Early changes in thyroid hormone metabolism in the heart, liver, and brown adipose tissue during the induction of low T₃ syndrome in streptozotocin-diabetic rats

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Abstract. In order to elucidate the day-by-day development of low T₃ syndrome, we made rats diabetic by an injection of streptozotocin. Untreated controls killed at day 0 and rats treated for 8 days with insulin after they had received streptozotocin served as controls. Subgroups of animals were killed 1, 2, 3, 4 and 8 days after streptozotocin. In serum, heart and liver, T₃ was depressed to less than 50% of controls at day 4, whereas the insulin-treated rats differed from controls only as to heart T₃. Heart iodothyronine 5'-deiodinase activity was depressed to a minimum at day 3 and depression was not prevented by insulin. Liver iodothyronine 5'-deiodinase activity had not reached a minimum at day 8, and again, insulin treatment did not normalize this parameter. T₃ contents and iodothyronine 5'-deiodinase activity in brown adipose tissue did not differ from controls at any point of time. Thus, in the rats with low T₃ syndrome induced by streptozotocin-diabetes, a lowered iodothyronine 5'-deiodinase activity is not fully inhibited by insulin treatment, whereas the T₃ content in the liver is re-established during an observation period of 8 days. A direct toxic effect of streptozotocin seems unlikely as an in vitro study showed no influence of streptozotocin on iodothyronine 5'-deiodinase activity in the liver. The study thus indicates that iodothyronine 5'-deiodinase activity in the heart and liver is depressed in experimental diabetes, despite near optimal regulation of blood glucose, and we suggest that lowered intracellular T₃ production could, after some time, result in a hypothyroid state in different tissues.

Uncontrolled diabetes as well as several other conditions with severe, non-thyroidal illness, alters the thyroid economy into the well-known state of low T₃ syndrome (1). The condition has been described in humans (1-4) as well as in various animal species (5,6) and is characterized by depressed serum levels of T₃, T₄ and TSH and increased levels of rT₃. This is due to a depressed outer ring deiodination of T₄ in peripheral tissues (4,5) as well as to a blunted TSH response in the pituitary (3,7). In addition, recent investigations indicate that there may exist a transmembraneous blockade of the transport of T₄ into peripheral cells (4).

The present study was designed to elucidate the day-by-day changes in T₃ in various tissues of streptozotocin-diabetic rats. Local contents of T₃ were compared with the changes in iodothyronine-5'-deiodinase activity (5'-D), and the role of insulin treatment in re-establishing thyroid homeostasis was evaluated.

Material and Methods
Male Wistar rats were purchased from Møllegaard's breeding laboratory (Eiby, Denmark), weight 210-225 g, and made diabetic by a single iv injection of streptozotocin, 55 mg/kg, pH 4.0 (Upjohn Co, Kalamazoo, MI). Tap water and normal lab chow (I⁻content 0.9 mg/kg) were provided ad libitum. Blood glucose was measured daily (Haemo-gluco test 1-44 and Refloulux II, Boehringer-Mannheim, FRG) and urine tested daily for glucose and ketone bodies with Neostix-4 (Ames, Stoke Pages, Slough, UK). The animals were weighed daily. Serum was col-
lected at day zero and at the time of sacrifice. Subgroups of rats were killed 0 (control, N=6), 1 (N=6), 2 (N=6), 3 (N=6), 4 (N=8), and 8 (N=6) days after the injection of streptozotocin. A matched group of diabetic animals (N=12) was treated for 8 days with a daily (08.00 h) injection of a long-acting heat-treated non-commercial ultralente insulin (Novo, Bagsværd, DK). Sacrifice and tissue sampling was performed between 08.00 and 09.00 h to prevent diurnal variation, shortly after an ip injection of sodium barbital (25 mg/kg). The heart, liver, and brown adipose tissue were collected and immediately frozen in liquid nitrogen. Tissues were stored at −70°C and assayed within 10 days.

**Preparation of tissue homogenates**
Tissue, 0.5 g, was washed twice in buffer and homogenized in 5 volumes (v/w) of ice-cold buffer (25 mmol/l TRIS-HCl, 5 mmol/l dithiothreitol, 1 mmol/l EDTA, pH 7.2) for 5 sec with Ultraturrax (T25, 24,000 rpm). Homogenate was centrifuged for 5 min at 4500 x g, 4°C (Sigma 3K1). After removing the supernatant, the pellet was resuspended in another 5 volumes of buffer and centrifugation repeated. The two supernatants were pooled and used for determining 5'-D and T3, respectively.

**Assays**
T3 and T4 in serum and T3 in tissues were measured in triplicate on undiluted serum and tissue homogenates by radioimmunoassay (8), with dog serum as standard.

5'-D was determined according to an earlier described method (9). In brief, 50 μl homogenate was incubated with 100 μl substrate [125I]reverse T3 (specific activity 5500 cpm per 3 pg, 70% counting efficiency); 100 μl incubate was applied to 1 ml Sephadex columns (G-25 Fine, Pharmacia). 125I derived by deiodinating activity was eluted by 0.5 + 1.5 ml 50 mmol/l phosphate buffer (pH 7.5). The last 1.5 ml eluate was collected and counted for 5 min (LKB-Wallac 1277 Gammamaster). The liberated 125I was taken as an index of relative changes in 5'-D. All experiments were carried out in triplicate.

Protein content was estimated by the Bio-Rad kit, with bovine serum albumin as standard.

Statistical analyses were performed using Student's two-tailed t-test. In case of variance inhomogeneity, the Mann-Whitney test was used. Comparisons are made between control values obtained at day 0 and at the time of sacrifice unless otherwise noted. Data are presented as mean ± SEM. Level of significance, p<0.05.

**Results**
All diabetic animals had a blood glucose exceeding 18.0 mmol/l at day 1; a mean level of 22.5 ± 1.5 mmol/l was maintained in the untreated group, whereas it was reduced to near-normal levels in the insulin-treated group (Table 1). None of the animals had ketonuria. Non-insulin-treated rats had a mean decrease of 26% in body weight after 8 days of diabetes, whereas the insulin-treated rats did not lose weight.

Serum levels of T3 and T4 are depicted in Fig. 1. In untreated diabetic rats, serum T3 and T4 declined steadily from control values until day 4 after streptozotocin, then apparently stabilizing at a

| Table 1. Day-by-day blood glucose in insulin-treated streptozotocin-diabetic rats. |
|------------------|------------------|
|                  | Blood glucose    |
| Day 0            | 4.9±0.3          |
| Streptozotocin   |                  |
| Day 1            | 20.5±1.8         |
| Day 2            | 2.1±0.2          |
| Day 3            | 4.0±0.9          |
| Day 4            | 6.8±1.4          |
| Day 5            | 5.2±1.3          |
| Day 6            | 3.1±0.8          |
| Day 7            | 3.8±0.8          |
| Day 8            | 4.2±1.1          |

Blood glucose levels before (Day 0) and after streptozotocin in rats (N=9) treated with a daily injection (08.00 h) of long-acting insulin for 8 days. Values are given in mmol/l. Mean ± SEM.

Serum concentrations of T4 and T3(nmol/l) in streptozotocin (Stz)-induced diabetes mellitus in rats. Day 0 represents concentrations before diabetes onset and serves as control values. Serum was collected at day 0 and at the day of sacrifice. Groups of non-insulin-treated rats (N=6-8) were killed on day 1, 2, 3, 4 and 8. Day 8 include an extra group of rats (N=12) treated with insulin from the day after streptozotocin was given (+), *: p<0.05. Mean ± SEM.

![Fig. 1.](image-url)}
The heart \( T_3 \) and \( 5'-D \) are depicted in Fig. 2. A reduction in \( T_3 \) was present from day 1 after streptozotocin (p<0.05), persisting at day 8 (p<0.01) in untreated but not in insulin-treated rats. The low content of \( T_3 \) stabilized after day 4 after streptozotocin, with a mean of 38% of control values. \( 5'-D \) in the heart of diabetic rats declined steadily until day 2 after streptozotocin (mean 33%, p<0.01). The insulin-treated group also differed from controls (p<0.01), although the enzyme activity was higher in the insulin-treated than in the non-insulin-treated (mean 46% and 29% of controls, respectively, p<0.01).

Liver \( T_3 \) in untreated diabetic rats showed the same tendency as was observed in the heart, with a steep initial decline (p<0.0005 at day 1), stabilizing at a minimum at day 4 after streptozotocin (mean 65% of controls, Fig. 3). Insulin-treated and controls did not differ as to liver \( T_3 \). On the other hand, \( 5'-D \) in the liver of untreated diabetic rats did not seem to have reached a minimum at the end of the observation period, showing a decrease (p<0.0005) from day 4 to day 8 after streptozotocin. Although \( 5'-D \) was lower in the untreated than in the insulin-treated rats (p<0.0001), the insulin-treated also differed from controls (mean 14% and 35%, respectively, p<0.01).

Brown adipose tissue, \( T_3 \) and \( 5'-D \) are depicted in Fig. 4. In contrast to the other tissues, no significant changes in either \( T_3 \) content or \( 5'-D \) was observed, although there seemed to be a tendency towards increased \( 5'-D \) in the non-insulin-treated rats at day 8.

**In vitro experiment with streptozotocin**

To evaluate the possible direct toxic effect of streptozotocin on \( 5'-D \), samples of liver from 3 control rats were minced into small pieces and incubated for up to 22 h at 25°C in buffer (25 mmol/l TRIS-HCl, 3 mmol/l dithiothreitol, 1 mmol/l EDTA, minimum at day 4 (mean 43% and 47%, respectively), (p<0.01 day 1 after streptozotocin). The rats treated with insulin for 8 days did not differ from the control animals in this respect.
Liver pieces from normal control rats were minced and incubated with streptozotocin (65 mg streptozotocin/g tissue in 1 ml buffer) or in buffer alone at room temperature for 1 to 22 h. 5'-deiodinase activity is expressed in percent of obtained control values. Each value represents triplicate experiments from 3 different rats. Mean ± SEM.

pH 7.2). Streptozotocin was added in parallel tubes (65 mg/kg wet weight tissue in 1 ml buffer) and tissue samples in triplicate were collected 1, 2, 4, 6 and 22 h later. No difference in 5'-D between controls and the tissues incubated with streptozotocin was observed during the 22 h (Table 2).

### Table 2.
In vitro experiment with streptozotocin.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Control</th>
<th>Streptozotocin</th>
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<tbody>
<tr>
<td>1</td>
<td>100±3</td>
<td>99±6</td>
</tr>
<tr>
<td>2</td>
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<td>100±3</td>
<td>113±2</td>
</tr>
<tr>
<td>6</td>
<td>100±9</td>
<td>108±14</td>
</tr>
<tr>
<td>22</td>
<td>100±3</td>
<td>100±8</td>
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Discussion

In order to create a state of low T₃ with a definite time of onset, we used the streptozotocin diabetic rat model. The present study thus describes the time scale for early changes in serum and tissue thyroid hormone metabolism reflected by serum T₃ and T₄ contents as well as by T₃ content and 5'-D in heart, liver and brown adipose tissue.

Four days after the injection of streptozotocin, serum and tissue levels of thyroid hormones had fallen to a minimum. While heart 5'-D was lowest at day 3, the activity of this enzyme in the liver persistently declined during the observation period. Insulin treatment leading to near-normalization of blood glucose could prevent changes in serum, liver and brown adipose tissue T₃ (but not in heart T₃), whereas a depression of 5'-D in heart and liver was not inhibited. The possibility that streptozotocin in itself could exert a toxic effect on the deiodinase enzyme was examined by an in vitro study. This showed that incubation with streptozotocin for up to 22 h did not result in any depression of the enzyme activity. This does not, however, totally exclude that there may be some toxic effect after in vivo treatment.

The mechanism behind the low T₃ syndrome may be related to changes in the hypothalamic-pituitary axis (3,7) as well as to a depression of the peripheral deiodination of T₄ (4). Whether depression of the 5'-D in diabetes in itself causes cellular dysfunction or sooner should be considered a nonspecific reaction to non-thyroidal illness, is a matter of debate (2,4,10). Thyroxine metabolism in the liver from streptozotocin-diabetic rats has earlier been studied in tissue slices by Balsam & Ingbar (11). They suggested that the metabolism of glucose is somehow related to the T₃ production of this organ. In vivo insulin treatment for 3 days could normalize the [¹²³]I-T₃ production from [¹²³]I-T₄, whereas in vitro addition of either insulin or glucose did not have this effect. The reason for the lack of normalization by insulin treatment in the present report might be a more sensitive assay for the 5'-D. Another possibility is that an 8-day treatment period could allow short periods of higher blood glucose temporarily depressing the enzyme activity. Measurement of serum T₃ has in fact been suggested as a reliable index of metabolic control in diabetes (3). According to the present results, 5'-D seems to be a parameter even more sensitive to changes in glucose homeostasis.

In contrast to the liver and heart, which mainly contain 5'-D Type I (propylthiouracil sensitive), (12), brown adipose tissue 5'-D in the rat is composed of mainly 5'-D type II, responding to stimuli such as catecholamines, feeding, high blood glucose, exposure to cold, and other forms of stress (13,14). We found no significant changes in the tissue content of T₃ or 5'-D following diabetes induction or insulin treatment of the diabetes. On the other hand, there seems to be a tendency towards higher 5'-D activity in the non-insulin-treated rats at day 8. It might be expected that a statistical significant difference would appear after some time, and that 8 days of untreated diabetes is too short a time for attaining steady state in this specific tissue. It should be noted that in this study we did not distinguish between 5'-D Type I and II. To our knowledge, there are no other reports on brown adipose tissue thyroid hormone homeostasis in experimental diabetes, and it would be of interest to examine changes in the tissue after in vitro treatment with propylthiouracil.

Undersupply of T₃ to tissues do not necessarily create a state of intracellular hypothyroidism, as stated by Ladenson et al. (15). They found that hypothyroidism in rats resulted in an upregulation of nuclear T₃ receptors in the myocardium. On the
other hand, Bernal et al. (16), found no difference in the T₃ binding capacity in the liver of euthyroid and hypothyroid rats. Whether the observed changes represent different tissue response to lowered T₃ is unclear. It is possible that an upregulation of T₃ nuclear receptors may keep the animal tissue euthyroid, at least for some period, despite lowered 5'-D. The long-term consequences of a lowered 5'-D on thyroid homeostasis in different tissues must await further studies. The present experiment shows, however, that a persistingly depressed tissue 5'-D may result in lowered tissue T₃.

Estimation of early changes in tissue 5'-D thus contributes to the understanding of thyroid hormone metabolism at the cellular level during induction of streptozotocin diabetes in rats. Day-by-day analysis of this enzyme shows an early and sensitive depression that does not seem to be totally prevented by insulin treatment.

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References

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