The Effect of Hypothyroidism and Thyroxine Replacement on Hepatic and Intestinal HMG-CoA Reductase and ACAT Activities and Biliary Lipids in the Rat

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Hepatic and intestinal cholesterol metabolism were investigated in the hypothyroid and thyroxine-treated hypothyroid rat. Plasma cholesterol levels were significantly increased in hypothyroid animals. After thyroxine administration, plasma cholesterol levels were reduced to levels observed in euthyroid controls. Hypothyroidism caused a significant decrease in biliary cholesterol output, which was reversed with thyroxine treatment. In contrast, biliary bile acid output was unchanged by the thyroid status. Cholesterol synthesis, as estimated by HMG-CoA reductase activity, was decreased in the liver of hypothyroid animals. Thyroxine administration, however, significantly increased reductase activity returning it to control levels. Hypothyroidism did not affect HMG-CoA reductase activity in the intestine, but thyroxine administration markedly stimulated the activity of this enzyme in this organ. Cholesterol esterification, as estimated by ACAT activity, was decreased in the liver of hypothyroid rats, while intestinal ACAT activity was greatly increased. Thyroxine treatment reversed these effects of hypothyroidism on ACAT activity in both organs. An increase in microsomal cholesterol content in the intestine of hypothyroid rats was associated with the observed increase in intestinal ACAT activity. The percent of cholesterol that was absorbed in the intestine was not changed by the thyroid status of the animal. The data suggest that the changes observed in cholesterol metabolism in hypothyroid rats or hypothyroid rats treated with thyroxine for 1 week cannot account for the increase in plasma cholesterol levels observed in the hypothyroid rat. This implies that other factors that were not studied, such as changes in lipoprotein catabolism, are likely to contribute to the hypercholesterolemia of hypothyroidism.

THE ETIOLOGY of the hypercholesterolemia, which has been identified in thyroid hormone deficiency both in man[1-4] and in rat,[5,6] is probably multifactorial. Thompson et al[7] observed that the catabolism of low density lipoproteins (LDL) is slower in hypothyroid patients compared to their euthyroid controls. Sykes et al[8] made similar observations in hypothyroid and euthyroid rats injected with labeled human LDL. The retardation of lipoprotein degradation in hypothyroidism is thought to be secondary to a defect in receptor-mediated lipoprotein catabolism.[7,9] Decreased activity of lipoprotein lipase has also been observed in thyroid hormone deficiency in man lending further support for a combined defect in peripheral lipoprotein catabolism.[10,11] Since hepatic secretion of lipoproteins in hypothyroid rats is thought to be decreased,[12] overproduction of lipoproteins by the liver cannot be implicated as a cause for the hypercholesterolemia of hypothyroidism. Abrams and Grundy[13] observed an increase in both cholesterol synthesis and cholesterol absorption associated with a reduction in fecal neutral sterols in some but not all patients with hypothyroidism. Others have reported a decrease in biliary bile acid concentration in thyroid hormone deficiency, suggesting a decrease in hepatic cholesterol catabolism to bile acids.[14-16]

It has long been recognized that the thyroid status of an animal affects many aspects of cholesterol metabolism.[17] It is still unclear, however, to what extent any of these changes might contribute to the increased plasma cholesterol levels in hypothyroidism. The present study was undertaken to investigate the effects of thyroid hormone deficiency and thyroid replacement on biliary lipids and the rates of cholesterol synthesis and esterification in the intestine and liver of propylthiouracil (PTU)-treated rats. The results suggest that although many aspects of cholesterol metabolism are altered by hypothyroidism, these changes probably contribute very little to the hypercholesterolemia observed in this state.

MATERIALS AND METHODS

Materials

[Oleoyl-1-^{14}C] CoA, 3-[glutaryl-3-^{14}C] hydroxy-3-methylglutaryl CoA, [1, 2-^{3}H] cholesterol, [4-^{14}C] cholesterol, and [5-^{3}H] mevalonolactone were purchased from New England Nuclear (Boston). β-[4-^{14}C] sitosterol was purchased from Amersham (Arlington Heights, Ill). Oleoyl-CoA, mevalonolactone, egg lecithin, PTU, thyroxine, trypsin inhibitor, cholesterol, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nucleotide adenine diphosphate, dithiothreitol, and fatty acid-poor bovine serum albumin were obtained from Sigma Chemical (St Louis). β-hydroxy-β-methylglutaryl CoA was from P.L. Biochemicals (Milwaukee). Mylar-backed silica gel-G chromatography sheets were obtained from Eastman Kodak (Rochester, NY). All other chemicals were reagent grade.

Animals and Diet

Eighteen male Sprague-Dawley rats were purchased from Harlan Industries (Madison, Wis). They were housed in a windowless room that was illuminated from 7 AM to 7 PM. After 1 week, they were divided into three different groups and weighed. All animals were fed normal ground rat chow (Teklad Mills Inc, Madison, Wis) contained in open glass jars. Two groups of animals received 0.1% PTU, which was pulverized and added to the chow. The remaining group was continued on the normal ground rat chow. The animals

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were allowed to eat and drink ad lib for 5 weeks. At the end of 4 weeks, one group receiving the 0.1% PTU was injected intraperitoneally with thyroxine, 0.02 mg/100 g in 0.5 mmol/L NaOH, daily for seven days. The others received the vehicle alone. Initial mean weights of the rats in each group (n = 6) prior to the start of the diets were: euthyroid, 212 ± 3 g; hypothyroid, 218 ± 5 g; and hypothyroid plus thyroxine treatment, 239 ± 2 g. As expected, the weights of the hypothyroid animals plateaued. At the time of death 5 weeks later, the mean weights were 268 ± 12 and 291 ± 3 g for hypothyroid and hypothyroid plus thyroxine-treated rats, respectively. The administration of thyroxine (0.02 mg/100 g body weight) during the last week of the dietary period to hypothyroid animals caused no untoward effects and resulted in an increase in food intake. These animals gained an average of 11 g during the week of thyroxine administration. During this same period, hypothyroid rats receiving the vehicle alone did not gain weight. Euthyroid animals weighed 329 ± 7 g just prior to death.

Although hypothyroid animals ingested less food over the 5-week dietary period and subsequently weighed less at the time of study, these animals were consuming sufficient calories to meet their daily metabolic requirements. No attempt was made to reduce the caloric intake of the euthyroid animals to match that consumed by the two hypothyroid groups. This would have resulted in the comparison of a "starved" to an "unstarved" animal model. The study, therefore, was designed to investigate the overall effects of hypothyroidism on cholesterol metabolism.

Biliary Drainage

At the end of 5 weeks, the animals were weighed and lightly anesthetized with ether. The bile ducts were cannulated with polyethylene-10 tubing as near to the ampulla as possible. The tubing was secured and the abdomen was closed. Light ether anesthesia was continued and the animals were placed under a heat lamp. The first ten minutes of biliary drainage ensured good bile flow. Bile was then collected for exactly one hour. At the end of the collection period, the animals were exsanguinated by aortic puncture at the iliac bifurcation. All animals were killed between 8:30 AM and 10:30 AM. The blood was collected in EDTA-containing tubes and centrifuged at 2,000 rpm × 15 min to obtain plasma.

Microsomal Preparation

After exsanguination, the entire small intestine and one g of liver were removed for the preparation of microsomes.[18]

Enzyme Assays

HMG-CoA reductase activity was determined as previously described.[19] The specific activity of the substrate was 21,750 dpm/nmol. ACAT activity was measured as before.[20] The specific activity of labeled oleoyl-CoA was 17,253 dpm/nmol and 24,634 dpm/nmol for hepatic and intestinal activities, respectively. The assays contained 0.075 mg to 0.150 mg microsomal protein.

Table 1. Plasma Thyroxine and Lipid Levels

	Thyroxine ($\mu g \cdot dL^{-1}$)	Cholesterol ($mg \cdot dL^{-1}$)	Triglyceride ($mg \cdot dL^{-1}$)
Euthyroid (6)	4.21 ± 0.28	64 ± 3	48 ± 6
Hypothyroid (6)	1.35 ± 0.05*	80 ± 3‡§	20 ± 2*
Hypothyroid + thyroxine (6)	15.9 ± 0.59†	68 ± 3	69 ± 6

Values represent mean ± SE. Number of animals in each group are given in parentheses.

*$P < 0.001$ *v* euthyroid and hypothyroid + thyroxine.

†$P < 0.001$ *v* euthyroid and hypothyroid.

‡$P < 0.005$ *v* euthyroid.

§$P < 0.05$ *v* hypothyroid + thyroxine.

Cholesterol Absorption

The percent of cholesterol absorbed was determined by the method of Borgstrom[21] using labeled β-sitosterol as a nonabsorbable marker. Animals were maintained in individual metabolic cages and stools were collected for four days following oral gavage. The percent recovery of β-sitosterol did not differ between the groups averaging 75 ± 2%.

Chemical Analysis

Plasma cholesterol was determined by gas-liquid chromatography.[18] Triglycerides were measured fluorometrically.[20] Plasma thyroxine levels were measured by radioimmunoassay using a kit from Tetra-Lab R1A, Nuclear-Medical Laboratories, Warner-Lambert Technologies, Inc (Irving, Tex). Lipids were extracted from bile and tissue using chlorofom-methanol, 2:1 (v/v). Cholesterol content was measured by gas-liquid chromatography using cholestane as an internal standard.[22] Phospholipids were determined using the method of Chalvardjian and Rudnicki.[23] Total bile acids in bile were measured according to the method of Turley and Dietschy.[24] Protein was determined according to the method of Lowry et al.[25]

Statistical Analysis

The unpaired Student's *t*-test was used to determine probability values.

RESULTS

Plasma Thyroxine and Lipid Levels

Plasma thyroxine levels were significantly decreased in animals ingesting PTU (Table 1). Hypothyroid rats that were injected daily with thyroxine for seven days had a substantial increase in their plasma thyroxine level.

Hypothyroidism also resulted in an increase in plasma

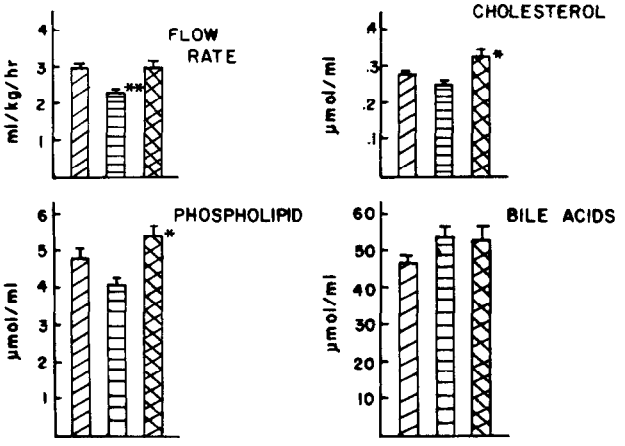

Fig 1. Effect of hypothyroidism and thyroxine replacement on the rate of bile flow and biliary lipid concentrations. Immediately prior to death, bile was collected for one hour. Biliary lipid concentrations were determined as described in "Materials and Methods." The rate of bile flow was calculated by weighing tared test tubes after biliary drainage. ▨, euthyroid; ▤, hypothyroid; ▩, hypothyroid + thyroxine. *$P < 0.02$ *v* hypothyroid. **$P < 0.05$ *v* euthyroid and hypothyroid + thyroxine.

cholesterol. With the administration of thyroxine, however, the levels decreased to those observed in the euthyroid group. Plasma triglycerides were significantly decreased in the hypothyroid rats and thyroxine administration increased them to control values.

Biliary Lipids

The bile ducts of all animals were cannulated just prior to death, and bile was collected for exactly one hour. As illustrated in Fig 1, the rate of bile flow was significantly less in the hypothyroid rats. In the hypothyroid animals injected with thyroxine, the flow rate returned to that observed in euthyroid rats. Although there was a small decrease in biliary cholesterol concentrations in hypothyroid animals *v* the euthyroid group, this difference was not significant. There was a significant difference, however, between the biliary cholesterol concentrations observed in hypothyroid animals compared to the biliary cholesterol concentrations in hypothyroid animals treated with thyroxine. This same relationship was observed for biliary phospholipid concentrations, whereas biliary bile acid concentrations were not affected in any of the dietary groups.

As shown in Fig 2, biliary cholesterol outputs, expressed as μmol/kg/h, were significantly reduced in hypothyroid animals. After thyroxine treatment, cholesterol outputs were the same as in euthyroid rats. Biliary phospholipid outputs showed essentially the same pattern as those observed for cholesterol outputs. Again, total bile acid outputs were not significantly affected.

HMG-CoA Reductase Activity

The relative rate of hepatic cholesterol synthesis in hypothyroid rats, as determined by the activity of microsomal HMG-CoA reductase, was significantly decreased compared to the rate of hepatic cholesterol synthesis in euthyroid animals (Fig 3). There was a marked stimulation of hepatic HMG-CoA reductase activity in hypothyroid animals after treatment with thyroxine.

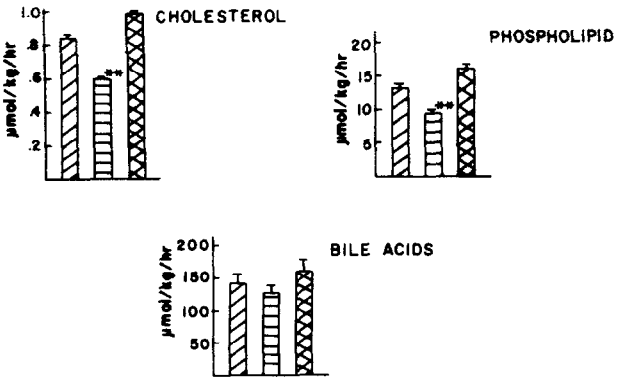

Fig 2. Effect of hypothyroidism and thyroxine replacement on biliary lipid output. ▨, euthyroid; ▤, hypothyroid; ▩, hypothyroid + thyroxine. **$P < 0.05$ *v* euthyroid and hypothyroid + thyroxine.

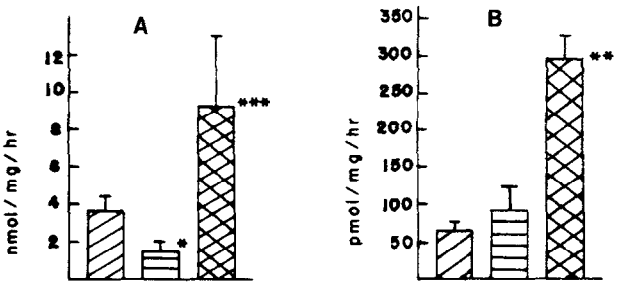

Fig 3. Effect of hypothyroidism and thyroxine replacement on HMG-CoA reductase activity in liver (A) and intestine (B). After biliary drainage, liver and intestinal microsomes were prepared and HMG-CoA reductase activity was measured. ▨, euthyroid; ▤, hypothyroid; ▩, hypothyroid + thyroxine. *$P < 0.05$ *v* euthyroid. **$P < 0.05$ *v* euthyroid and hypothyroid. ***$P < 0.05$ *v* hypothyroid.

In the intestine, HMG-CoA reductase activity was not significantly altered by hypothyroidism. However, much like the liver, the administration of thyroxine to the hypothyroid rats caused a significant increase in the activity of this enzyme in the intestine.

Acylcoenzyme A:Cholesterol Acyltransferase Activity

As shown in Fig 4, hypothyroidism caused a significant decrease in the activity of hepatic ACAT. Following the administration of thyroxine, hepatic ACAT activity returned to the activity observed in livers of euthyroid animals.

In distinct contrast, the intestine of hypothyroid rats had significantly higher ACAT activity than the intestine of euthyroid rats. This marked increase in ACAT activity was significantly reduced by the administration of thyroxine.

Hepatic and Intestinal Cholesterol Content

The amount of cholesterol in liver whole homogenate and microsomes was not changed by the thyroid status of the animal (Table 2). Although the same was true for the whole homogenate of intestine, microsomal cholesterol content of intestine obtained from hypothyroid rats was significantly greater than in intestinal microsomes prepared from euthyroid animals or thyroxine-treated hypothyroid animals.

Cholesterol Absorption

In another group of 18 animals, cholesterol absorption was estimated. As before, the dietary period was 5 weeks. Twelve animals were rendered hypothyroid by adding 0.1% PTU to their diet. A subset of six hypothyroid rats were injected intraperitoneally with 0.02 mg/100 g thyroxine for 1 week. The others received vehicle alone. The plasma cholesterol levels were 83 ± 4, 125 ± 4, and 106 ± 5 mg/dL for euthyroid, hypothyroid, and thyroxine-treated rats, respectively. The percent of cholesterol that was absorbed for the three groups did not differ, 77.3 ± 0.82%, 76.6 ± 0.39%, and 76.9 ± 0.56%, respectively.

DISCUSSION

The data exclude the possibility that an overproduction of cholesterol by the liver or intestine plays a significant role in

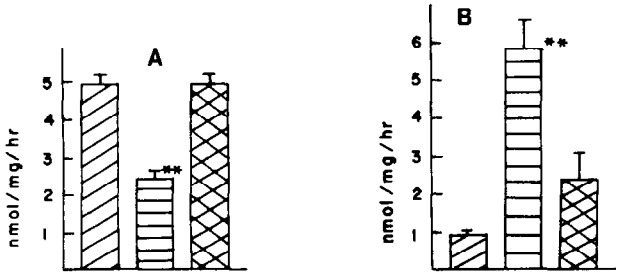

Fig 4. Effect of hypothyroidism and thyroxine replacement on ACAT activity in liver (A) and intestine (B). ▨, euthyroid; ▤, hypothyroid; ▩, hypothyroid + thyroxine. **$P < 0.01$ *v* euthyroid and hypothyroid + thyroxine.

Table 2. Total Cholesterol Content of Whole Homogenates and Microsomes From Liver and Intestine

	Liver		Intestine	
	Whole Homogenate	Microsomes	Whole Homogenate	Microsomes
Euthyroid (6)	6.7 ± 0.1	23 ± 1	17 ± 0.4	39 ± 1
Hypothyroid (6)	6.3 ± 0.4	22 ± 1	17 ± 0.3	44 ± 1*
Hypothyroid + thyroxine (6)	8.2 ± 0.8	21 ± 1	18 ± 0.4	39 ± 1

Values represent mean ± SE and are given as $\mu g \cdot mg\ protein^{-1}$. Number of animals in each group are given in parentheses.

*$P < 0.05$ *v* euthyroid and hypothyroid + thyroxine.

the hypercholesterolemia that is observed in the thyroid-deficient rat. Hepatic HMG-CoA reductase activity was significantly decreased in hypothyroid animals, whereas in the intestine it was not changed. More convincingly, however, is the observation that thyroxine administration to hypothyroid rats resulted in lowering of plasma cholesterol levels despite producing a marked stimulation of HMG-CoA reductase activity in both organs. Since the liver and intestine contribute significantly to newly synthesized cholesterol in the body, the data are in agreement with others suggesting that in thyroid deficiency states, overall rates of cholesterol synthesis are decreased.[26–29] Because of the inability to control the many different variables that occur in rats during thyroxine deficiency and thyroxine administration in vivo, it is impossible to implicate a direct regulation of HMG-CoA reductase by thyroid hormone per se. The data, however, support earlier observations that demonstrate that HMG-CoA reductase activity is regulated by the thyroid status of the animal.[31,32]

The effect of thyroxine deficiency and thyroxine administration on ACAT activity has not been previously studied. Hepatic ACAT activity was decreased in hypothyroid rats, whereas in the intestine it was markedly increased. Thyroxine administration reversed these effects produced by thyroxine deficiency on ACAT activity in both organs. Because thyroxine deficiency and thyroxine administration produced opposite effects on ACAT activity in the two organs, it is difficult to postulate a direct regulation of ACAT activity by this hormone. It is more reasonable to assume that other factors related to the consequences of hypothyroidism or thyroxine administration affect ACAT activity. The activity of this enzyme is very responsive to increases in microsomal cholesterol content.[22,33] In microsomes prepared from the intestine of hypothyroid animals, cholesterol content and ACAT activity were significantly increased. The reason for the increase in intestinal microsomal cholesterol content in hypothyroid rats is uncertain; however, Helgerud et al[34] have observed an increase in microsomal cholesterol content and ACAT activity in intestines of rats that have been fasted. Although hypothyroid animals are not fasting, the caloric intake of these rats is substantially reduced to meet their lower metabolic requirements. A significant reduction in caloric intake could perhaps have a tendency to increase microsomal cholesterol content and result in an increase in ACAT activity in the intestine. With resumption of eating following thyroxine administration, microsomal cholesterol and ACAT activity return to their euthyroid levels.

It has been suggested that hypothyroidism results in a decrease in biliary lipid concentrations, particularly bile acids[15,35] and cholesterol.[14,35,36] Our results do not confirm those observations. Biliary bile acids and cholesterol were not significantly different from the concentrations observed in bile from euthyroid rats. Biliary cholesterol output was decreased in hypothyroid rats, but this was secondary to a decrease in the rate of bile flow. In the earlier studies, total biliary drainage was sustained for up to seven days, conditions which will completely disrupt the enterohepatic circulation. In the present study, biliary drainage was performed for only one hour, a period of time that has been shown to reflect changes that would occur in animals with an intact enterohepatic circulation.[37]

An increase in the percent absorption of cholesterol by the intestine of hypothyroid rats could potentially contribute to the hypercholesterolemia observed in these animals.[17] Preliminary results using the dual isotope plasma ratio method[38] suggested this may be occurring.[39] However, in the present study using a different technique to measure cholesterol absorption, the thyroid status of the animal had no effect on the percent absorption of cholesterol. Thus, with a decrease in cholesterol intake, a decrease in biliary cholesterol excretion, and no change in cholesterol absorption, there is little to suggest an increased flux of cholesterol through the intestine of thyroid-deficient rats.

The dose of thyroxine, which was used to replace the thyroid-deficient animals, was chosen after reviewing previous literature.[17,31,36] It cannot be ascertained with certainty that 1 week of replacement with thyroxine is adequate to return the animal to a euthyroid state; however, plasma levels of thyroxine in these animals would suggest more than adequate amounts of circulating thyroxine. The fact that these animals resumed eating and had gained 11 g during thyroxine administration suggests a return to a more normal metabolic status.

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