[a]	2.91 ± 0.61[a,b]	0.54 ± 0.15[b]
Glycine: taurine	0.34 ± 0.12[a]	0.86 ± 0.40[a,b]	0.43 ± 0.25[b]
Hydrophobicity index	0.28 ± 0.01[a,b]	0.17 ± 0.01[a,c]	0.33 ± 0.02[b,c]

Values are mean ± SD with $n = 5$.
[a,b,c]Values sharing a common superscript are significantly different ($P < 0.05$) using one-factor ANOVA and Scheffe's F-test.

whereas hypothyroid rats revealed an increase in the ratio.[10] Also, in rats the thyroid hormone stimulation of bile acid synthesis favored cheno formation, while cholate synthesis decreased.[9] Depressed cholate synthesis in rats given thyroid hormone is explained by an inhibition of 12α hydroxylase[11] and stimulation of 26-hydroxylase,[48] both enzymes exerting considerable impact on the cholate to cheno ratio. In humans, hyperthyroidism also depressed cholate synthesis, but both cheno and total bile acid synthesis were unaffected.[2] Thyroxine treatment of hypothyroid humans significantly stimulated cheno synthesis, but did not affect cholate synthesis.[41]

Our previous hamster data found cholesterol gallstone induction was associated with an increased HI, reflecting a predominant cheno profile and a diminished cholate to cheno ratio.[17] On this basis, hamsters treated with PTU should have developed cholesterol gallstones because they had an exaggerated cheno profile and an extremely low cholate to cheno ratio. However, this was not the case, as only one of 10 hamsters had cholesterol gallstones.

Thyroid hormone status also influenced the pattern of bile acid conjugation. In hamsters fed the gallstone-inducing diet the glycine to taurine ratio was depressed because taurine-conjugated bile acids, especially taurocheno, predominated. On the other hand, thyroxine increased the glycine to taurine ratio to that usually found in hamsters fed a cholesterol-free diet,[47] where bile acids are almost equally conjugated with glycine and taurine. In contrast, the PTU-treated hamsters had the highest percentage of taurine-conjugated bile acids, resulting in an extremely low glycine to taurine ratio. Again, the impact of thyroxine on the conjugation pattern in hamsters conflicts with that observed in humans. For instance, in human hyperthyroidism, taurine conjugation increased and the glycine to taurine ratio decreased, whereas in hypothyroid patients glycine conjugation and the glycine to taurine ratio were increased.[49]

The changes observed in the glycine to taurine ratio of our hamsters paralleled the shift in the cholate to cheno ratio. Thus, whenever chenodeoxycholic acid dominates, taurine conjugation tends to be high. When cholate is the principal bile acid, glycine conjugation predominates, confirming the previously posited link between taurine conjugation and cheno synthesis or glycine conjugation and cholate synthesis.[50]

Thus, the impact of thyroid hormone on the cholesterol-fed hamster model in the present study appears opposite to humans in many ways, presumably reflecting differences in the basal rate of cholesterol synthesis, capacity for bile acid synthesis, and LDL receptor regulation. Nonetheless, like humans, thyroxine-treated hamsters seem to be protected against cholesterol gallstone formation, in part, by their ability to maintain favorable biliary lipids and a bile acid profile in which cholate predominates.

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