EFFECTS OF EXPERIMENTAL HYPOTHYROIDISM ON SKELETAL MUSCLE METABOLISM IN THE RAT

By

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ABSTRACT

Hind-limb perfusion was used to study the effect of thyroidectomy on some metabolic parameters in the skeletal muscle of the rat. A week after thyroidectomy obtained by one dose of 3/4 mCi $^{131}$I, neither T$_4$ nor T$_3$ was detected in the blood. Lactate production and glycerol production were already decreased a week after the treatment and reached a base level at two weeks. At that time, the oxygen consumption was significantly lower (70% of initial level) than in the control animals and decreased further in the third week to nearly 50% of the control level.

Glucose consumption and alanine release were decreased three weeks after thyroidectomy. One dose of T$_3$ (10 $\mu$g/100 g b. w.), administered to animals two weeks after the injection of $^{131}$I, restored the oxygen consumption, lactate production, and glycerol production to normal levels in 24 h. After 48 h, the glucose consumption was normal. Glycerol production was already significantly increased 6 h after T$_3$ injection in animals one week after thyroidectomy, and in another group of animals two weeks after thyroidectomy. Apparently the diminished oxygen consumption in the latter group does not retard the lipolytic response to T$_3$. No direct relationship could be found between the activity of lipolytic process and the thyroid hormone controlled oxygen consumption.

The study of skeletal muscle as a target organ of thyroid hormone is of interest for at least two reasons: firstly, because skeletal muscle and brown adipose tissue (not well developed in the rat) play the leading role in non-shivering thermogenesis (Himms-Hagen 1976; Jansky 1973), and secondly, because of the well-known structural and biochemical changes developing.
during thyroidal pathogenesis (Ramsay 1974). The role of thyroid hormone in non-shivering thermogenesis is established but has not been fully elucidated. Much progress in the understanding of the subject has been brought in recent years by Edelman and coworkers (Edelman & Ismail-Beigi 1974; Lo et al. 1976; Lo & Edelman 1976; Philipson & Edelman 1977a,b) and their results were confirmed by other investigators (Curfman et al. 1977). Asano et al. (1976) showed that ouabain sensitive oxygen consumption (Na⁺ transport dependent respiration) in skeletal muscle accounted only for 47% of the increase in O₂ consumption in the transition from the hypothyroid to the euthyroid state. So the remaining 53% needs further explanation. Furthermore in an extensive review on cellular thermogenesis (Himms-Hagen 1976) it was pointed out that ouabain has many other actions which may alter cellular metabolism of which alteration of Ca⁺⁺ distribution in the cell may be of special relevance to skeletal muscle. Very little is known about the biochemical changes that take place in skeletal muscle in hypothyroidism. However, it is accepted that the abnormalities of skeletal muscle in hypo- and hyperthyroidism are of biochemical origin (Ramsay 1974) and lie in the muscle rather than in the nerve tissue.

The analysis of metabolic interrelationships in hypothyroid rats is complicated, because energy expenditure is reduced and thermogenesis decreased. The slowing of cardiovascular haemodynamics (Scott et al. 1961) may in itself jeopardize oxidative events at appropriate sites. Similarly, the decreased generation of heat due to hypometabolism may cause further retardation of those metabolic activities that are most sensitive to the prevailing body temperature. In a previous study (van Hardeveld & Kassenaar 1977a) we showed that hind-limb perfusion is a convenient way to study the effects of thyroid hormone on skeletal muscle. The main advantage of this preparation is the maintenance of tissue integrity, especially the relationship between muscle and the nervous system, the importance of which is shown by the biochemical alterations occurring immediately after denervation (van Hardeveld & Kassenaar 1977b). Furthermore, the aforementioned complications related to primary and secondary effects of hypothyroidism can be circumvented by using a perfused isolated organ.

The present report extends our earlier findings in hyperthyroid rats. A study of the literature shows that the hypothyroid state, like the thyrotoxic state, is still poorly defined. Experiments on "hypothyroid" animals have been carried out 1 to 8 weeks after thyroidectomy (Asano et al. 1976; Bouknik et al. 1975; Correze et al. 1974; Frumess & Larsen 1975; Grill & Rosenquist 1975; Gustafsson et al. 1965; Surks et al. 1975; Tata et al. 1963), whereas the investigations discussed here deal with the chronological effects of short-term depletion (1 day to 2 weeks) of thyroid hormone on some metabolic parameters of importance for the energy economy of skeletal muscle.
MATERIAL AND METHODS

Animal experiments
Female rats of a Wistar strain, 7 weeks old and weighing 130–140 g, were divided into a control group and an experimental group. Both were put on a low-iodine diet and given bi-distilled water, the drinking water of the control group being supplied with 15 mg KI/litre. After 2 weeks, the experimental group received an intraperitoneal injection of 3/4 mCi $^{131}$I. This time point of injection will be referred to as the moment of thyroidectomy. These animals were used for perfusion experiments performed 1, 2, or 3 weeks later. Completeness of thyroidectomy was routinely checked by $T_4$ RIA done in plasma obtained by retro-orbital eye puncture before perfusion was started.

Perfusion
The perfusion apparatus was a modification of a cyclic closed system described by Hems et al. (1966) for liver perfusion, the main alteration being the addition of a second Whatson-Marlow roller pump to introduce the oxygenated medium into the animal’s aorta. For further details of the perfusion and the surgical preparation, see van Hardeveld & Kassenaar (1977b). The perfusion medium consisted of Krebs-Heinseleit buffer (pH 7.4), 4 g/100 ml BSA (Sigma Chemical Company), washed bovine erythrocytes (8 g Hb/100 ml), 12 mmol/l glucose, and 0.15 mmol/l sodium pyruvate. The initial lactate concentration was 1–2 mmol/l. To correct, when necessary, for lactate production by the erythrocytes (which sometimes occurred), blank samples were collected before the perfusion of the hind-limb was started or the perfusion procedure was performed without an animal. In these blank perfusions glucose consumption was negligible during the perfusion period.

Analytical methods
The perfusate was deproteinized in iccd 3.5% w/v HClO$_4$. Glucose was determined by the method of Krebs et al. (1963), adapted for the auto-analyzer. Lactate was determined enzymatically with an UV test set manufactured by Boehringer Mannheim N. V. (Amsterdam). The method of Williamson (1970) was used for alanine determinations. Oxygen consumption was measured with a radiometer 13 MS 13 (Copenhagen) and PO$_3$ electrode type E 5047 (Copenhagen) and calculated from arteriovenous differences in O$_2$ content multiplied by the flow rate. $T_4$ and $T_3$ concentrations in rat plasma were measured by RIA. The samples were stored (–20°C) until the $^{131}$I in the plasma was sufficiently decayed (2–4 weeks) to allow determination of $T_4$ and $T_3$. Assay conditions were mainly according to Larsen (1972).

Statistical calculations
Significance of differences between group means was calculated with Student’s $t$-test.

RESULTS

I. Course of hormonal and metabolic alterations after thyroidectomy

1. Thyroid hormone concentrations. – Figs. 1 and 2 show the alterations occurring in plasmas $T_4$ and $T_3$ after the administration of one dose of 3/4 mCi $^{131}$I. The $T_4$ level rose steeply after one day and reached a maximum on the
T₄ concentrations in rat plasma at several time points after one intraperitoneal injection of 3/4 mCi ¹³¹I. The blood sampled at t = 0 was taken just prior to injection of the radioactivity. Figures shown above the mean values ± so give the number of experiments.

**Fig. 1.**

T₃ concentrations in rat plasma at several time points after one intraperitoneal injection of 3/4 mCi ¹³¹I. The blood sampled at t = 0 was taken just prior to injection of the radioactivity. Figures shown above the mean values ± so give the number of experiments.

**Fig. 2.**

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third day, although large variations were found from animal to animal. The T₃ level followed the same pattern, but compared with the doubling of the T₄ concentration by day 3, the mean rise of the T₃ concentration was much lower. In this case, too, large variations were observed from animal to animal. After 5 to 6 days, neither T₄ nor T₃ was detectable in the plasma.

2. Metabolic alterations. – Table 1 shows the oxygen consumption, lactate production, glucose consumption, glycerol production, and alanine release one week (Tₓ(1)), two weeks (Tₓ(2)) and three weeks (Tₓ(3)) after thyroidectomy. Two weeks after the injection the oxygen consumption was significantly decreased and decreased further to about 50% of the control level three weeks after thyroidectomy (P < 0.001). The effect of thyroid hormone depletion on lactate and glycerol production was immediately observable. One week after thyroidectomy the production of lactate and glycerol was significantly de-

<table>
<thead>
<tr>
<th></th>
<th>Oxygen consumption</th>
<th>Lactate production</th>
<th>Glucose consumption</th>
<th>Glycerol production</th>
<th>Alanine production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g muscle/min</td>
<td>µmol/g muscle</td>
<td>µmol/g muscle</td>
<td>µmol/g muscle</td>
<td>µmol/g muscle</td>
</tr>
<tr>
<td>Control</td>
<td>0.62 ± 0.06</td>
<td>11.8 ± 5.1</td>
<td>12.0 ± 3.1</td>
<td>0.64 ± 0.11</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>Tₓ(1)</td>
<td>0.62 ± 0.06</td>
<td>6.3 ± 3.6</td>
<td>9.5 ± 1.1</td>
<td>0.42 ± 0.09</td>
<td>0.76 ± 0.05</td>
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<tr>
<td></td>
<td>(6)</td>
<td>(7)</td>
<td>(5)</td>
<td>(5)</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>N. S.</td>
<td>P &lt; 0.05</td>
<td>N. S.</td>
<td>P &lt; 0.005</td>
<td>N. S.</td>
</tr>
<tr>
<td>Tₓ(2)</td>
<td>0.47 ± 0.06</td>
<td>4.7 ± 1.2</td>
<td>8.3 ± 1.6</td>
<td>0.13 ± 0.11</td>
<td>0.71 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(7)</td>
<td>(5)</td>
<td>(7)</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>N. S.</td>
</tr>
<tr>
<td>Tₓ(3)</td>
<td>0.36 ± 0.05</td>
<td>4.8 ± 1.7</td>
<td>7.9 ± 1.9</td>
<td>0.22 ± 0.05</td>
<td>0.58 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(6)</td>
<td></td>
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<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

* The oxygen consumption represents the mean oxygen consumption of the perfused hind-limb (± 30 g of muscle mass) during 90 min of perfusion. The lactate, glycerol, and alanine production and glucose consumption are represented by the cumulative values after 90 min of perfusion. Where significant differences were found between the treated animals and the controls, the P-value is given. Figures in brackets give the number of experiments.
Table 2.
Effect of a single dose of T₃ (10 μg/100 g b.w.) on the mean oxygen consumption in the perfused hind-limbs during 60 min of perfusion and the cumulative lactate production, glucose consumption, and glycerol production after 60 min of perfusion.

<table>
<thead>
<tr>
<th>Tₓ(2)</th>
<th>Hours after injection</th>
<th>Euthyroid controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td>0.45 ± 0.04</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>μmol/g muscle/min</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>Lactate production</td>
<td>2.9 ± 1.2</td>
<td>3.3 ± 1.4</td>
</tr>
<tr>
<td>μmol/g muscle</td>
<td>(10)</td>
<td>(7)</td>
</tr>
<tr>
<td>Glucose consumption</td>
<td>4.9 ± 1.1</td>
<td>5.7 ± 2.9</td>
</tr>
<tr>
<td>μmol/g muscle</td>
<td>(9)</td>
<td>(7)</td>
</tr>
<tr>
<td>Glycerol production</td>
<td>0.07 ± 0.05</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>μmol/g muscle</td>
<td>(5)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

* The T₃ dose was administered two weeks after thyroidectomy (Tₓ(2)). Where significant differences were found between T₃-treated animals and controls (Tₓ(2) animals (t = 0)), the P-value is given. Figures in brackets give the number of experiments.
Table 3.
Effect of a single dose of T₃ (10 µg/100 g b. w.) on the mean oxygen consumption in the perfused hind-limbs during 60 min of perfusion and the cumulative lactate production, glucose consumption, and glycerol production after 60 min of perfusion*.

<table>
<thead>
<tr>
<th>Time after thyroidectomy</th>
<th>Tₓ(1)</th>
<th>Tₓ(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours after injection</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Oxygen consumption µmol/g muscle/min</td>
<td>0.60 ± 0.08 (11)</td>
<td>0.55 ± 0.05 (7)</td>
</tr>
<tr>
<td>Lactate production µmol/g muscle</td>
<td>2.8 ± 1.6 (11)</td>
<td>3.7 ± 1.2 (7)</td>
</tr>
<tr>
<td>Glucose consumption µmol/g muscle</td>
<td>5.8 ± 1.3 (10)</td>
<td>6.5 ± 2.2 (7)</td>
</tr>
<tr>
<td>Glycerol production</td>
<td>7.7 ± 2.6 (7)</td>
<td>0.27 ± 0.07 (6)</td>
</tr>
</tbody>
</table>

* The T₃ dose was administered one week (Tₓ(1)) and two weeks (Tₓ(2)) after thyroidectomy. Where significant differences were found between T₃-treated animals and controls (Tₓ(1) and Tₓ(2) animals (t = 0)), the P-value is given. Figures in brackets give the number of experiments.
creased. A further decrease occurred in the second and third weeks when a base level seemed to be reached. The effect of thyroidectomy on glucose consumption and alanine production was much slower, the decrease of both parameters only becoming significant three weeks after thyroidectomy.

II. Effect of one dose of $T_3$ on the skeletal muscle metabolism of thyroidectomized rats

A second series of experiments was performed with thyroidectomized rats to determine the time course of restoration of the affected metabolic parameters after one subcutaneous injection of 10 $\mu$g $T_3$/100 g b. w. For this purpose, rats were used two weeks after thyroidectomy ($T_x(3)$). The results are shown in Table 2. Oxygen consumption, lactate production, and glycerol production were restored to normal levels within 24 h. Glucose consumption increased more slowly. Forty-eight hours after the $T_3$ injection, a significant increase was observable relative to the ($T_x(3)$) controls. Furthermore, it seemed of interest to us to find out whether the rapidity with which the $T_3$-induced effects appeared was influenced by the oxygen consumption of the hind-limb. To answer this question, rats were used one week after thyroidectomy ($T_x(1)$), these animals having unimpaired oxygen consumption and, compared with ($T_x(3)$) animals, diminished oxygen consumption. The results are shown in Table 3.

Lactate production was still not significantly increased in the ($T_x(1)$) and ($T_x(3)$) groups 6 h after $T_3$ injection, but at this time both groups exhibited a significantly increased glycerol production.

**DISCUSSION**

$T_4$ and $T_3$ levels in the peripheral blood were followed after thyroidectomy because this enabled us to choose the moment for perfusions that were to be carried out just after thyroid hormone had disappeared from the blood. All experiments were done within a month after thyroidectomy and maximally two weeks after thyroid hormone had been cleared from the blood. This experimental design was chosen for two reasons. First, to permit observation of the early effects of thyroid hormone depletion, and secondly, to avoid structural alterations in the muscle mass as much as possible.

The transient rise in thyroid hormone levels after thyroidectomy is a well-known phenomenon, and is observed in man after ablation of the thyroid gland with $^{131}$I (Lamberg 1959). The larger rise we observed in the $T_4$ level as compared with the $T_3$ level, can be explained by the much higher $T_4$ content of thyroid colloid found in rats (Abrahams & Larsen 1973). Most of the published studies on hypothyroidism in rats were performed a month or more after
thyroidectomy, the main criterion being the time point at which the animals stop growing. However, the data in Table 1 show that the metabolic alterations at least in skeletal muscle occur much earlier. About a week after the disappearance of thyroid hormone from the blood, the oxygen consumption has already diminished markedly. We suppose that this phenomenon is reflected in our earlier finding (van Hardeveld et al. 1976) of a decrease in mitochondrial protein in skeletal muscle after thyroidectomy. The rather rapid effect on oxygen consumption is in accordance with Terjung’s observation (Terjung 1975) that the half-life of cytochrome C in skeletal muscle is approximately 8 days instead of the hitherto accepted half-life of 30 days. This means that the turnover of mitochondrial elements in skeletal muscle is much faster than was previously supposed. Terjung (1975) also found that thyroidectomy caused a 25% decrease in the cytochrome C content after one week. Three weeks after this treatment the oxygen consumption of isolated muscle mitochondria is 50–60% of the normal level (Gollnich & Lanazzo 1972; Gustafsson et al. 1965). This seems to be in accordance with the oxygen consumption in our perfused hind-limb preparation three weeks after thyroidectomy. One day after thyroid hormone had disappeared from the blood, the lactate production was significantly diminished. As previously reported (van Hardeveld & Kassenaar 1977b), lactate production is controlled by the activity of the nervous system. The activity of the sympathetic nervous system may be an especially important determinant of the amount of lactate produced, because the catecholamines produced by sympathetic nerve endings are known to be stimulators of glycogenolysis in skeletal muscle (Helmreich & Cori 1965; Krebs 1970; Villar-Palasi 1968). Relating these facts to the reported potentiating action of thyroid hormone to catecholamine effects in heart muscle (Bray & Goodman 1965; Levey & Epstein 1969), we attribute the decrease in lactate production to the absence of thyroid hormone which may play a permissive role in catecholamine induced glycogenolysis. This is in accordance with the findings of McDaniel et al. (1977), who reported on carbohydrate metabolism in hypothyroid myopathy. They observed 35% lower blood lactate levels in untreated hypothyroid patients. The investigators suggest impaired glycogenolysis from the skeletal muscle in the absence of thyroid hormones. Glycerol production, like lactate production, is markedly decreased one week after thyroidectomy. We attribute this to a similar mechanism as outlined above, in view of the considerable evidence indicating a permissive role of thyroid hormone in catecholamine-induced lipolysis (Bray & Goodman 1965; Krishna et al. 1968).

In sum, a distinction might be made in our skeletal muscle preparation between a slow effect of thyroid hormone on substrate oxidation, as reflected by oxygen consumption, and a rapid effect on substrate mobilization (lipolysis, glycogenolysis). The multiple effects of the thyroid state on glucose metabolism (insulin secretion, absorption of glucose in the small intestines) (Malaisse et
by process both oxygen and glucose. One of those affected processes might have a rate-limiting effect on the glucose uptake. Karl et al. (1976) suggest that the release of alanine can serve as a measure of the rate of skeletal muscle protein metabolism. Table 1 shows that alanine release too is influenced by the thyroid state, but apparently very slowly because the release is not significantly lowered until three weeks after thyroidectomy. The administration of one dose of T₃ to thyroidectomized animals (Tₓₒ) already stimulates lipolysis after 6 h. However, at this time, oxygen consumption was still not affected. Conversely a 30% decrease in oxygen consumption does not alter the T₃-induced lipolytic response (Table 3). Apparently there is no direct relationship between the activity of the lipolytic process and the oxygen consumption in this hind-limb preparation, although both processes are under thyroid hormone control. In view of the fact that such a direct relationship between thyroid hormone induced oxygen consumption and the effect of thyroid hormone on Na⁺/K⁺ transport has been established by several investigators (e.g. Asano et al. 1976) it will be investigated whether such a relationship is demonstrable in our preparation.

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