Carnitine Metabolism in Thyroid Hormone Treated Rats and Mice

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Summary This study was undertaken to investigate the changes in carnitine metabolism in rats and mice injected with T₄ for 3 days and 10 days, respectively, and in rats fed a T₃ and T₄-supplemented diet for 6 weeks. Thyroid hormone administration brought about a significant increase in urinary excretion of total carnitine. In T₃+T₄-treated rats urinary esterified-carnitine to free-carnitine ratio increased significantly in the later phase of administration. Carnitine pool size in the body was significantly decreased in both T₄-injected mice and T₃+T₄-fed rats. In the latter animals, this decrease was due to the reduced carnitine contents in organs other than the liver, especially in skeletal muscle. The amount of carnitine synthesized by control and T₃+T₄-treated rats was calculated from the data on carnitine intake, urinary carnitine excretion and carnitine pool size in the body over the 6-week period. Values obtained were 66.2 ± 3.2 (mean ± SEM) μmol/rat and 28.5 ± 4.9 μmol/rat, respectively, and the difference was significant (p < 0.05). These results indicate that carnitine synthesis is depressed by thyroid hormone, however, some possibilities that thyroid hormone may increase carnitine synthesis were also discussed.

Key Words thyroid hormone, carnitine metabolism, free- and esterified-carnitine, urinary carnitine, carnitine body pool size, carnitine synthesis, rat, mouse

L-Carnitine exists in free and esterified (acetyl and acyl) forms in organisms and plays an essential role in the transport of activated long-chain fatty-acyl groups into the site of β-oxidation in the mitochondria (1). Carnitine has been established to be synthesized from lysine and methionine (2–7) through the peripheral tissues—liver relay system in vertebrates (8–14). Carnitine pool size in the body depends on

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not only carnitine synthesis but also carnitine intake and urinary excretion. Maebashi et al. have reported the increased urinary excretion of total carnitine after thyroid hormone administration to normal persons (15) and to patients with hypothyroidism (16) and also the decreased total carnitine excretion after the treatment of patients with hyperthyroidism (15). Cederblad and Engström (17) have observed decreased concentration of free- and acetyl-carnitine in the skeletal muscle of thyroxine treated rats. Contrarily, Bressler and Wittels (18) have reported the increased heart carnitine levels in T₄-treated guinea pigs. It has been also reported that there are changes in carnitine metabolism in animal and human tissues under conditions of increased fatty acid oxidation and elevated plasma thyroid hormone, such as high fat feeding (19–24), chronic exercise training (25, 26), starvation (19, 20, 27–33) and cold exposure (34–36). These findings suggest that thyroidal function is intensely related to carnitine metabolism.

The present study was undertaken to investigate the effects of thyroid hormone administration on carnitine metabolism in rats and mice. Previous studies on carnitine synthesis and its turnover rate have been conducted using ¹⁴C-labeled carnitine in its precursors and provided variable results (22, 36–38). Therefore, in our study, carnitine intake and urinary carnitine excretion were measured besides changes in carnitine pool size in the body during the thyroid hormone administration. In addition, turnover time of carnitine in rats has been estimated to be 5 to 50 days (36), suggesting that a long-term administration of thyroid hormone is required to see its effect on the pool size of carnitine in the body. Thus, in this study, chronic effects as well as acute effects of thyroid hormone administration were also examined.

**EXPERIMENTAL**

**Animals.** Male JCL-Sprague Dawley rats (experiments 1 and 3) and male ICR mice (experiment 2) (CLEA Japan Inc., Tokyo) were used. One rat or 5 mice were housed in usual cage during preliminary feeding periods, and then animals were transferred to metabolism cages for the collection of urine samples during the

| Table 1. Composition of a low carnitine diet (experiments 1, 2, and 3). |
|-----------------|-----------------|-----------------|
|                | (g/100 g diet)  | (I.U./100 g diet) |
| Wheat flour (Hard) | 85.0            |                  |
| Soybean oil      | 10.0            |                  |
| l-Lysine-HCl     | 0.4             |                  |
| Salt mixture⁴    | 4.0             |                  |
| Vitamin mixture⁴ | 0.85            |                  |
| Choline-Cl       | 0.15            |                  |

Total l-carnitine content of this diet is 2 nmol/g diet. ⁴Purchased from Tanabe Amino Acid Foundation, Tokyo.
period of hormonal treatment. Animal room was lit between 08.00–20.00 h (experiment 1) or 07.00–19.00 h (experiments 2 and 3) and maintained at 23°C. A low carnitine diet (Table 1) for all experiments was meal-fed (experiments 1 and 2) or fed ad libitum (experiment 3), and water was provided freely in all experiments. Body weight and food consumption were recorded every day (experiments 1 and 2) and periodically (experiment 3) during the hormonal treatment.

Experiment 1. Eighteen rats weighing 100–110 g were used to determine the acute effects of L-thyroxine-Na ($T_4$, Wako Pure Chemicals Ind. Co., Tokyo) administration on body pool size of carnitine and its urinary excretion. The low carnitine diet was meal-fed to rats 2-h each day (20.00–22.00 h) for 4 days, then the animals were divided at random into 3 groups: a) control group, b) saline-group and c) $T_4$-group. Rats of control group were killed by decapitation after 5-day meal feeding, and liver and the remaining carcass excluding digestive tract were obtained. Rats of saline- and $T_4$-groups were fed for 3 days, while they were intraperitoneally injected at 13.00 h every day with 0.2 ml of saline (pH 10.7) and 20 µg of $T_4$ dissolved in 0.2 ml of saline (pH 10.7) per 100 g body weight, respectively. The 24-h urine samples were collected into bottles containing 0.5 ml of 6 N HCl solution on the days before and during the thyroxine treatment. Rats were killed 24 h after the final injection, and liver and carcass samples were obtained as described above.

Experiment 2. To further elucidate the acute effects of the administration of thyroxine on body pool size of carnitine and its urinary excretion, seventy-five mice weighing 28–34 g were divided into 5 equal groups and meal-fed the low carnitine diet during the dark period (19.00–07.00 h) for 10 days. On these days each group was intraperitoneally injected daily at 10.00–11.00 h with 0, 5, 10, 20 or 50 µg of $T_4$ in 0.2 ml of saline per 100 g of body weight as described in experiment 1. Five mice of each group were housed together in a metabolism cage, and 24-h urine was collected per group for the 10-day hormonal treatment period.

Experiment 3. To see the chronic effects of thyroid hormone, three weeks old rats, weighing 45–55 g, were divided into 5 rats of control group and 6 rats of hormone treated group, and they were housed individually in metabolism cages. Control rats were fed a low carnitine diet ad libitum, and rats of thyroid hormone treated group were fed a low carnitine diet added with 3 mg of L-thyroxine-Na and 1 mg of L-triiodothyronine ($T_3$, Wako Pure Chemicals Ind. Co., Tokyo) per kg diet according to Winder et al. (39). Twenty-four h urine was collected as described in experiment 1 for 3 consecutive days in the middle of every week, and food intake was also measured on these days. Body weight was recorded every week at the start and the end of the urine collection. On the final day of the 6-week feeding, rats were killed by decapitation, and blood, heart, liver, testes, kidneys, epididymal adipose tissues, gastrocnemius muscles and superficial-white and deep-red portions of the quadriceps muscle were taken. The remaining tissue excluding digestive tract was also obtained as the carcass. To determine the basal body pool size of carnitine, 5 untreated rats of 3 weeks old were killed.

Samples of all experiments were stored at $-80^\circ$C until analyses.
Carnitine extraction from tissues, serum and urine. Tissue samples were hydrolyzed at 50°C in 5–10 vol of 0.1 N KOH solution according to the method by Tanphaichitr et al. (4). Carcass was homogenized in 200–300 ml of chloroform-methanol (C–M; 3:2, v/v) and then extracted with Soxhlet apparatus at 65°C for 10 h. The residue on the filter was homogenized and extracted for an additional 8 h as described above. The extracts were served for total carnitine assay.

Urine was neutralized with NaOH, concentrated to about 2 ml with rotary evaporator, mixed well in about 15 vol of C–M (3:2) solvent, and then centrifuged at 840 × g for 10 min. The upper layer was obtained, and the residue was extracted and centrifuged again. The solvent of combined upper layer was evaporated to dryness and its residue was dissolved in water. A portion of the solution was used for free-carnitine determination. Potassium hydroxide was added to the other portion to a concentration of 0.1 N KOH and then hydrolyzed at 50°C for 30 min. After cooling to room temperature and neutralization, the samples were analyzed for total carnitine.

Carnitine was extracted from serum samples which were pooled per group (experiment 3) and hydrolyzed according to the method of Mikhail and Mansour (40). Analysis of free- and total carnitine was carried out before and after the hydrolysis.

L-Carnitine assay. L-Carnitine assays were conducted by a modification (33) of the method by Pearson et al. (41) using carnitine acetyltransferase (EC 2.3.1.7; Boehringer Mannheim Co., New York). Esterified-carnitine levels in serum and urine samples were obtained by subtracting the values of free-carnitine from those of total carnitine.

Free fatty acid and glycogen assay. In experiment 3, heart and serum free fatty acid (FFA) levels and fatty acid composition of total lipids in liver and epididymal adipose tissue were determined. A portion of the C–M extracts from heart for carnitine assay was used for FFA determination according to the method by Trout et al. (42). Serum FFA and liver glycogen were analyzed as described elsewhere (43). Total lipids were extracted from liver and epididymal adipose tissue with C–M (2:1), and their fatty acids were methylated and analyzed gas-chromatographically.

RESULTS

Experiment 1

The 3-day administration of T4 (20 μg/100 g body weight) had no effect on body weight and food consumption in rats but gave smaller liver sizes (Table 2). Body and liver carnitine pool size was not influenced by T4 administration. However, urinary excretion of free- and total carnitine increased progressively during the T4 treatment period, and the increase was statistically significant on days-2 and 3 (p < 0.05). The sum of carnitine per whole body after the T4 treatment with dietary and urinary carnitine per 3 days provides 68.92 μmol for saline-treated rats and 67.65 μmol for T4-treated rats. Thus, carnitine synthesis was not signifi-
Table 2. Effects of the 3-day administration of thyroid hormone on body pool size of L-carnitine and its urinary excretion in rats (experiment 1).

| Experimental groups | Before treatment (6) | Saline (5) | T₄-treated (6) |
|---------------------|---------------------|-----------|---------------|
| Body weight (g)     |                     |           |               |
| Initial             | 149 ± 3             | 150 ± 3   | 147 ± 2       |
| Final               | 157 ± 2             | 156 ± 2   |               |
| Food intake (g/3 days) | 37.3 ± 1.3     | 39.3 ± 1.4 |             |
| Liver weight (g)    | 7.70 ± 0.33         | 8.19 ± 0.15 | 7.14 ± 0.21*  |
| Total carnitine content (μmol) |       |           |               |
| Whole liver         | 2.46 ± 0.08         | 2.76 ± 0.12 | 2.67 ± 0.16   |
| g of liver          | 0.32 ± 0.02         | 0.33 ± 0.01 | 0.37 ± 0.02   |
| Whole rat           | 64.6 ± 2.4          | 67.6 ± 2.1 | 65.8 ± 1.0    |
| 100 g of rat        | 44.6 ± 1.2          | 44.5 ± 1.1 | 43.4 ± 0.5    |
| Urinary carnitine excretion (μmol) |       |           |               |
| Free-carnitine:     |                     |           |               |
| Day-0               | 0.14 ± 0.01         | 0.16 ± 0.05  |       |
| Day-1               | 0.15 ± 0.01         | 0.21 ± 0.03  |       |
| Day-2               | 0.14 ± 0.01         | 0.32 ± 0.04* |       |
| Day-3               | 0.16 ± 0.02         | 0.43 ± 0.12* |       |
| Total carnitine:     |                     |           |               |
| Day-0               | 0.43 ± 0.03         | 0.39 ± 0.03  |       |
| Day-1               | 0.41 ± 0.03         | 0.49 ± 0.03  |       |
| Day-2               | 0.37 ± 0.02         | 0.53 ± 0.06* |       |
| Day-3               | 0.36 ± 0.03         | 0.75 ± 0.11* |       |
| Day-1–Day-3         | 1.25 ± 0.11         | 1.77 ± 0.23* |       |

Number of rats is shown in parentheses. Values are means ± SEM. Day-0 means the day before the treatment. *Significantly different from saline group (p < 0.05).

Significantly affected by the 3-day administration of T₄ to rats.

**Experiment 2**

In general, administration of T₄ for 10 days resulted in decrease of liver weight in mice (Table 3). The changes were significant when T₄ was administered daily at the levels of 20 and 50 μg/100 g body weight. Cardiac hypertrophy was observed in mice administered with 50 μg of T₄ per 100 g of body weight. Carnitine pool size in the body was significantly decreased with the 10-day administration of T₄. On the other hand, total amounts of carnitine excreted into urine during the T₄ treatment was increased significantly.

**Experiment 3**

As shown in Fig. 1, the food consumption of T₃+T₄-treated rats was initially smaller but later it became significantly (p < 0.05) larger than that of control rats.

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Table 3. Effects of the 10-day administration of thyroid hormone on body pool size of L-carnitine and its urinary excretion in mice (experiment 2).

| µg of T₄ dosed/100 g body weight/day | 0  | 5  | 10 | 20 | 50 |
|------------------------------------|----|----|----|----|----|
| Body weight (g) | Initial | 31.8 ± 0.4 | 30.8 ± 0.6 | 30.3 ± 0.4 | 31.0 ± 0.3 | 32.0 ± 0.4 |
| Final | 27.9 ± 0.3 | 28.3 ± 0.6 | 26.9 ± 0.7 | 25.3 ± 0.2ab | 23.6 ± 0.4a |
| Food consumption (g/5 mice/10 days) | 131.0 | 156.5 | 160.0 | 167.0 | 249.5 |
| Heart weight (mg) | 135 ± 2 | 137 ± 4 | 139 ± 3 | 138 ± 4 | 147 ± 3b |
| Liver weight (mg) | 1,327 ± 27 | 1,223 ± 42 | 1,216 ± 42 | 1,107 ± 36a | 1,147 ± 50b |
| Total carnitine content (µmol) | 9.40 ± 0.32 | 8.43 ± 0.25a | 7.81 ± 0.30a | 7.78 ± 0.20a | 7.72 ± 0.20c |
| Whole mouse 100 g of mouse Urine/5 mice per 10 days | 33.68 ± 0.98 | 29.96 ± 1.12a | 30.00 ± 1.44 | 30.73 ± 0.84a | 32.86 ± 0.81 |
| 13.6 | 17.8 | 20.1 | 20.7 | 20.1 |
| (12.2) | (12.3) | (13.1) | (13.0) | (13.3) |

Values are means or means ± SEM for 5 mice of each group. Free-carnitine, µmol/5 mice/10 days. Significantly different from control (0 µg of T₄) (a, p < 0.05; b, p < 0.01; c, p < 0.001).

However, body weight of T₃+T₄-treated rats was lower than that of controls throughout the 6-week feeding period. Thyroid hormone treatment caused hypertrophy of liver, heart and kidney and weight reduction of gastrocnemius muscle, testis and epididymal adipose tissue (Table 4).

Cardiac FFA concentration was not influenced by thyroid hormone treatment, but serum FFA concentration was increased significantly (p < 0.05) (Table 4). Liver glycogen store of rats treated with T₃ and T₄ decreased to one-twentieth of the level of control rats (p < 0.05).

Thyroid hormone supplementation caused similarly significant (p < 0.05) changes in fatty acid composition of total lipids in both liver and epididymal adipose tissue (Table 5) such as increased proportions of palmitic and oleic acids and decreased proportions of linoleic and linolenic acids. There were increases in myristic, palmitoleic and stearic acids in both tissues, but the increase was significant (p < 0.05) only in the latter.

As a consequence of thyroid hormone administration, carnitine contents were significantly (p < 0.05) decreased in heart, kidney, testis and two skeletal muscles (Table 6). In contrast, liver carnitine showed an insignificant but slight increase with thyroid hormone treatment. The red portion of the quadriceps muscle had a significantly (p < 0.05) higher carnitine content than the white portion.

With thyroid hormone administration, urinary free-carnitine excretion was
Increased in week-1 of experiment but maintained a lower level than that of controls thereafter (Fig. 2). Urinary esterified-carnitine, being about 60% of total urinary carnitine in both groups of rats, was always higher in T3+T4-treated rats than in control rats and the difference was significant (p<0.05) at week-6. In addition, thyroid hormone treated rats showed higher urinary total carnitine levels than controls, but the difference was significant (p<0.05) only at week-1. Esterified-carnitine to free-carnitine ratio increased with thyroid hormone treatment, and the increase was significant at weeks-5 and 6 when food consumption became considerably large in T3+T4-treated rats (Fig. 1).

As compared with control rats, free- and total carnitine concentrations in pooled serum samples were lower in T3+T4-treated rats, and those of esterified-carnitine were slightly higher (Table 7). Esterified-carnitine to free-carnitine ratio in serum was considerably higher than in urine in both groups of rats, and the ratio in both were increased when treated with thyroid hormone.

Renal carnitine clearance was calculated from the data of carnitine levels in urine and serum at week-6 of the experiment 3. The clearance of esterified-carnitine was extremely larger than that of free-carnitine and increased with thyroid hormone.
Table 4. Effects of the 6-week administration of thyroid hormone on tissue weight, liver glycogen content and serum and heart FFA level in rats (experiment 3).

| Experimental groups          | Control             | T3 + T4-treated          |
|-----------------------------|---------------------|--------------------------|
|                            | (g)                 | (g/100 g b.w.)           | (g)                     | (g/100 g b.w.) |
| Liver                       | 7.81 ± 0.02         | 4.48 ± 0.17              | 8.14 ± 0.59             | 5.40 ± 0.14a  |
| Heart                       | 0.66 ± 0.02         | 0.42 ± 0.04              | 1.23 ± 0.14a            | 0.78 ± 0.14a  |
| Kidneys                     | 1.41 ± 0.04         | 0.81 ± 0.01              | 2.10 ± 0.11a            | 1.37 ± 0.04a  |
| Testes                      | 2.63 ± 0.09         | 1.51 ± 0.05              | 1.92 ± 0.09a            | 1.28 ± 0.11   |
| Gastrocnemius               | 2.07 ± 0.06         | 1.17 ± 0.03              | 1.44 ± 0.13a            | 0.92 ± 0.04a  |
| Epididymal fat              | 1.68 ± 0.14         | 0.97 ± 0.07              | 0.67 ± 0.13a            | 0.42 ± 0.07a  |
| Liver glycogen              | (mg/g)              | (mg/liver)               | (mg/g)                  | (mg/liver)    |
|                            | 43.7 ± 3.9          | 340 ± 30                 | 2.1 ± 1.1a              | 15 ± 7a       |
| Heart FFA                   | (μmol/g)            | (μmol/heart)             | (μmol/g)                | (μmol/heart)  |
|                            | 10.7 ± 0.2          | 7.0 ± 0.3                | 11.2 ± 1.7              | 13.8 ± 1.6a   |
| Serum FFA                   | (μmol/liter)        | (μmol/liter)             | 430 ± 38                | 452 ± 35a     |

Values are means ± SEM for 5 control and 6 rats treated with T3 and T4. *Significantly different from control (p < 0.05).

Table 5. Effects of the 6-week administration of thyroid hormone on fatty acid composition of total lipid in liver and epididymal adipose tissue in rats (experiment 3).

| Fatty acids     | Liver                  | Epididymal adipose tissue |
|-----------------|------------------------|---------------------------|
|                 | Control                | T3 + T4-treated          | Control                | T3 + T4-treated |
| Myristic        | 0.3 ± 0.0              | 0.4 ± 0.0                 | 0.9 ± 0.0              | 2.1 ± 0.1a     |
| Palmitic        | 24.3 ± 0.9             | 28.3 ± 0.9a               | 18.9 ± 0.4             | 30.9 ± 0.7a    |
| Palmitoleic     | 1.8 ± 0.2              | 2.0 ± 0.1                 | 3.0 ± 0.2              | 7.5 ± 0.3a     |
| Stearic         | 15.8 ± 1.3             | 17.6 ± 1.2                | 2.5 ± 0.1              | 4.1 ± 0.3a     |
| Oleic           | 12.1 ± 0.5             | 15.5 ± 0.9a               | 23.5 ± 0.1             | 28.5 ± 0.5a    |
| Linoleic        | 32.2 ± 1.5             | 24.5 ± 0.8a               | 46.8 ± 0.5             | 25.0 ± 0.4a    |
| Linolenic       | 1.8 ± 0.1              | 1.1 ± 0.1a                | 4.5 ± 0.2              | 2.0 ± 0.2a     |
| Arachidonic     | 11.8 ± 0.9             | 10.4 ± 1.1                |                          |                |

Values are percentages as means ± SEM for 5 controls and 6 rats treated with T3 and T4. *Significantly different from control (p < 0.05).

treatment (Table 7). Assuming the glomerular filtration rate as 2,550 ml/150 g rat/day (44), 97.5%–99.9% of carnitine were reabsorbed, regardless of the carnitine form.

Table 8 describes the changes in pool size of carnitine in the body during the 6-
Table 6. Effects of the 6-week administration of thyroid hormone on total L-carnitine levels in several organs and muscles in rats (experiment 3).

| Tissues          | Control          | T3 + T4-treated |
|------------------|------------------|-----------------|
|                  | (μmol carnitine per) |                |
|                  | (g tissue)       | (whole tissue)  |
| Liver            | 254 ± 22         | 2,021 ± 210     |
|                  | 286 ± 18         | 2,410 ± 220     |
| Heart            | 734 ± 29         | 481 ± 25        |
|                  | 337 ± 30         | 426 ± 76        |
| Kidneys          | 471 ± 15         | 674 ± 35        |
|                  | 236 ± 16         | 497 ± 52        |
| Testes           | 133 ± 5          | 355 ± 12        |
|                  | 107 ± 13         | 204 ± 18        |
| Gastrocnemius    | 770 ± 40         | 1,613 ± 96      |
|                  | 363 ± 40         | 539 ± 101       |
| Quadriceps       |                  |                 |
| Superficial      | 668 ± 10         | 347 ± 39 \textsuperscript{a} |
|                  | 866 ± 22 \textsuperscript{b} | 403 ± 40       |
| Deep             |                  |                 |

Values are means ± SEM for 5 controls and 6 rats treated with T\textsubscript{3} and T\textsubscript{4}. Values in parentheses are % of body pool size. \textsuperscript{a} Significantly different from control (p < 0.05). \textsuperscript{b} Significantly different from superficial portion (p < 0.05).

Fig. 2. Effects of the 6-week administration of thyroid hormone on urinary profile of L-carnitine and its derivatives in rats (experiment 3). Each point and vertical bar represent mean and SEM, respectively, for 5 controls and 6 rats treated with T\textsubscript{3} and T\textsubscript{4}. \textsuperscript{a} Significantly different from control (p < 0.05).
Table 7. Effects of the 6-week administration of thyroid hormone on serum and urinary concentrations of free-, esterified- and total L-carnitine and their renal handling in rats (experiment 3).

| L-Carnitine | Experimental groups |  |  |
|-------------|---------------------|-----|-----|
|             | Control             | T3 + T4-treated |
|             | Serum (nmol/ml)     | Serum (nmol/ml) | Urine (nmol/day) | Urine (nmol/day) |
| Free        | 41.1                | 20.1 | 66 ± 19 |
| Esterified  | 4.4                 | 5.6  | 353 ± 37* |
| Total       | 45.5                | 25.7 | 419 ± 53 |
| Esterified/Free ratio | 0.11 | 0.28 | 7.70 ± 1.77* |

Renal clearance (ml/day) |  |  |
| Free | 3.1 | 3.2 |
| Esterified | 53.2 | 63.0 |
| Total | 8.0 | 16.3 |

Urine samples collected at the week-6 of experiment were used. Values are means or means ± SEM for 5 controls and 6 rats treated with T3 and T4. *Significantly different from control (p<0.05).

Table 8. Effects of the 6-week administration of thyroid hormone on body pool size and biosynthesis of L-carnitine in rats (experiment 3).

| Experimental groups | Control | T3 + T4-treated |
|---------------------|---------|-----------------|
|                     | (μmol carnitine) | (μmol carnitine) |
| Initial body pool size (per 100 g b.w.) (per rat) | 29.4 ± 1.3 | 15.3 ± 0.6 |
| Final body pool size (per 100 g b.w.) (per rat) | 39.6 ± 0.9* | 19.3 ± 1.4*a,b |
| Δ Body pool size (per rat) | 70.1 ± 2.9a | 30.9 ± 4.4a,b |
| Dietary intake (per rat per 6 weeks) | 54.8 ± 2.9 | 15.6 ± 4.4b |
| Urinary excretion (per rat per 6 weeks) | 1.1 ± 0.0 | 1.2 ± 0.1 |
| Biosynthesis (per rat per 6 weeks) | 66.2 ± 3.2 | 28.5 ± 4.9b |

Values are means ± SEM for 5 controls and 6 rats treated with T3 and T4. *Significantly different from initial body pool size (p<0.05). aSignificantly different from control (p<0.05). Initial body pool size was obtained from 5 untreated rats of 3 weeks old.

week of experimental period. In control rats during the growing period, carnitine pool size per rat increased about 4.5 times of the initial pool size obtained at 3 weeks of age. Thyroid hormone treatment depressed the increase of the carnitine pool size.

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with growth, expressed as whole body or 100 g body weight basis, markedly or significantly ($p < 0.05$), respectively. Carnitine biosynthesis during the 6-week feeding period, calculated by subtracting total urinary carnitine excretion from carnitine body pool size plus total carnitine intake, was $66.2 \, \mu\text{mol}$ and $28.5 \, \mu\text{mol}$ for control and $\text{T}_3 + \text{T}_4$-treated rats, respectively. The difference between the two groups was significant ($p < 0.05$).

**DISCUSSION**

In the present study, the effects of short- and long-term administration of thyroid hormone were studied in rats and mice fed a low carnitine diet. In all 3 experiments, urinary excretion of total carnitine was significantly increased with thyroid hormone administration. The decreased pool size of carnitine in the body was found in both mice injected with $\text{T}_4$ for 10 days and rats fed on $\text{T}_3$ and $\text{T}_4$ for 6 weeks, but this was not detected in rats injected with $\text{T}_4$ for 3 days. Thus, thyroid hormone seems to affect rapidly the urinary excretion of carnitine but carnitine pool size in the body is affected after a relatively long time. Any dose between 5–50 $\mu\text{g}$ of $\text{T}_4$ per 100 g body weight seemed to be effective on carnitine metabolism of mice, but significant effects could be observed when either level of 20 or 50 $\mu\text{g}$ of $\text{T}_4$ per 100 g body weight was injected for 10 days.

It is generally accepted that heart and skeletal muscle contain relatively higher concentrations of carnitine than liver (20), and that carnitine contents of heart exceed those of skeletal muscle (6, 20, 37). However, in both control and $\text{T}_3 + \text{T}_4$-treated rats of the present study, carnitine contents in gastrocnemius and quadriceps muscles were comparable to or slightly higher than in heart. This finding is in accord with that by Tanphaichitr and Broquist (6) who have found almost equal carnitine contents in either heart or skeletal muscle of rats fed a low carnitine diet (0.6 nmol carnitine/g diet). Thus, in rats, when dietary carnitine level is modified, skeletal muscle, rather than heart, is likely to be affected in its carnitine store. A deep-red portion of quadriceps muscle had more carnitine than a superficial-white portion, which is in agreement with results by Baldwin and Tipton (45). Total skeletal muscle per rat may hold $92.2 \pm 2.9\%$ of total body carnitine pool, when 100 g rat is assumed to have 47 g of skeletal muscle (46).

Thyroid hormone administration has been reported to increase the utilization of fatty acids by animal body. In the present study, rats treated with $\text{T}_3$ and $\text{T}_4$ showed significant increase in serum FFA concentration and depletion of liver glycogen stores. In liver, fatty acid oxidation is high when glycogen store is low (47). Thus, during the later phase of the 6-week experiment, rats of $\text{T}_3 + \text{T}_4$-treated group might be increased in their ability to oxidize fatty acids.

The increased percentages of palmitic and oleic acids in the total lipids in liver and adipose tissue from $\text{T}_3 + \text{T}_4$-treated rats suggest that de novo synthesis of fatty acids is stimulated with thyroid hormone administration. This finding supports Diamant et al. (48) and Landriscina et al. (49) who have reported the increased fatty acid synthesis under thyroid hormone stimulation.
acid synthesis in liver from thyroid hormone administered rats.

Thyroid hormone administration for 10 days caused a slight increase in urinary excretion of esterified-carnitine in rats, but a greater one when treated for 6 weeks. Similar increases in urinary esterified-carnitine have been reported previously in starved human (33), where energy metabolism depends much on fatty acids as the fuel. Since esterified-carnitine to free-carnitine ratio increased dramatically in T₃+T₄-treated rats at weeks-5 and 6 of experiment 3, a significant elevation in fatty acid oxidation of their bodies might occur with thyroid hormone treatment. At weeks-5 and 6, rats of T₃+T₄-treated group also showed significant increase in food consumption.

During the thyroid hormone treatment, mice showed markedly higher urinary total carnitine than control animals, and in rats of experiment 3, this difference was significant. This is in agreement with our previous findings in T₄-treated human (15, 16). It has been reported in starved persons (31) and in rats and mice administered with large amounts of carnitine (50) that changes in urinary excretion of carnitine were parallel with those in plasma carnitine concentrations. However, the present study clearly indicated that renal control is one of the important factors regulating urinary excretion of carnitine derivatives, likewise in starved normal persons and ACTH administered patients with myopathy (33). Thus, alterations in plasma profile of carnitine and its derivatives besides total carnitine concentration probably affect the urinary level of total carnitine.

In untreated control rats of experiment 3, an estimated carnitine biosynthesis was 66.2±3.2 µmol/rat/6 weeks. A sum of the ingested and synthesized carnitine during 6 weeks of experiment was about 68 µmol per rat, of which 80% was deposited in body and the remaining 20% was excreted into urine during the 6-week period. The decreased pool size of carnitine in the body of rats treated with T₃ and T₄ was determined to be due to the decreased carnitine contents in heart, kidney, testis and skeletal muscle. The increase in urinary excretion of total carnitine with the 6-week thyroid hormone administration was too small to account for the decreased carnitine body pool size in T₃+T₄-treated rats.

The results obtained in the present study seem to indicate that thyroid hormone administration may depress carnitine synthesis in rats and mice. However, some possibilities that thyroid hormone may increase carnitine synthesis still remain to be further investigated because of several reasons described below. a) Conversion of carnitine to β-methylcholine, which was reported from one laboratory (51), has not been examined in the present study. This might be responsible for the under-estimation of carnitine synthesis in rats of both control and thyroid hormone treated groups, especially in the rats of the latter group. b) The decrease in carnitine contents in cardiac and skeletal muscle from T₃+T₄-treated rats was in accord with the observation in mice by Cederblad and Engström (17) which were injected with 20 µg of T₄ twice a day for 4 days. Contrary, Bressler and Wittels (18) found increased carnitine levels in hearts from guinea pigs treated with thyroid hormone. In addition, it has been reported (52) that metabolic effects of thyroid hormone
depends on its dose levels. Thus, different levels of thyroid hormone supplementation than those used in the present study could enhance carnitine synthesis in rats and mice. c) Carnitine synthesis in the present study was determined from the data on carnitine intake, urinary carnitine excretion and changes in carnitine pool size in the body over a 6-week period of thyroid hormone administration. As mentioned above, physiological and nutritional states of the animals, however, altered dramatically after 4 weeks administration of T3 and T4. Furthermore, among tissues of T3+T4-treated rats, liver, the site of carnitine synthesis, contained a little more total carnitine as compared untreated controls. These findings could suggest a hypothesis that carnitine synthesis might have decreased in the earlier phase of thyroid hormone administration but enhanced later, especially at weeks-5 and 6 of the administration period.

In conclusion, the present study clearly demonstrated that thyroid hormone intensely influence the metabolism of carnitine and its renal clearance, however, the question of whether thyroid hormone would stimulate or depress carnitine synthesis has not been dissolved but remains to be elucidated by further investigations.

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