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Effect of Thyroidectomy on Circulating Components and Liver Metabolism in Fed and Fasted Rats

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Abstract. To study intermediary metabolism in hypothyroidism, thyroidectomized rats were compared with thyroidectomized rats daily injected with 1.5 µg of thyroxine and with intact controls. When fed, thyroidectomized animals show decreased plasma protein-bound iodine (PBI) and immunoreactive insulin (IRI) levels and body and liver weights, normal blood glucose and ketone bodies, liver DNA-P concentration and in vitro liver (14C) glucose synthesis from (U-14C) alanine, and elevated liver glycogen concentration and in vivo (U-14C) alanine and (1-14C) glucose uptakes and (14C) lactate from both substrates. After 48 h of starvation, PBI and IRI remain lower in thyroidectomized rats than in the other groups, normal blood glucose and glycogen concentration fall more in this than in the other groups while the rise in ketone bodies does not differ among the groups. The uptake of (U-14C) alanine by the liver slices remains elevated in fasted thyroidectomized rats while the other parameters, studied on in vitro liver metabolism, are equal to the other groups. Thus, different from what it was thought, liver metabolic activity is increased in thyroidectomized rats when corrected by their smaller liver and body weights, allowing them to maintain a normal homeostasis of glucose in the fed state although not when food is withheld.

Introduction

In hypothyroidism, carbohydrate metabolism is very much altered but an equilibrium is established between decreased anabolism and diminished catabolism [19] which allows to maintain normal concentrations of liver glycogen and blood glucose [2, 13, 17]. This equilibrium is broken after prolonged fasting where both parameters fall in hypothyroid animals below those in normal controls [2]. These alterations seem to be mainly localized in the liver where the activity of glycolytic and gluconeogenic enzymes is diminished in hypothyroid animals [3, 7, 18, 24]. Actually, the rate of liver
gluconeogenesis has been reported to be decreased in these animals [18], but the greater production of urea by their perfused livers [18] and the normal steady-state concentration in the liver of regulatory metabolites for gluco-
neogenesis in them [2] would point to a different conclusion. To gain a better understanding of these alterations in hypothyroidism, we have investigated in the present work the in vitro utilization of alanine and glucose by livers from thyroidectomized rats. For a better characterization of these changes, the study was performed in animals both fed and starved for 48 h.

**Materials and Methods**

Female Wistar rats, weighing 75 ± 5 g, were fed a low-iodine diet (0.04–0.09 μg of iodine/g) [9], surgically thyroidectomized and injected daily intraperitoneally thereafter with either 0 or 1.5 μg of l-thyroxine/100 g body weight for 45–60 days. They were compared with age-matched intact female controls under the same diet supplemented with 1.7 μg of KI03/g and injected daily with 0.9% NaCl during the same period of time. Animals were killed by decapitation and without anaesthesia. Blood was collected into heparinized chilled beakers and a piece of liver was rapidly placed in liquid N2. The rest of the liver was placed in fresh Krebs-Ringer bicarbonate buffer, pH 7.4 [22]. Deproteinized blood [21] was used to analyze glucose [16] and total ketone bodies [5]. Immunoreactive insulin was measured in the plasma by a double-antibody technique [12] by using a radioactive insulin kit obtained from The Radiochemical Centre, Amersham, Bucks, UK. Rat insulin (kindly given by Novo Industries, Copenhagen, Denmark) was used as standard. Plasma was used for determination of the protein-bound iodine [4].

Portions of the frozen liver were digested with KOH for precipitation of glycogen with ethanol [11]. The purified precipitate was hydrolysed (2.5 M H2SO4; 2 h; 100 °C) and analyzed enzymatically with glucose oxidase [16]. DNA-P was isolated from the residual pellet after lipid extraction with chloroform-methanol (2:1, v/v) of another portion of frozen liver [20]; inorganic phosphorus was determined [10] after digestion with 72% HClO4.

The fresh liver was used for the preparation of slices and incubation during 90 min in Krebs-Ringer bicarbonate medium, pH 7.4, supplemented with 0.5 μCi/ml of either (U-14C) alanine (10-5 M) or (1-14C) glucose (1 mg/ml), as described previously [6, 8]. The incubations were stopped by injecting 0.1 ml of 1N H2SO4 into the medium and after centrifugation, aliquots of the medium were used for the isolation of radioactive metabolites by paper ascending chromatography [6].

**Results**

*Circulating components.* After 45–60 days of being thyroidectomized and fed a low-iodine diet, the rats show a great reduction in the plasma protein-
Table 1. Effect of thyroidectomy and starvation on blood components in the female rat. p denotes the significance of the differences between mean ± SEM values of thyroidectomized rats and its respective controls. The significance of the differences between the mean for fed animals and animals starved for 48 h is denoted by asteriks: * = p < 0.05; ** = p < 0.02; *** = p < 0.01; **** = p < 0.001; N.S. = not significant, i.e. p > 0.05. The number of rats in each group is shown in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>Starved for 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intact controls</td>
<td>thyroidectomized + 1.5 μg L-T4</td>
</tr>
<tr>
<td>Plasma protein-bound iodine, μg/100 ml</td>
<td>5.7 ± 0.3 (13)</td>
<td>4.0 ± 0.5 (13)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood glucose mg/100 ml</td>
<td>123 ± 6 (15)</td>
<td>97 ± 5 (10)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Plasma insulin μU/ml</td>
<td>47 ± 4 (20)</td>
<td>53 ± 3 (16)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood ketone bodies, μM/ml</td>
<td>579 ± 127 (7)</td>
<td>502 ± 77 (10)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
bound iodine concentration when compared with thyroidectomized animals
daily injected with 1.5 μg of thyroxine/100 g body weight and with intact
controls under the same diet (table I). After 48 h of fasting, the relative dif-
f erences between the groups remained the same as in the fed state (table I).
Blood glucose is the same in the thyroidectomized rats as in the intact con-
trols while in the thyroidectomized animals treated with 1.5 μg of thyroxine,
it was lower than in the other groups (table I). After 48 h of starvation,
blood glucose concentration fell in all the groups but the greatest fall was
observed in the thyroidectomized rats, the difference with the controls being
statistically significant (table I). Plasma concentration of insulin in the fed
state is slightly augmented in the thyroidectomized rats treated with 1.5 μg
of thyroxine and, as in similar conditions [2], this could explain the fall in
blood glucose in these animals. The concentration of plasma insulin is,
however, significantly decreased in the thyroidectomized rats when com-
pared to the other two groups (table I). After 48 h of fasting, the plasma
insulin concentration fell in all groups when compared with fed rats, and it
remains lower in the thyroidectomized animals than in the controls. Blood
total ketone bodies concentration is similar in all the groups with the same
dietary status, and starvation made this parameter increase in all the expe-
rimental conditions (table I).

Body and liver weights and liver DNA-P and glycogen. Although there
were no differences in the weight of the rats of the different groups before
thyroidectomy, the growth rate was slowing down in the thyroidectomized
rats from the 19th day after the surgery, obtaining, at the time of the sacri-
fice, values of body weight significantly lower than when treated with 1.5 μg
of thyroxine and than in the intact controls (table II). In all groups, there is
a significant fall in the body weight with 48 h of starvation, and the decrease
in percent does not differ among the groups. Parallel changes are found in
the liver weight in such a way that the difference among the groups disappears
when the liver weight is expressed per 100 g body weight (table II). This
differs from that we have previously found in male animals maintained under
similar conditions where liver weight/100 g of body weight is lower in the
thyroidectomized rats than in the controls [2] and could be due to the differ-
ent sex of the animals. Despite these differences in the absolute weight of
the livers, the concentration of DNA-P in liver does not differ among the
groups when fed (table II). After 48 h of starvation, the concentration of
DNA-P rose in all groups (table II) and as on other occasions [2, 14, 15], the
total amount of DNA-P in the whole liver was found to be the same in
Table II. Effect of thyroidectomy and starvation on body and liver weights and liver components in the female rat. Statistical comparisons among the groups are as described in Table I.

<table>
<thead>
<tr>
<th></th>
<th>Fed intact controls</th>
<th>thyroidectomized + 1.5 μg L-T₄</th>
<th>Starved for 48 h intact controls</th>
<th>thyroidectomized + 1.5 μg L-T₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>183 ± 5 (13)</td>
<td>181 ± 5 (19)</td>
<td>99 ± 3 (19)</td>
<td>163 ± 4 (13)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>5.2 ± 0.1 (19)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>7.4 ± 0.3 (16)</td>
<td>7.1 ± 0.2 (13)</td>
<td>4.0 ± 0.2 (14)</td>
<td>5.2 ± 0.1 (19)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>5.7 ± 0.2 (18)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Liver weight g/100 g body weight</td>
<td>4.12 ± 0.2 (16)</td>
<td>4.30 ± 0.15 (13)</td>
<td>4.10 ± 0.10 (14)</td>
<td>3.33 ± 0.07 (19)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>3.37 ± 0.06 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver DNA-P μg/g</td>
<td>232 ± 18 (17)</td>
<td>251 ± 9 (12)</td>
<td>212 ± 8 (4)</td>
<td>318 ± 18 (6)</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>N.S.</td>
<td>**</td>
<td>N.S.</td>
</tr>
<tr>
<td>Liver glycogen, %</td>
<td>4.0 ± 0.5 (15)</td>
<td>2.8 ± 0.9 (18)</td>
<td>6.1 ± 0.8 (9)</td>
<td>N.S.</td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td>&lt;0.05</td>
<td>0.17 ± 0.04 (16)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* N.S. = Not significant

** Additional statistical comparisons not shown in the table.
Table III. Effect of thyroideotomy and starvation on the disposal of (U-\(^{14}\)C) alanine (10\(^{-3}\)m) and (1-\(^{14}\)C) glucose (1 mg/ml) in vitro by liver slices from female rats. The disposal of labelled substrates to the different metabolites during the 90 min of incubation has been expressed as a function of the total counts initially present within each flask. Statistical comparisons among the groups are as described in Table I.

<table>
<thead>
<tr>
<th></th>
<th>Fed intact controls</th>
<th>thyroidectomized + 1.5 (\mu)g L-T(_4)</th>
<th>Starved for 48 h intact controls</th>
<th>thyroidectomized + 1.5 (\mu)g L-T(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disposal of (U-(^{14})C) alanine Uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p ((^{14})C) Glucose</td>
<td>3.9 ± 0.8 (7)</td>
<td>N.S.</td>
<td>5.5 ± 1.1 (11)</td>
<td>N.S.</td>
</tr>
<tr>
<td>p ((^{14})C) Lactate</td>
<td>14.7 ± 2.1 (8)</td>
<td>N.S.</td>
<td>7.4 ± 0.6 (9)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Disposal of (1-(^{14})C) glucose Uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p ((^{14})C) Lactate</td>
<td>1.51 ± 0.19 (6)</td>
<td>N.S.</td>
<td>1.37 ± 0.13 (7)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
starved as in fed animals. These data suggest that the number of cells in the liver of the thyroidea
tomized rats is reduced, although the size of the hepa
toocyte is the same as in the controls and that fasting does not produce a change in
the total number of cells in neither group. The concentration of glycogen
is higher in the liver of the fed thyroidea
tomized rats than in those treated
with 1.5 μg of thyroxine and than in the intact controls (table II). This fact
is also different from that of male thyroidea
tomized animals where the liver glycogen concentration is the same as in the intact controls [2]. Fasting
produces a fall in the glycogen concentration of the liver from all the groups,
the differences disappearing among them (table II) which means that the
fall is maximal in the thyroidea
tomized animals as they started from a
higher level when fed.

Alanine and glucose utilization by liver slices. The uptake of (U-14C)
alanine by liver slices incubated in vitro is higher in the thyroidea
tomized rats than in their controls (table III). Fasting makes this parameter decrease
in all the groups, and the tissues from the thyroidea
tomized animals remain
taking up more 14C-labelled alanine than those from the other two groups.
The percentage of initial radioactivity converted to (14C) lactate is also aug
mented in the thyroidea
tomized animals when fed although after fasting,
this parameter falls more in these animals, the differences disappearing
among the groups (table III). The formation of (14C) glucose from (U-14C)
alanine is not different in the livers from thyroidea
tomized rats injected with either 0 or 1.5 μg of thyroxine and in the intact controls in either fed or
fasted state, suggesting a preservation of liver gluco
neogenesis in the former
group, even when food is withheld.

The uptake of (1-14C) glucose and the formation of 14C-labelled lactate
from this substrate is also augmented in the liver from fed thyroidea
tomized rats when compared with those receiving 1.5 μg of thyroxine and with those
of intact controls, the difference is disappearing when the animals are under
fasting 48 h before the sacrifice (table III).

Discussion

In the present study, we have seen that the growth rate is very much
decreased in thyroidea
tomized animals fed on a low iodine diet, and this is
accompanied by a parallel decrease in the liver weight and in the plasmatic
immunoreactive insulin concentration. These changes are primarily induced
by the decrease in the supply of thyroid hormones to the tissues as thyroidectomized rats fed on the same diet, but daily injected with substitutive doses of exogenous thyroxine do not show such alterations. Despite these changes that demonstrate an intense alteration in the whole endocrine system in the thyroidectomized rats, these animals are able to maintain normal levels of circulating glucose and ketone bodies and even to accumulate in their liver a greater percentage of glycogen than their controls. The liver should be playing a very important role in the maintenance of this balanced equilibrium of the carbohydrate metabolism in the hypothyroid animals. Actually, we have seen here that the hepatic metabolism of these animals is quite active, as shown by the increased uptake of labelled alanine and glucose by liver slices incubated in vitro and the increased conversion of these two substrates to $^{14}$C-labelled lactate. The conversion of (U-$^{14}$C) alanine to glucose by liver slices from thyroidectomized animals is the same as that in the intact controls which agrees with the normal steady-state concentration in the liver, from animals under similar conditions, of regulatory metabolites for gluconeogenesis, as previously reported [2]. The low concentration of circulating insulin might contribute to the maintenance of liver gluconeogenesis in the hypothyroid animals. The preferential conversion of alanine and glucose to lactate in the thyroidectomized animals agrees with the elevated lactate/pyruvate ratio found in the liver of hypothyroid rats [1] and would suggest a reduced cytoplasmic potential that would facilitate the reduction of 1,3-diphosphoglycerate for its conversion to glucose [23]. It might be inferred that the whole liver gluconeogenic capacity in these rats is lower in the thyroidectomized rats than in the controls due to the smaller livers of the former group, but this difference disappears when it is taken into account of the smaller body weight of these animals.

After 48 h of starvation, the peripheral lipids are mobilized in the thyroidectomized animals at least as much as in the controls, as suggested by previous findings [2] and by the normal rise of blood ketone bodies found here. Despite this lipid availability, the glycaemia is no longer preserved indicating that the utilization of glucose is greater than its synthesis. We have seen, however, that the uptake of (U-$^{14}$C) alanine is higher, and its conversion to ($^{14}$C) glucose and ($^{14}$C) lactate is the same in the fasted thyroidectomized rats than in their controls, and the uptake of (1-$^{14}$C) glucose and its conversion to ($^{14}$C) lactate do not differ between both groups. The preservation of liver gluconeogenesis in the fasted thyroidectomized animals would agree with the increased production of urea by the perfused liver of rats under comparable conditions [18] and with the normal liver steady-state
concentration of regulatory metabolites for gluconeogenesis in hypothyroid animals [2]. It has been shown, however, that the activity of key gluconeogenic enzymes is reduced in the liver of the fasted hypothyroid animals [3, 7, 18, 24], but they seem to be compensated by a parallel reduction in the activity of some glycolytic enzymes [3, 7, 24].

Although, as pointed out above, the primary defect of the thyroidectomized animals is the reduction in the supply of thyroid hormones to the peripheral tissues, we must emphasize that most of the metabolic changes observed in these animals are probably secondary to the other endocrine alterations that accompany this situation of extreme hypothyroidism. In agreement with this point are our previous results showing that when the thyroid hormone deficiency is not high enough to alter the growth rate and the fed and fasted plasma insulin levels, as indexes of an unimpaired endocrine system, none of the changes here observed in the thyroidectomized animals are present [8].

References