JCI The Journal of Clinical Investigation

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J Clin Invest. 1980;66(4):646-654. https://doi.org/10.1172/JCI109900.

Research Article

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Influence of Thyroid Hormone Status on Mevalonate Metabolism in Rats

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ABSTRACT Mevalonate, an essential intermediate in cholesterol synthesis, is metabolized either to cholesterol or, by the shunt pathway, to CO_2. Previous investigations have demonstrated that the kidneys are the chief site of circulating mevalonate metabolism and that sex hormones as well as insulin markedly influence circulating mevalonate metabolism. The present study examined in rats the influence of thyroid hormone status on mevalonate metabolism in vivo and in vitro. L-thyroxine administration increased renal conversion of circulating mevalonate to cholesterol, 41% in the females and 22% in the males. Conversely, hypothyroidism induced by 6 N propyl-2-thiouracil reduced renal conversion of circulating mevalonate to cholesterol by 45% in females and 27% in males; thyroid hormone replacement in these animals returned cholesterogenesis in the kidneys to supranormal levels. Neither L-thyroxine nor hypothyroidism altered circulating mevalonate conversion to cholesterol in the liver or carcass. In vitro studies confirmed the in vivo observations. Changes in thyroid hormone produced only minor changes in the shunt pathway of mevalonate metabolism. This study demonstrates that the major effect of the thyroid hormone on the metabolism of circulating mevalonate is to alter the conversion of mevalonate to cholesterol, an effect localized solely to the kidneys.

INTRODUCTION

Thyroid hormone strongly influences cholesterol degradation and synthesis. Plasma cholesterol levels decrease with hyperthyroidism and increase with hypothyroidism. Paradoxically, however, *de novo* cholesterol synthesis is augmented in the hyperthyroid state, and the observed reduction in plasma cholesterol levels occurs secondarily to an increase in cholesterol degradation. Conversely, hypothyroidism causes a reduction in *de novo* cholesterol synthesis, and the elevated plasma cholesterol values occur because of a decrease in cholesterol degradation (1). The step(s) in cholesterol biosynthesis that is regulated by thyroid hormone has not been investigated extensively.

Fletcher and Myant (2) demonstrated that cholesterol synthesis from acetate was increased in liver slices from thyroid-treated rats, whereas cholesterol synthesis from mevalonate was unchanged. Ness et al. (3) have further shown that thyroid hormone stimulated the activity of hepatic β-hydroxy-β-methylglutaryl CoA (HMG CoA)[1] reductase, the enzyme that converts HMG CoA to mevalonate. These results suggest that thyroid hormone affects cholesterogenesis by increasing the conversion of acetate to mevalonate and that the post-mevalonate biosynthetic steps of cholesterogenesis are unaffected by thyroid hormone.

As noted above, mevalonic acid is formed from HMG CoA; this reaction is catalyzed by HMG CoA reductase and is the site of feedback control of cholesterogenesis (4, 5). In 1974, Edmond and Popjak (6) demonstrated that the label from [^{14}C]mevalonate can be diverted to long chain fatty acids, and in 1975, Fogelman et al. (7) published evidence that, in rats and in man, mevalonate is directly oxidized to CO_2. The results of these two studies demonstrated conclusively that mevalonate is metabolized by an alternative, or shunt, pathway other than that leading to cholesterol production. Previous studies from this and other laboratories have shown that the kidneys, rather than the liver, are the chief site of mevalonate metabolism by both the sterol and shunt pathways (8–14). Further, it has been observed that there are major sex differences in mevalonate metabolism. The female rat oxidizes circulating mevalonate to CO_2 by the shunt pathway at twice

A portion of this work was presented at the Annual Meeting of the Western Society for Clinical Research. 1980. *Clin. Res.* 28: 80A. (Abstr.)

Received for publication 26 February 1980 and in revised form 4 June 1980.

[1] *Abbreviations used in this paper*: HMG, β-hydroxy-β-methylgluaryl; PTU, propyl-2-thiouracil; T_4, thyroxine.

the rate of the male; conversely, the male rat converts circulating mevalonate to cholesterol to a significantly greater extent than the female. These sex differences in the metabolism of circulating mevalonate are due entirely to differences in renal metabolism (15). Recently, a sex difference in circulating mevalonate metabolism has also been demonstrated in humans; premenopausal women oxidize 68% more circulating mevalonate to CO_2 than age-matched males (16).

Studies of immature female rats and castrated male rats treated with estrogens have suggested that the observed sex differences in circulating mevalonate metabolism are mediated by sex hormones (15). Studies using streptozotocin-induced diabetic rats have shown that insulin also influences circulating mevalonate metabolism, with insulin deficiency reducing the shunt pathway and leading to an increase in sterolgenesis (17). Because of the above hormonal effects on mevalonate metabolism, and because thyroid hormone is known to have a marked influence on cholesterol synthesis, the present study was initiated to investigate the effect of thyroid hormone on mevalonate metabolism by both the sterol and shunt pathways in the kidneys, liver, and peripheral tissues of the rat.

MATERIALS

Materials. (*R,S*)-[5-^{14}C]mevalonate (15 mCi/mmol) was purchased from Research Products International Corp. (Elk Grove Village, Ill.). The [1α,2α-^{3}H]cholesterol (31 Ci/mmol) used as an internal standard and [^{14}C]sodium acetate (56.2 mCi/mmol) were purchased from New England Nuclear (Boston, Mass.). The *R,S*-[1-^{14}C]mevalonate (6 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. The thin layer polygram Sil G plates were purchased from Brinkmann Instruments, Inc. (Westbury, N. Y. The counting solution used for $^{14}CO_2$ and blood samples contained 300 ml of Beckman Bio-Solv III (Beckman Instruments, Fullerton, Calif.) 1,000 ml of Packard scintillation grade toluene (Packard Instrument Co., Inc., Downers Grove, Ill.), 100 ml of glass-distilled water, and 6.0 g of 2,5-diphenyloxazole (PPO) (Amersham/Searle Corp., Arlington Heights, Ill.). The thin-layer strips were counted in a solution containing 1.133 of 1,4-bis-[2-(5-phenyloxazolyl)]benzene (Amersham/Searle Corp.), 9.33 g of PPO, 1,333 ml of scintillation grade toluene, and 666 ml of Triton X-100. 6 N propyl-2-thiouracil (PTU) was purchased from Sigma Chemical Co. (St. Louis, Mo.) and L-thyroxine (T_4) from Flint Laboratories (Deerfield, Ill.). Radioautography was carried out on Kodak RP-14 x-ray film (Eastman Kodak Co., Rochester, N. Y.).

Animal procedures. Male and female Sprague-Dawley rats (200 g) were purchased from Simonson Animal Vendors (Gilroy, Calif.). The animals were maintained on a reverse 12-h light cycle and were fed Simonson rat and mouse diet and water ad lib. The hyperthyroid state was induced by the intraperitoneal injection of L-T_4 and the hypothyroid state by feeding 0.2% PTU mixed with rat chow for 1 mo. Control and PTU animals in the L-T_4 studies were injected intraperitoneally with an equal volume of saline for the same number of days as the thyroid-treated animals.

Kinetics studies. Control and thyroid-treated animals were anesthetized with diethyl ether and injected via the tail vein with 1 μCi [1-^{14}C]mevalonate. At 10, 20, 30, 45, and 60 min, blood was obtained and 100-μl samples were added to 20 ml of scintillation counting solution. The ^{14}C in the blood samples was determined on a Beckman LS-330 scintillation counter (Beckman Instruments).

In vivo. Between 8:00–9:00 a. m. on the day of study, the rats were anesthetized with diethyl ether and injected via the tail vein with 5 μCi, 1.05 μmol potassium (*R,S*)-[5-^{14}C]-mevalonate in 0.4 ml of a 0.9% saline solution. The animals were placed in 2-liter wide-mouth Erlenmeyer flasks fitted with two-hole stoppers. Air was drawn through the bottles at the rate of 2 liters/min, and $^{14}CO_2$ was collected continuously in gas-washing bottles containing 180 ml of 1 N NaOH. To ensure complete trapping of CO_2, a second gas-washing bottle also containing 180 ml of 1 N NaOH was connected in tandem with the primary collecting bottle. At the times noted, 0.2-ml samples of the 1 N NaOH were added to 10 ml of scintillation counting solution and the ^{14}C was determined on a Beckman LS-330 scintillation counter. At the times noted, the animals were killed and weighed, and the livers, kidneys, and carcasses were saponified separately by refluxing overnight in a solution of 90% KOH, H_2O, and 70% ethyl alcohol (1:2:5).

In vitro. The animals were anesthetized with diethyl ether and the liver and kidneys rapidly removed. Slices of each tissue (0.5 mm thick) were prepared with a McIlwain tissue slicer, and 200 mg of tissue was placed in the outer well of a 25-ml center-well flask containing 2 ml of Krebs-Ringer PO_4 buffer and 1 μCi, 262 nmol (*R,S*)-[5-^{14}C]mevalonate. Where indicated, [1-^{14}C]acetate (10 μCi/10 μmol) was used in place of [5-^{14}C]mevalonate. The flasks were gassed with 95% O_2/5% CO_2 for 15 s, stoppered with serum caps, and incubated for 1 h at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. At the end of this incubation period, 0.9 ml of 1 N NaOH was injected through the serum cap into the center well and 1 ml of 1 N H_2SO_4 injected through the serum cap into the outer well. $^{14}CO_2$ was then collected in the inner well by reincubating the samples for 15 min in the Dubnoff shaker. The incubation flasks were then opened and 0.1 ml of the center-well NaOH solution was added to scintillation tubes containing 10 ml of counting solution. The ^{14}C content was determined with a Beckman LS 330 liquid scintillation counter. All values were corrected for the small amounts of ^{14}C recovered from flasks incubated simultaneously with boiled tissues. The tissue was saponified by refluxing overnight in a solution of 90% KOH, H_2O, and 70% ethyl alcohol (1:2:5) and then analyzed for ^{14}C lipids.

Lipid analysis. The flasks were cooled and an internal standard of [^{3}H]cholesterol was added before extracting the nonsaponifiable material three times with 25 ml of petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and then applied to thin layer chromatographic plates. The plates were developed in ethyl acetate/benzene (1:5) for 50 min, and the radioactive bands were located by radioautography (3–7 d). The bands corresponding to standards of cholesterol were cut from each plate and placed in scintillation vials containing 10 ml of counting solution and then counted in a Beckman LS 330 scintillation counter. The gain and discriminator window settings of the scintillation counter were adjusted so that <0.2% of the ^{3}H counts were recorded in the ^{14}C window and ~10% of the ^{14}C counts were recorded in the ^{3}H window. The amount of [^{3}H]cholesterol added as an internal standard was adjusted so that ^{3}H counts were approximately five times greater than ^{14}C counts. Calculations were corrected for spillover of ^{3}H and ^{14}C and for background. Because only the *R* isomer of mevalonate is metabolized, all calculations assume that half the administered mevalonate is inactive.

RESULTS

Effects of L-T_4 on mevalonate metabolism in vivo. The disappearance rates of circulating mevalonate in two female control and two female animals injected with 50 μg L-T_4 i.p. for 3 d were similar. The half-life was 27 min in the control animals and 25 min in the thyroid-treated animals. This demonstrated that thyroid administration did not alter the rate of disapperance of circulating mevalonate. The half-lives observed were similar to those previously reported by this and other laboratories (8, 18).

Table I illustrates the affects of administering L-T_4 (50 μg for 8 d) to female rats. The shunt pathway, represented as $^{14}CO_2$ production, was significantly decreased in the thyroid-treated animals (control 94±7 SE vs. thyroid 73±2 nmol of mevalonate oxidized to CO_2 in 6 h; $P < 0.05$). The oxidation of circulating mevalonate in the control female animal was similar to values previously observed. Whereas the shunt pathway was decreased in the thyroid-treated animals, total body cholesterol synthesis from circulating mevalonate was increased (control 218±11 vs. thyroid 268±14 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.05$). This elevation in total body cholesterol synthesis in the thyroid-treated animals was due entirely to an increase in renal cholesterogenesis (control 148±7 vs. thyroid 208±17 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.05$). Kidney weight was increased in the thyroid-treated animals in this particular experiment, but in other experiments there was no consistent relationship between kidney weight and the activity of either the shunt or sterol pathway. In the liver and carcass, thyroid hormone did not significantly alter cholesterol synthesis from circulating mevalonate. These results demonstrate that the major effect of thyroid hormone administration on circulating mevalonate metabolism is to induce an increase in total body cholesterol synthesis and, moreover, that this increase is wholly accounted for by an elevation in renal cholesterogenesis from circulating mevalonate.

To clarify further the effects of thyroid hormone on circulating mevalonate metabolism in female animals, L-T_4 was administered at three different dose levels for either 3 or 8 d. Because cholesterol synthesis varied slightly in different groups of animals, and to facilitate comparison between several separate experiments, the results were standardized by dividing the mean values for a thyroid-treated group by the mean value obtained for the simultaneously studied control group. As illustrated by the results in Table II, 10 μg of L-T_4, whether given for 3 or 8 d, did not cause significant alterations of circulating mevalonate metabolism, although there was a tendency for an increase in renal cholesterol synthesis. Larger doses of L-T_4 induced a consistently altered pattern in circulating mevalonate metabolism, elevating renal and total body cholesterol synthesis and reducing shunt activity. These alterations in circulating mevalonate metabolism were maximal after the administration of 25 μg of L-T_4 for 3 d; increasing either the dosage or duration of thyroid hormone administration had no further effect.

The effect of L-T_4 administration on the time course of cholesterol synthesis from circulating mevalonate is shown in Table III. At all time points, female animals treated with 50 μG of L-T_4 for 3 d demonstrated an increase in the synthesis of cholesterol from circulating mevalonate.

Because earlier studies in this laboratory have demonstrated sex differences in the metabolism of circulating mevalonate, the effect of thyroid hormone administration was next investigated in male rats. After administration of L-T_4 (50 μg) for 3 d, the shunt pathway was reduced in the thyroid-treated animals (control 68±5.2 vs. thyroid 56±3.8 nmol of mevalonate oxidized

TABLE I

Effect of L-T_4 Administration on Mevalonate Metabolism in Female Rats In Vivo

	Body wt.	Liver wt.	Kidney wt.	$^{14}CO_2$	Cholesterol			
					Liver	Kidney	Carcass	Total body
	g				*nmol of mevalonate±SE converted in 6 h*			
Control (n = 4)	196±7	7.4±0.1	1.58±0.05	94±7	21.8±2.2	148±7	48±4	218±11
50 μg T_4 × 8 d (n = 3)	191±7	7.5±0.4	2.00±0.20	73±2	21.8±0.4	208±17	38±4	268±14
	NS	NS	$P < 0.10$	$P < 0.05$	NS	$P < 0.05$	NS	$P < 0.05$

Control females and females administered 50 μg of L-T_4 for 8 d were injected intravenously with 5 μCi, 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH; at 6 h the animals were killed and the organs and carcass weighed and then saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

TABLE II

Effect of L-T_4 Dose and Duration on Mevalonate Metabolism in Female Rats In Vivo

Dose and duration	$^{14}CO_2$	Cholesterol			
		Liver	Kidney	Carcass	Total body
10 μg × 3 d (n = 6)	0.85*	0.96*	1.07*	—	—
10 μg × 8 d (n = 6)	1.04*	0.97*	1.06*	—	—
25 μg × 3 d (n = 3)	0.78 ($P < 0.10$)	0.78 ($P < 0.10$)	1.43 ($P < 0.05$)	0.86*	1.22 ($P < 0.10$)
25 μg × 8 d (n = 5)	0.85*	0.99*	1.32 ($P < 0.01$)	0.90*	1.22 ($P < 0.05$)
50 μg × 3 d (n = 3)	0.79 ($P < 0.05$)	0.86*	1.39 ($P < 0.05$)	0.94*	1.19 ($P < 0.02$)
50 μg × 8 d (n = 3)	0.78 ($P < 0.05$)	1.00*	1.41 ($P < 0.05$)	0.79*	1.23 ($P < 0.05$)

Control females and females administered various doses of L-T_4 for either 3 or 8 d were injected intravenously with 5 μCi, 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH; at 6 h the animals were killed and the organs and carcass weighed and then saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography. Because of slight variations in cholesterol synthesis in different groups of animals, and to facilitate comparison between several separate experiments, the results are standardized by dividing the mean value for the thyroid-treated group by the mean value obtained for the simultaneously studied control groups. The statistics presented in parentheses represent the significance of the difference between the mean value for the thyroid-treated groups and the mean value for the simultaneously studied control groups.

* NS.

to CO_2 in 6 h), but this difference was not statistically significant ($P > 0.10$) (Table IV). The shunt pathway activity in the control males was similar to that previously observed and was substantially less than values seen in control females. As was observed in the female rats, renal cholesterol synthesis was significantly increased ($P < 0.05$) in thyroid-treated males. Likewise, some increase in total body cholesterol synthesis was noted ($P < 0.10$). Once again, little effect on liver or carcass cholesterol synthesis from circulating mevalonate was seen. These results demonstrated that circulating mevalonate metabolism in male rats was altered by thyroid hormone administration and that the changes observed were qualitatively similar in both sexes.

TABLE III

Renal Cholesterol Synthesis in Control and Thyroid-treated Female Rats

	Control	Thyroid	
	nmol of mevalonate±SE converted to cholesterol		
1 h (n = 3)	29±2	55±3	$P < 0.01$
3 h (n = 3)	79±10	133±12	$P < 0.05$
6 h (n = 5)	127±5	174±12	$P < 0.01$

Control females and females administered 50 μg of L-T_4 for 3 d were injected intravenously with 5 μCi, 1.05 nmol of [5-^{14}C]-mevalonate. At the times indicated, the animals were killed and the kidneys saponified in a KOH-ethanol solution. [^{14}C]-Cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

Effects of L-T_4 on mevalonate metabolism in vitro. After administration of 50 μg of L-T_4 to female rats for 3 d, mevalonate metabolism was studied in liver and kidney slices. The shunt pathway of mevalonate metabolism in both tissues and cholesterol synthesis from mevalonate in the liver were unaffected by prior thyroid hormone treatment (Table V). However, cholesterol synthesis from mevalonate in the kidney of the thyroid-treated animals was increased by 79% (thyroid 7.01±0.6 vs. control 3.9±0.4 nmol of mevalonate converted to cholesterol by 200 mg of tissue in 1 h; $P < 0.001$). This result confirmed the in vivo observation that the major effect of thyroid hormone on mevalonate metabolism is to increase cholesterol synthesis from mevalonate, an action localized to the kidney.

In females the incorporation of [1-^{14}C]acetate into cholesterol was also accelerated in the kidneys of the thyroid-treated animals (control 0.35±0.03 vs. thyroid 0.84±0.18 nmol of acetate converted to cholesterol by 200 mg of tissue in 1 h; $P < 0.05$). This demonstrated that both *de novo* synthesis and the postmevalonate steps of renal cholesterogenesis were enhanced by thyroid hormone.

L-T_4 (50 μg) was administered to male rats for 3 d,

TABLE IV

Effect of L-T_4 Administration on Mevalonate Metabolism in Male Rats In Vivo

	Body wt.	Liver wt.	Kidney wt.	$^{14}CO_2$	Cholesterol			
					Liver	Kidney	Carcass	Total body
		g			*nmol of mevalonate ± SE converted in 6 h*			
Control (n = 4)	258±10	11.2±0.8	2.22±0.11	68±5.2	21.8±1.4	171±9	42±2	234±12
50 μg T_4 × 3 d (n = 4)	246±5 NS	9.9±0.4 NS	2.48±0.10 NS	56±3.8 NS	19.1±0.4 NS	208±9 $P < 0.05$	48±4 NS	275±12 $P < 0.10$

Control males and males administered 50 μg of L-T_4 i.p. for 8 d were injected intravenously with 5 μCi, 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH; at 6 h the animals were killed and the organs and carcasses weighed and then saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

after which mevalonate metabolism was studied in liver and kidney slices. As was observed in the female, prior administration of L-T_4 did not alter the shunt pathway in either tissue or cholesterol synthesis from mevalonate in the liver (Table V). Cholesterol synthesis from mevalonate in the kidney was also increased in the males receiving thyroid hormone (7.9±0.7 vs. 5.4±0.4 nmol of mevalonate converted to cholesterol by 200 mg of tissue in 1 h; $P < 0.01$).

The effect of hypothyroidism on mevalonate metabolism in vivo. To study the effects of hypothyroidism, PTU was fed to female and male rats for 1 mo. Animals on the PTU diet did not grow as rapidly as the control animals, and at death the thyroid glands were markedly hypertrophied. The shunt pathway in the PTU-treated animals, especially in the females, was reduced in comparison with controls (control 111±3 vs. PTU 87±4 nmol of mevalonate oxidized to CO_2 in 6 h; $P < 0.02$) (Table VI). Surprisingly, L-T_4 (10 μg for 8 d, a dose that would be expected to render the animals euthyroid and not affect shunt activity) lowered the shunt pathway activity further (control 111±3 vs. PTU

TABLE V

Effect of T_4 Administration on Mevalonate Metabolism in Male and Female Rats In Vitro

	Liver		Kidney	
	CO_2	Cholesterol	CO_2	Cholesterol
	nmol of mevalonate ± SE converted to CO_2 or cholesterol/200 mg tissue/1 h			
Male				
Control (n = 8)	0.75±0.08	16.2±1.5	6.3±0.4	5.4±0.4
T_4 (n = 8)	0.67±0.07	15.9±1.4	6.0±0.5	7.9±0.7
	NS	NS	NS	$P < 0.05$
Female				
Control (n = 7)	0.70±0.08	15.6±0.9	5.6±0.5	3.9±0.4
T_4 (n = 7)	0.66±0.06	17.6±1.2	5.7±0.4	7.0±0.6
	NS	NS	NS	$P < 0.001$

200 μg of liver and kidney slices from control animals and animals administered 50 μg of L-T_4 i.p. for 3 d were placed in center-well flasks containing 2 ml of Krebs-Ringer phosphate buffer and 1 μCi, 262 nmol of [5-^{14}C]mevalonate. The flasks were gassed with 95% O_2/5% CO_2 and then incubated at 37°C in a Dubnoff shaker for 1 h. At the end of the incubation, 0.9 ml of 1 NaOH was injected into the center well and 1 ml of 1 N H_2SO_4 was injected into the outer well. The flasks were reincubated for 15 min and then the center well was assayed for $^{14}CO_2$ and the slices were saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

TABLE VI

Effect of Hypothyroidism on Mevalonate Metabolism in Female Rats In Vivo

	Body wt.	Liver wt.	Kidney wt.	$^{14}CO_2$	Cholesterol		
					Liver	Kidney	Carcass
		g			*nmol of mevalonate ± SE converted in 6 h*		
Control (n = 4)	204±5	6.4±0.3	1.58±0.12	111±3	15.8±1.5	129±14	56±13
PTU (n = 5)	182±1*	6.1±0.2	1.24±0.02‡	87±4§	20.2±2.4	71±3*	63±3
PTU + T_4 (10 μg × 8 d) (n = 4)	182±4§	6.2±0.3	1.48±0.03‖	68±2*¶	16.8±1.6	201±4*	52±6

Control, PTU(0.2%)-fed, and PTU(0.2%)-fed animals administered 10 μg of L-T_4 i.p. for 8 d were injected intravenously with 5 μCi, 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH; at 6 h the animals were killed and the organs and carcasses weighed and then saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

* $P < 0.01$ difference between control.

‡ $P < 0.05$ difference between control.

§ $P < 0.02$ difference between control.

‖ $P < 0.001$ difference between PTU.

¶ $P < 0.01$ difference between PTU.

plus L-T_4 68±2 nmol of mevalonate oxidized to CO_2 in 6 h; $P < 0.01$). This unexpected further reduction in shunt pathway activity suggests that hypothyroidism per se does not explain the decrease in shunt activity seen in PTU-fed animals, but rather that PTU directly reduces the oxidation of mevalonate to CO_2. Cholesterol synthesis from circulating mevalonate was also reduced in the kidneys of the female PTU-fed animals (control 129±14 vs. PTU 71±3 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.01$). Moreover, L-T_4 treatment (10 μg for 8 d) not only returned cholesterol synthesis to normal but increased cholesterol synthesis to supranormal values (control 129±14 vs. PTU plus L-T_4 201±4 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.01$). These results again demonstrated the marked influence of thyroid hormone status on renal cholesterol synthesis from mevalonate. A decrease in thyroid hormone markedly reduces cholesterol synthesis, and in the hypothyroid state small doses of thyroid hormone (which in a normal rat do not significantly affect mevalonate metabolism) caused almost a threefold increase in renal cholesterogenesis. Mevalonate metabolism in the livers and carcasses of the three groups of animals was unaffected by thyroid hormone status.

Male animals fed a PTU diet also demonstrated a decrease in cholesterol synthesis from mevalonate in the kidneys (control 116±7.5 vs. PTU 85±4.7 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.02$) (Table VII). Administering 10 μg of L-T_4 for 8 d again restored cholesterol synthesis to supranormal levels (control 116±7.5 vs. PTU plus L-T_4 132±3.4 nmol of mevalonate converted to cholesterol in 6 h; $P < 0.10$). Shunt activity in both PTU-fed and PTU plus L-T_4 males was reduced but the decline was not statistically significant. Liver and carcass cholesterol synthesis from mevalonate was similar in the three groups of male animals.

Effects of PTU diet on mevalonate metabolism in vitro. Mevalonate metabolism was studied in liver and kidney slices from female animals after their ingesting the PTU diet for 1 mo. The shunt pathway in both the liver and kidney was significantly decreased in the PTU-fed animals, confirming in vivo results (Table VIII). Once again, cholesterol synthesis from mevalonate in the liver slices was unaffected by thyroid hormone status; however, cholesterol synthesis from mevalonate in the kidney slices was somewhat reduced (control 2.70±0.39 vs. PTU 2.03±0.15 nmol of mevalonate converted to cholesterol in 6 h; $P > 0.10$). This in vitro experiment supported the in vivo studies demonstrating that hypothyroidism reduced the renal synthesis of cholesterol from mevalonate.

DISCUSSION

Mevalonic acid is an essential intermediate in cholesterol synthesis (19, 20). In 1974, Edmond and Popjak (6) first presented evidence that mevalonic acid is metabolized in the intact rat by a nonsterol, or shunt, pathway that ultimately results in the oxidation of mevalonate to CO_2. The following year, Fogelman et al. (7) further demonstrated that this pathway is present in humans. Surprisingly, both in vivo and in vitro investigations have demonstrated that the kidneys, rather than the liver, are the most active site of mevalonate metabolism by both the sterol and shunt pathways (8–14). Subsequent studies from our laboratory have shown

TABLE VII

Effect of Hypothyroidism on Mevalonate Metabolism in Male Rats In Vivo

	Body wt.	Liver wt.	Kidney wt.	$^{14}CO_2$	Cholesterol		
					Liver	Kidney	Carcass
	g			nmol of mevalonate±SE converted in 6 h			
Control (n = 3)	300±2	11.4±0.6	2.36±0.13	62±9	14.1±2.1	116±7.5	34±4.3
PTU (n = 5)	157±2*	7.1±0.27*	1.26±0.02*	46±4.5	16.54±0.4	85±4.7‡	37±2.3
PTU + T_4 (10 μg × 8 d) (n = 7)	178±3*,§	7.5±0.14*	1.67±0.05§,‖	45±3.7	17.71±1.6	132±3.4§,¶	37±4.1

Control, PTU(0.2%)-fed, and PTU(0.2%)-fed animals administered 10 μg of L-T_4 i.p. for 8 d were injected intravenously with 5 μCi, 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH; at 6 h the animals were killed and the organs and carcasses weighed and then saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

* $P < 0.001$ difference between control.

‡ $P < 0.02$ difference between control.

§ $P < 0.001$ difference between PTU.

‖ $P < 0.01$ difference between control.

¶ $P < 0.10$ difference between control.

that, in rats and man, there is a marked sex difference in circulating mevalonate metabolism (15, 16). First, the female rat metabolizes circulating mevalonate by the shunt pathway at twice the rate of the male, a difference accounted for almost entirely by the greater ability of the female kidney to oxidize mevalonate to CO_2. Second, the male rat converts significantly more circulating mevalonate to cholesterol than the female. Observations of circulating mevalonate metabolism in castrated male rats and immature female rats have implied that these sex differences are secondary to the effects of sex hormones (15). In humans, premenopausal females oxidize 68% more circulating mevalonate to CO_2 than age-matched males (16). Insulin-deficient, streptozotocin-treated rats also demonstrate a marked reduction in shunt activity and an increase in sterologenesis (17). These findings indicate that hormonal alterations greatly affect the metabolism of circulating mevalonate.

Thyroid hormone state significantly affects cholesterol synthesis. In vivo studies using deuterium-labeled water, tritium-labeled water, or [^{14}C]acetate have demonstrated that cholesterol synthesis is increased in the hyperthyroid and decreased in the hypo-

TABLE VIII

Effect of Hypothyroidism on Mevalonate Metabolism in Female Rats In Vitro

	Liver		Kidney	
	CO_2	Cholesterol	CO_2	Cholesterol
	nmol of mevalonate±SE converted to CO_2 or cholesterol/200 mg tissue/1 h			
Control (n = 4)	1.05±0.09	14.42±1.19	6.91±0.58	2.70±0.39
Hypothyroid (n = 4 for liver; n = 5 for kidney)	0.71±0.04 $P < 0.02$	14.26±0.80 NS	4.93±0.42 $P < 0.05$	2.03±0.15 NS

200 μg of liver and kidney slices from control animals and animals fed PTU (0.2%) for 1 mo were placed in center-well flasks containing 2 ml of Krebs-Ringer phosphate buffer and 1 μCi, 262 nmol of [5-^{14}C]mevalonate. The flasks were gassed with 95% O_2/5% CO_2 and then incubated at 37°C in a Dubnoff shaker for 1 h. At the end of the incubation, 0.9 ml of 1 N NaOH was injected into the center well and 1 ml of 1 N H_2SO_4 into the outer well. The flasks were reincubated for 15 min and then the center well was assayed for $^{14}CO_2$ and the slices were saponified in a KOH-ethanol solution. [^{14}C]cholesterol was assayed after extraction with petroleum ether and thin layer chromatography.

thyroid animal (21–24). In vitro investigation by Fletcher and Myant (2), using rat liver slices, has shown that thyroid hormone increases the conversion of acetate to cholesterol, thereby confirming the in vivo studies, but that the conversion of mevalonate to cholesterol is unaffected. Additional studies by Ness et al. have shown that liver HMG CoA reductase, the enzyme that converts HMG CoA to mevalonate, is stimulated by thyroid hormone (3). These investigations suggest that thyroid hormone increases cholesterol synthesis by increasing the conversion of acetate to mevalonate, but does not alter the postmevalonate pathway of cholesterogenesis (20). The present study, however, demonstrates that thyroid hormone status also affects the in vivo and in vitro metabolism of mevalonate by both the sterol and shunt pathways. Because plasma levels of circulating mevalonate are low, i.e., in the range of 0.2 nmol/ml (8, 18), alterations in circulating mevalonate metabolism are unlikely to have a significant effect on sterol balance; therefore the observed thyroid hormone-induced increase in *de novo* cholesterogenesis is probably not primarily the consequence of changes in circulating mevalonate metabolism.

Thyroid hormone status was found to induce minor changes in the shunt pathway of circulating mevalonate metabolism. In both males and females, the shunt activity was reduced in PTU-induced hypothyroid animals; however, L-T_4 administration to the PTU-fed animals, while leading to supranormal elevations in the sterol pathway, did not increase the activity of the shunt pathway. In fact, in the PTU-fed female rats, T_4 administration further reduced the shunt pathway. We interpret these data as indicating that the observed depression in shunt activity is a direct toxic effect of PTU. The further decrease in shunt pathway activity in the females was perhaps secondary to thyroid hormone-induced hyperphagia, leading to an increase in PTU ingestion and toxicity. Whether hypothyroidism per se alters the metabolism of circulating mevalonate by the shunt pathway is therefore unanswered. Thyroid hormone administration resulted in a minor reduction in shunt pathway activity (12 nmol in males and 21 nmol in females). Quantitatively, this reduction in the shunt pathway was considerably less than the increase observed in the sterol pathway under similar circumstances. The effect of thyroid hormone on the shunt pathway appears, therefore, to be of secondary importance as compared with alterations induced in the sterol pathway.

The present study demonstrated that the major effect of thyroid hormone on circulating mevalonate metabolism is to alter significantly the conversion of mevalonate to cholesterol and, furthermore, that this effect occurs solely in the kidneys, the organ previously identified as the chief site of circulating mevalonate metabolism (8–14). Interestingly, the alterations of circulating mevalonate metabolism induced by sex steroid hormones have also been shown to occur primarily in the kidneys (15). In female rats administered L-T_4 (50 μg/8 d), total body cholesterol synthesis from circulating mevalonate increased 23% (50 nmol) and renal cholesterol synthesis from circulating mevalonate increased 41% (60 nmol). The entire elevation in total body cholesterol synthesis could, therefore, be accounted for by the increase in renal cholesterogenesis. Several different doses and durations of L-T_4 administration were studied in female animals, and a maximal effect on renal conversion of mevalonate to cholesterol was induced by 25 μg of L-T_4 administered intraperitoneally for 3 d. Further increases in either dose or duration of thyroid hormone administration did not significantly alter the response. In male rats administered L-T_4, similar increases in the conversion of mevalonate to cholesterol were also observed. These in vivo results were confirmed by the in vitro studies that demonstrated in kidney slices a 79% increase in mevalonate conversion to cholesterol in females and a 46% increase in males. Moreover, in addition to the above data demonstrating a thyroid-induced stimulation of cholesterol synthesis from mevalonate, studies in hypothyroid animals conversely revealed a decrease in cholesterol synthesis from mevalonate. In PTU-induced hypothyroidism, renal cholesterol synthesis from mevalonate decreased 27% in males and 22% in females. Thyroid hormone administration in a dose and duration that, in normal animals, does not alter the sterol pathway of circulating mevalonate metabolism led, in PTU-induced hypothyroid animals, to supranormal increases in the shunt pathway. These results clearly indicate that thyroid status has a marked effect on renal cholesterol synthesis from mevalonate and that the hypothyroid state sensitizes the kidney to the influence of thyroxine.

By contrast with these major and consistent effects of thyroid hormone on cholesterol synthesis in the kidney, neither thyroid hormone administration nor PTU-induced hypothyroidism significantly affected the conversion of mevalonate to cholesterol in liver or in any other tissue studied. These results indicated that the thyroid-induced changes in the postmevalonate synthesis of cholesterol were localized solely to the kidney, the organ chiefly responsible for metabolizing circulating mevalonate. These findings extend the conclusion that sterol metabolism in the kidney is under major endocrine controls that now must include thyroid as well as polypeptide and steroid hormones.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants CA-15979 and HL-06285, the Kroc Foundation, and the Veterans Administration. Dr. Feingold is the recipient of a National Institutes of Health Special Emphasis Research Career Award.

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