The effect of fasting on thyroidal T₄-5' monodeiodinating activity in mice

Sing-yung Wu

Nuclear Medicine and Medical Service, Veterans Administration Medical Center Long Beach, and University of California Irvine, CA, USA

Abstract Complete fasting induces a significant decrease of serum T₃ and a fall in TSH in rodents and man. To evaluate the effect of starvation on thyroidal T₄, 5' monodeiodinating activity, in vitro conversion of T₄ to T₃ by thyroid and liver homogenate from one to three days fasted mice was compared with homogenates from control mice on animal chow. 5' monodeiodinating activity was significantly lower in thyroid homogenates of fasted mice than in those of chow-fed control [100±5.0 and 92±5.0 pmol T₃/(mg protein)⁻¹·h⁻¹ at 48 and 72 h fasting, respectively, vs 132±5.0 pmol T₃/(mg protein)⁻¹·h⁻¹ of fed control, p<0.01]. A similar decrease in thyroidal 5' monodeiodinating activity was seen in the liver. The decrease in thyroidal 5' monodeiodinating activity induced by fasting was not reversed by the supplementation of homogenates with the thiol-protecting agent, dithiothreitol (0.2-4.0 mmol/l). Physiological replacement of T₄, 0.58 nmol-(100 g)⁻¹·day⁻¹, did not alter the effect of starvation in either the thyroid or liver. TSH (0.02 IU/day) injection, on the other hand, stimulated 5' monodeiodinating activity in homogenates of thyroids from 3 days fasted mice which was no different from TSH-treated fed control. It is postulated that starvation-induced decrease in thyroidal T₄ to T₃ converting activity may play a role, together with decreased hepatic 5' monodeiodinating activity, in fasting-induced low serum T₃ in mice.

Complete fasting induces a significant reduction of serum T₃ (1-3) and a fall in TSH which is transient in man (4) but sustained in rats (5). Kinetic studies in man showed that the decrease in serum total and free T₃ was mainly a result of decreased T₃ production (6) which was associated with decreased or suppressed activity of 5' monodeiodination of T₄ in liver (5,7,8). The effects of fasting on deiodination in other organs are variable. T₄ 5' monodeiodination in tissue homogenate from fasted rats showed no change in the kidney (9) and brain (10) and only a 10-20% decrease in the anterior pituitary (11). No information is available concerning the effect of fasting on thyroid where active iodothyronine 5' monodeiodination is present (12-14). The evidence suggests that thyroidal 5' monodeiodination is maintained and can be stimulated by TSH (15,16). More recently, we have shown that 5' monodeiodination in the thyroid is extremely sensitive to TSH stimulation and the increase is dose-dependent in mice (16). We also demonstrated that long-acting thyroid stimulator-rich patient serum may stimulate T₄ 5' monodeiodination in mouse thyroid (17). These findings may be relevant to the kinetic studies in man which suggest a preferential secretion of T₃ from the thyroid in hypo- and hyperthyroid patients (18,19). The present studies examine the effect of complete fasting on thyroid 5' monodeiodination in comparison with hepatic changes.

Materials and Methods

Animal preparation
Female Swiss-Webster albino mice (20-25 g) were maintained on regular animal chow. In the case of fasted animals, the food was removed 24 to 72 h before sacrifice. Water was available to all animals ad libitum. Bovine TSH (Thytopar® from Armour Pharmaceutical Co, Phoenix, AZ; 0.02 IU/day) was administered ip for 3 consecutive days at the time of fasting as specified in some experi-
ments. In experiments with T4 supplement T4 (0.58-5.8 nmol/100 g) was given daily, ip through the 3-day fasting period. Fed controls received the same T4 treatment.

**Measurement of tissue 5’monodeiodination activity**

T4 5’monodeiodination in the thyroid and liver was assayed according to the methods reported previously (16). The reaction mixture (final volume 250 μl) consisted of 0.5 mg wet tissue weight equivalent homogenate suspended in 120 mmol/l phosphate buffer (pH 7.0) containing 4 mmol/l dithiothreitol and 2.5 μmol/l T4. All incubations were at 37°C for 30 min, after which 2 vol 95% ethanol were added during mixing. Incubation vessels were kept at 4°C overnight and then centrifuged at 5000 xg for 20 min. T3 produced was measured in the supernatant by RIA. The zero time incubation tubes were prepared by adding ethanol before addition of the enzyme preparation. The amount of endogenous free T4 in the thyroid homogenates (containing approximately 100 μg protein) was insignificant, since the in vitro production of T3 in the absence of added exogenous T4 was negligible. To test for the stability of the product of deiodination, 5-50 ng T3 were added at time zero, and the amounts remaining after incubation were determined; less than 5% of T3 was degraded. Results are expressed as mean ±SEM pmol produced per mg protein per h.

**Statistical analysis**

Statistical analyses were performed with Student’s two-tailed t-test for unpaired data modified with Bonferroni’s inequality for multigroup comparison (20).

## Results

**Effect of starvation on body weight, tissue weight, protein content, serum T3, T4 concentrations, and tissue monodeiodinating activity**

As shown in Table 1 a 21.8% reduction of body weight was found in the 72 h-fasted group (p<0.01 vs chow fed control). Thyroid weight did not decrease significantly in starved animals, but hepatic weight decreased by 22% from fed controls. Liver protein was not significantly altered in the fasted group except at 72 h (p<0.05 vs control). Both serum T4 and T3 were reduced significantly after fasting for 24 to 72 h (Table 1). T3 was reduced to 56.5% of control level in 72 h fasted mice (p<0.001 vs chow-fed).

T4 to T3 monodeiodinating activity was significantly lower in thyroid homogenates of fasted mice than in those of chow-fed controls (100±5.0 and 92±5.0 pmol at 48 and 72 h fasting, respectively vs 132±5.0 pmol of fed control, p<0.01). A similar decrease in monodeiodinating activity was seen in the liver (Table 1). The decrease in thyroidal T4 to T3 converting activity induced by fasting was not reversed by the supplementation of the homogenates with the thiol-protecting agent dithiothreitol (0.2-4.0 mmol/l) (results not shown).

### Table 1.


<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Body weight (g)</th>
<th>Tissue weight Thyroid(mg)</th>
<th>Liver(g)</th>
<th>Tissue protein (mg/g)</th>
<th>Thyroid Liver</th>
<th>Deiodinase activity †</th>
<th>Serum concentration T3 (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed control</td>
<td>6</td>
<td>23.9±0.5</td>
<td>2.6±0.1</td>
<td>1.05±0.05</td>
<td>218±9.4</td>
<td>215±6.8</td>
<td>132±5.0</td>
<td>135±15</td>
</tr>
<tr>
<td>Fast – 24 h</td>
<td>5</td>
<td>21.4±0.6*</td>
<td>2.7±0.2</td>
<td>0.84±0.02*</td>
<td>224±8.0</td>
<td>235±12</td>
<td>132±9.2</td>
<td>–</td>
</tr>
<tr>
<td>Fast – 48 h</td>
<td>5</td>
<td>19.5±0.3*</td>
<td>2.4±0.2</td>
<td>0.84±0.02*</td>
<td>228±7.2</td>
<td>229±7.1</td>
<td>100±5.0*</td>
<td>–</td>
</tr>
<tr>
<td>Fast – 72 h</td>
<td>6</td>
<td>18.7±0.3*</td>
<td>2.5±0.1</td>
<td>0.82±0.02*</td>
<td>226±3.2</td>
<td>240±6.0*</td>
<td>92±5.0*</td>
<td>89±6.0*</td>
</tr>
</tbody>
</table>

† The converting activity [pmol T3(mg protein)⁻¹h⁻¹] was measured in a mixture containing 0.5 mg equivalent of tissue homogenate, 4 mmol/l dithiothreitol, 2.5 μmol/l T4 and 120 mmol/l phosphate buffer (pH 7.0). The reactions were carried out at 37°C for 30 min, after which 2 volumes of 95% ethanol were added during mixing. T3 was measured in the supernatant by radioimmunoassay.

*p<0.05 represents comparison with fed control.

(N) number of groups of mice (usually 3 mice per group).
(40±2.6 vs 48±3.9 nmol/l, p>0.1), whereas serum T₃ remained lower in T₄-replaced fasted mice (0.98±0.03 vs 1.66±0.08 nmol/l, p<0.001) (Fig. 1). With increasing daily doses of T₄ (1.74 and 5.8 nmol/100 g), there was a progressive decrease in thyroid T₄ to T₃ converting activity in both fed and fasted animals. The effect of fasting on thyroidal activity could no longer be seen in these mice treated with higher doses of T₄. It is noteworthy that hepatic T₃ to T₄ converting activity was increased in 3-day fasted mice receiving a daily injection of T₄ (1.74 nmol/100 g), and it reached a level [229±18 pmol T₃/(mg protein)⁻¹ h⁻¹] even higher than similar T₄-treated fed control [153±11 pmol T₃/(mg protein)⁻¹ h⁻¹, p = 0.012]. Further increase in T₄ dosage to 5.8 nmol/100 g per day stimulated even higher hepatic converting activities in starved animals but also increased the activity in control to the same extent (Fig. 1).

**Effect of TSH treatment in fasted mice on tissue monodeiodinating activity**

As shown in Fig. 2, conversion of T₄ to T₃ in thyroids from 72 h fasted mice was stimulated with daily injection of TSH (0.02 IU/day) during starvation [240±32 vs 97±7.1 pmol-(mg protein)⁻¹ h⁻¹ in fasted mice without TSH, p<0.01]. The stimulated value was not different from that in fed animals receiving TSH [222±31 pmol mg-(protein)⁻¹ h⁻¹]. In fed animals, TSH treatment did not produce any significant change in serum T₄ (57±3.9 vs 55±2.6 nmol/l of untreated control) or T₃ (1.57±0.09 vs 1.60±0.08 nmol/l of untreated control). In 72-h fasted animals, however, TSH treatment resulted in an increase in serum T₃ (1.67±0.32 vs 0.91±0.08 nmol/l of untreated 72 h fasted mice, p<0.05) and serum T₄ (45±6.4 vs 30±3.9 nmol/l of untreated 72-h fasted mice, not statistically significant, p>0.05).

**Discussion**

Kinetic studies of thyroid hormone metabolism in total food deprivation revealed that T₃ production is markedly decreased in euthyroid obese man (7). In the rat, hepatic 5’monodeiodination is decreased as shown by others (5,8) as well as in the present study in mice, and is thought to be responsible for the resulting "low T₃ syndrome" in total fasting. The present study, however, provides an additional possible mechanism, i.e. decreased thyroidal
Thus, thyroidal similar than rectly nal uncertain. deprivation decreased monodeiodination (5). monodeiodination of T4 whether intra- or extra-thyroidal in origin (22). Even though we have no serum TSH data in the present mice study, the fact that the conversion defect can be corrected by endogenous TSH suggests that it may be mediated through TSH, which is decreased in total energy deprivation in man (3), but not known in mice. However, since pharmacological doses of heterologous (bovine) TSH is used in the present experiments, the exact role of TSH in vivo is somewhat uncertain. Other hormones such as growth hormone (23), somatostatin (24) and/or gastrointestinal hormone (25) may also play a role either directly or indirectly.

Mouse hepatic weight is 300-400 times greater than that of the thyroid gland, whereas the in vitro specific deiodinating activity in these two organs is similar in both fed and fasted states (Table 1). Thus, it would be reasonable to assume that the thyroidal deiodination of circulating T4 is of little quantitative importance for total T3 production in either fed or starved mice. This conclusion would be true if the thyroid gland only takes up and converts circulating T4 (or exogenous T4) to T3 in a similar manner as the liver. On the other hand, 5’monodeiodination of endogenous T4 before its liberation from thyroglobulin into the blood stream has been suggested by Laurberg in studies with perfused dog thyroid lobes (22). However, the quantitative contribution of thyroidal 5’monodeiodination of endogenous T4 to total T3 production is uncertain. Nevertheless, the contribution should be less than the total thyroidal secretion of T3, which has been estimated to be 20 (26) and 32% (27) of the total production of T3 in the rat. In addition, the early decrease in serum T4 and T3 (40% by 24 h of fasting) cannot be accounted for by thyroidal conversion of T4 to T3 which remains unaltered (Table 1).

In fasted mice, as shown in the present study, T4 is also decreased as it is in fasted rats. It has been shown in fasted rats, which were on physiological

---

Fig. 2.
TSH treatment on the effect of fasting on tissue T4 5’monodeiodination. Mice were given saline (-) or TSH (+) (0.02 IU/day, ip) during the 72-h fast (shaded bar). The reaction mixture is the same as in the legend to Table 1. P values represent comparisons either with fed (open bar) control (* p<0.05) or fed with TSH-treated mice (+ p<0.04; # p<0.015) as specified. The results are expressed as pmol T3 produced per mg tissue in 60 min at 37°C. Values are mean ± SEM (represented by vertical lines at the top of bars).
replacement of T₄, that raising T₄ to normal levels did not result in a normalization of the serum T₃ (23,24). The present study also demonstrated that physiological dose of T₄ (0.58 nmol/100 g daily, 21) did not normalize the thyroidal 5' monodiodination or serum T₃. A higher dose of T₄ (1.74 nmol/100 g daily) reduced 5' monodeiodination in thyroids from both fed and fasted to a similar degree. It is surprising to find that at this dosage of T₄, hepatic 5' monodeiodination in fasted mice was paradoxically increased as compared with that in fed animals receiving the same amount of T₄. This change was reproducible and was subsequently repeatedly confirmed. In starved mice, the hepatic 5' monodeiodination appeared to be "sensitive" to the slight increase in serum T₄ and became readily "inducible"; this mechanism is unclear and remains to be elucidated. By comparison, monodeiodination in the thyroid is markedly suppressed with higher doses of exogenous T₄.

A recent study showed a paradoxically greater decrease in serum T₃ than in serum T₄ level and the direct chromatographic analysis of total body homogenates after the injection of [¹²⁵I] T₄ suggested that decreased serum T₃ in starving rats was due primarily to diminished thyroidal secretion of T₄ (28). However, these data with a far greater decrease in serum T₃ than in serum T₄ level are somewhat different from a previous report (29). In addition, T₄ replacement in fasted rodents usually did not normalize serum T₃ level as demonstrated in the present study in mice (Fig. 1), as well as in rats (23,24); this appeared to suggest the role of peripheral deiodination in fasting-induced "low T₃ state." Furthermore, their data suggest all serum T₃ in starved rats was derived from peripheral conversion of T₄ (28); thus, it certainly does not exclude the possibility that the thyroid gland itself may act as an organ of peripheral deiodination (22). However, whether or not thyroidal monodeiodinase plays any significant role in the decreased serum T₃ level in vivo in fasting awaits further kinetic studies.

References
15. Erickson VJ, Cavalieri RR, Rosenberg LL. Thyroxine-5'-deiodinase of rat thyroid, but not that of liver is dependent on thyrotropin. Endocrinology 1982;111:434-40.
16. Wu SY, Reggio R, Florsheim WH. Characterization...

Received July 27th, 1989.
Accepted October 17th, 1989.

Dr Sing-yung Wu,
Nuclear Medicine Service,
VA Medical Center,
Long Beach,
California 90822,
USA.