Alterations in hepatic nuclear binding of triiodothyronine in experimental diabetes mellitus in rats

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Abstract. The interrelationship between hepatic nuclear T₃ receptors and glucose metabolism was studied in 8 diabetic rats and 8 paired control animals. Serum glucose (mean ± SEM, normal vs. diabetic, 167 ± 11 vs. 470 ± 41 mg/100 ml, P < 0.001) and plasma glucagon (183 ± 8.5 vs. 370 ± 29 pg/ml, P < 0.001) were higher in diabetic animals than in controls; serum insulin was lower but not significantly (59 ± 19 vs. 24 ± 12 µU/ml). Serum T4 (4.1 ± 0.53 vs. 0.8 ± 0.27 µg/100 ml, P < 0.005) and T₃ (77.3 ± 3.2 vs. 41.7 ± 12.1 ng/100 ml, P < 0.05) were lower in diabetic rats than in controls. Hepatic concentration of non-protein sulfhydryl-groups was also moderately (~19%) lower in diabetic rats than in controls (4.62 ± 0.11 vs. 3.75 ± 0.24 µmol/g, P < 0.02).

The maximal binding capacity (MBC) of the binding of [¹²³T]T₃ to isolated rat liver nuclei was significantly decreased in the diabetic rats (368 ± 37 vs. 232 ± 36 fmol/mg DNA, P < 0.01; mean decrease 38%); the affinity constant Kₐ was also lower but not significantly (1.60 ± 0.13 vs. 1.25 ± 0.15 10⁻⁹ L/M, 0.05 < P < 0.1). Addition of 1 mM dithiothreitol (DTT) enhanced the Kₐ of nuclear binding of T₃ similarly in the controls and the diabetics. However, it did not restore the decreased MBC of nuclear binding of T₃ in diabetic rats to the level of the controls.

The decrease in MBC of hepatic nuclei for T₃ did not correlate (P < 0.05) with the decrease in serum T₃, serum T₄, or hepatic non-protein sulfhydryl-groups. There was, however, a significant negative correlation of MBC of T₃ with serum glucose and with plasma glucagon. These data suggest a regulatory role of glucose metabolism (or glucagon) in modulation of binding of T₃ by hepatic nuclear T₃ receptors.

Diabetes mellitus is associated with several changes in thyroid hormone physiology. Although the serum thyrotrophin (TSH) level is generally normal in diabetic patients, a blunted TSH response to thyrotrophin-releasing hormone (TRH) has been observed (Naeye et al. 1978; Pittman et al. 1979a). The extrathyroidal deiodination of thyroxine (T₄) is consistently impaired. The ‘low T₃, high reverse T₃ (rT₃)’ syndrome of non-thyroid illness has been documented in several studies of diabetic patients (Naeye et al. 1978; Saunders et al. 1978). Its occurrence has been explained on the basis of an impaired production of triiodothyronine (T₃) from metabolism of T₄ and a decreased metabolic clearance rate of rT₃, both apparently due to a reduction in the activity of iodothyronine 5'-monodeiodinase (Pittman et al. 1979b). Studies of experimental diabetes mellitus have demonstrated both a decrease in 5'-deiodination (Balsam et al. 1978) and 5-deiodination of iodothyronines (Chopra et al. 1981). In view of these changes in thyroid physiology, it was of interest to examine whether or not there is a change in the binding of T₃ by nuclear receptors in the tissues in the diabetic syndrome. We have also investigated the influence of possible changes in tissue sulfhydryl (SH)-groups on the nuclear binding of T₃ in diabetes mellitus. This was done because previous studies have shown a marked enhancement in the binding of T₃ to

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nuclear receptors in the presence of dithiothreitol (DTT), a potent donor of SH groups (DeGroot & Torresani 1975). Additionally, since some studies (Dillmann et al. 1978a) have documented a reduction in hepatic nuclear T₃ binding capacity following administration of glucagon to the rat, we have evaluated the possibility of a relationship between nuclear T₃ receptors on the one hand and circulating glucagon, insulin or glucose, on the other.

Materials and Methods

Eight pairs of male Sprague-Dawley rats, matched for age and weight, were individually caged and maintained on Purina Rat Chow and water ad libitum. One rat from each pair was rendered diabetic by injecting 65 mg/kg streptozotocin into the tail vein (streptozotocin, a gift of the Upjohn Company, Kalamazoo, Michigan, was dissolved in 0.1 M sodium citrate buffer, pH 4.5) (Junod et al. 1969); the other rat was similarly injected with an equivalent volume of the buffer solution alone. After 7 days the 2 rats were killed between 8 and 10 a.m. (without any prior period of fasting) by cervical dislocation, and blood was collected from the trunk. The liver was removed and weighed. A portion of the liver was saved for measurement of the tissue concentration of non-protein sulphydryl (NP-SH) groups according to the method of Sedlak & Lindsay (1968). The remaining liver tissue was used for the isolation of liver nuclei as described by Spindler et al. (1975).

The liver was put into ice-cold solution A (20 mM Tricine, 0.25 M sucrose, 2 mM CaCl₂, 1 mM MgCl₂ and 5% glycerol (v/v), pH 7.6), finely minced with a pair of scissors, and thereafter homogenized in 4 volumes (w/v) of solution A in a Potter-Elvehjem type 'Wheaton' tube by a motor-driven Teflon pestle (grinding chamber clearance 0.004"-0.006") in 12-15 slow up and down strokes, separated from each other by 30 s of cooling; the whole procedure was carried out at 0-4°C. The homogenate was filtered through two, and subsequently through four layers of gauze, centrifuged for 7 min at 1000 × g, and the supernatant was discarded. The pellet was resuspended in 5 volumes (w/v) of solution A containing 0.5% Triton X-100, and centrifuged for 7 min at 800 × g; this was repeated twice. Finally, the pellet was suspended in 2 volumes (w/v) of solution B (20 mM TRIS, 0.25 M sucrose, 1 M MgCl₂ and 5% glycerol (v/v), pH 7.6). Smears of this nuclear suspension, stained with 0.1% crystal violet, showed under the light microscope no whole cells, and abundance of intact nuclei with clearly visible nucleoli, and a minimal amount of cell debris. The DNA concentration of the nuclear suspensions was determined according to the method of Webb & Levy (1955), and protein measurements were done by the Bio-Rad assay (Bradford 1976); the mean ratio of protein to DNA was 3.7 ± 0.27.

Binding of radioactive T₃ to the isolated rat liver nuclei was studied by the addition of approximately 15 pg of [¹²⁵I]T₃ (specific activity ~ 550 µCi/µg) to 0.2 ml of the nuclear suspension (representing approximately 100 mg of starting tissue). The final concentrations of various agents in the incubation mixtures (total volume 0.4 ml) were 20 mM TRIS, 0.25 M sucrose, 1 mM MgCl₂, 2 mM EDTA, 50 mM NaCl and 5% glycerol (v/v) at a pH 7.6. After incubating for 1 h in a shaking waterbath at 22°C, the tubes were chilled in ice-cold water. Two ml of solution B containing 0.5% Triton X-100 were then added to each tube and tubes were mixed and centrifuged at 4°C for 15 min at 1250 g. The supernatant was decanted, and the radioactivity in the pellet (the hormonal fraction bound to the nuclei) was counted in a gamma-scintillation counter. Specific binding was calculated by the difference between the counts bound to the nuclei in the absence and the presence of an excess of non-radioactive T₃ (10⁻⁸ M). The non-specific binding averaged 4.60 ± 0.18% of total radioactivity.

Scatchard plots (Scatchard 1949) were prepared from data on bound and free T₃ obtained in the absence and in the presence of increasing quantities of non-radioactive T₃ (ranging from 1.0 to 33 × 10⁻¹₀ M). Similar studies were also conducted after adding 1 mM DTT to the incubation mixtures. Each pair of rats (the control animal and its diabetic counterpart) were studied simultaneously. All measurements were done in quadruplicate.

The effect of thiol groups on the binding of [¹²⁵I]T₃ to rat liver nuclei was evaluated in separate experiments by the addition of several SH-reagents in various concentrations to the incubation mixtures. The results were expressed as per cent change from the specific binding of [¹²⁵I]T₃ observed in incubation mixtures in which no SH-reagent was added.

Serum glucose was measured with the Beckman glucose analyzer 2 (Kadish et al. 1968), serum insulin (Soeldner & Stone 1965) and plasma glucagon (Unger et al. 1970) by radioimmunoassay (the glucaon assays using the 31K antibody were kindly performed by Dr. Seymour Levin, V.A. Hospital Wadsworth Medical Center, Los Angeles, California). Thyroid function was evaluated by measurement of the concentration of serum T₄ (Chopra 1972) and serum T₃ (Chopra et al. 1972).

The results in the control rats and in the diabetic rats were evaluated statistically by Student's two-tailed paired t-test; the level of significance was taken as α = 0.05. The results are presented as the mean ± SEM, unless stated otherwise.

Results

The streptozotocin-treated rats gained less weight during the study period than the control animals,
Table 1.
Effect of streptozotocin treatment (65 mg/kg iv) on various parameters of glucose metabolism and thyroid function, and on liver non-protein SH-groups in 8 pairs of rats (values as mean ± SEM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Buffer (group A, controls, n = 8)</th>
<th>Streptozotocin (group B, diabetic, n = 8)</th>
<th>P-value (A vs. B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>186 ± 21</td>
<td>193 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>63.4 ± 11.7</td>
<td>9.5 ± 11.4</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Liver wet weight (g)</td>
<td>10.1 ± 0.4</td>
<td>8.2 ± 0.7</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Serum glucose (mg/100 ml)</td>
<td>167 ± 11</td>
<td>470 ± 41</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serum insulin (μU/ml)*</td>
<td>57 ± 19</td>
<td>24 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma glucagon (pg/ml)**</td>
<td>183 ± 8.5</td>
<td>370 ± 29</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serum T₄ (μg/100 ml)</td>
<td>4.1 ± 0.53</td>
<td>0.80 ± 0.27</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Serum T₃ (ng/100 ml)***</td>
<td>77.3 ± 3.2</td>
<td>41.7 ± 12.1</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Liver SH groups (μmol/g)

<table>
<thead>
<tr>
<th></th>
<th>Buffer (group A, controls, n = 8)</th>
<th>Streptozotocin (group B, diabetic, n = 8)</th>
<th>P-value (A vs. B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>18.8 ± 1.2</td>
<td>20.5 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Protein bound</td>
<td>14.2 ± 1.2</td>
<td>16.7 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Non-protein bound</td>
<td>4.62 ± 0.11</td>
<td>3.75 ± 0.24</td>
<td>P &lt; 0.02</td>
</tr>
</tbody>
</table>

* 7 pairs; ** 5 pairs; *** 7 pairs.

although the initial body weight in the two groups was similar (Table 1). Streptozotocin treatment was further associated with an increased consumption of water and food, and with markedly elevated serum glucose concentrations. The post-prandial serum insulin levels were lower in diabetic animals than in controls in 6 out of 7 instances so studied; the mean insulin level was lower in diabetics than in controls but the difference was not significant statistically (Table 1). The mean serum glucagon level was, however, clearly higher in diabetics than in controls. Serum T₄ and T₃ concentrations were lower in diabetic animals than in controls. The concentration of non-protein SH-groups in the liver of diabetic rats was also decreased compared to control rats (Table 1). The concentration of total SH-groups and protein-bound SH-groups in the liver were, however, unaltered in diabetics. The concentration of DNA in the suspension of liver nuclei was also similar in control and diabetic rats (558 ± 50 vs. 554 ± 82 μg DNA/ml, NS) as was the protein content in the two groups (1.88 ± 0.018 vs. 1.91 ± 0.035 ng/ml, NS).

The Scatchard plots of the binding of [¹²⁵I]T₃ to the isolated rat liver nuclei are depicted in Fig. 1.

The maximal T₃ binding capacity (MBC) of the nuclear receptors was lower in the diabetic group than in controls; this was the case whether the data were expressed as moles T₃ bound/liter of incubation mixture (0.97 ± 0.06 vs. 0.59 ± 0.08 10⁻¹⁰ M/L, P < 0.005) or as moles T₃ bound/mg DNA (368 ± 37 vs. 292 ± 36 fmol T₃/mg DNA, P < 0.01). The affinity constant (Kᵦ) of the [¹²⁵I]T₃ binding to its nuclear receptors in diabetic animals was slightly but not significantly lower than that in controls (1.60 ± 0.13 vs. 1.25 ± 0.13 10⁹ L/M, NS); it is possible that a study of more animals may show a significant reduction in affinity constant of the binding in diabetic animals. Addition of 1 mM DTT to the incubation mixture greatly enhanced the Kᵦ of T₃ binding to nuclei in both diabetics and controls. The absolute and relative increase in Kᵦ was similar in the two groups (Δabs. 0.63 ± 0.15 vs. 0.54 ± 0.16, NS; Δrel. 37 ± 8 vs. 49 ± 15%, NS). Addition of DTT did not restore the decreased MBC of T₃ in diabetic rats to the level in controls (Fig. 2). The data on relative influence of various SH reagents on nuclear binding of T₃ are shown in Fig. 3. Consistently, SH-reducing agents increased the binding of T₃ and SH-oxidizing or binding
agents decreased the binding of T₃. Clearly, DTT is the most potent agent in stimulating binding of T₃ to nuclei.

The MBC of T₃ in diabetic and control rats did not correlate significantly with the concentration of liver non-protein SH-groups ($r = 0.45$, NS), serum T₃ ($r = 0.30$, NS), or serum insulin ($r = 0.22$, NS); its correlation with serum T₄ was of borderline significance ($r = 0.51$, $P = 0.05$). A statistically significant negative correlation was found between

### Fig. 1.

Scatchard plots of the binding of $[^{125}I]T_3$ to isolated rat liver nuclei of 8 diabetic rats and 8 paired control animals, in the absence (closed circles) and in the presence (open circles) of 1 mM DTT. Each point represents the mean of 8 animals.

### Fig. 2.

Relative changes in maximal binding capacity (MBC) and affinity constant (Ka) of the binding of $[^{125}I]T_3$ to isolated rat liver nuclei of 8 diabetic rats (closed bars) and 8 paired control animals (open bars). Data are presented as mean ± SEM.
the MBC of T₃ and the serum glucose concentration \( y = -410x + 639, \ r = -0.65, P < 0.01 \) and between the MBC of T₃ and plasma glucagon concentration \( y = -339x + 533, \ r = -0.92, P < 0.01, \) Fig. 4).

Discussion

The present study describes a clear reduction in hepatic nuclear binding of T₃ in the diabetic rat. This observation and that of reduced serum thyroid hormone levels in the diabetic rat are reminiscent of those in the fasting rat (Burman et al. 1977; DeGroot et al. 1977; Schlusser & Orlando 1978). This analogy suggests that reduction in nuclear binding of T₃ in diabetes mellitus may result from a state of intracellular starvation.

Since thiol groups are so important to the binding of T₃ by hepatic nuclear receptors (Fig. 3 and DeGroot & Torresani 1975), we examined the importance of alterations in hepatic concentration of NP-SH groups in reducing nuclear binding of T₃. Although we did observe a modest reduction in hepatic concentration of NP-SH groups in the diabetic rat, this did not appear to be the major factor responsible for reduced nuclear binding of T₃. Thus, addition of a potent SH-group donor, DTT, to nuclear suspensions from diabetic rats did

![Graph](https://via.placeholder.com/150)

Fig. 3.
The effect of sulphydryl reagents on the binding of \[^{125}I\]T₃ to isolated rat liver nuclei. Results are plotted as percent change from baseline levels vs. log-dose concentration of the test agent. DTT: dithiothreitol; MCE: 2-mercapto-ethanol; GSH: reduced glutathione; N-EM: N-ethyl-maleimide; GSSG: oxidized glutathione; DIA: diamide; HgCl₂: mercuric chloride.

![Graph](https://via.placeholder.com/150)

Fig. 4.
The relation between plasma glucagon concentration and maximal binding capacity (MBC) of \[^{125}I\]T₃ binding to isolated rat liver nuclei (in the absence of DTT) of 5 diabetic rats (●) and 5 paired control animals (×) \( y = -339x + 533; \ r = -0.92, P < 0.001 \).
not restore the decreased MBC for T\textsubscript{3} to normal. Additionally we were unable to detect a significant correlation between hepatic concentration of NP-SH groups and nuclear binding of T\textsubscript{3}.

Several studies have documented a reduction in hepatic monodeiodination of iodothyronines in experimental diabetes mellitus (Balsam et al. 1978; Chopra et al. 1981) and it, too, is not normalized by in vitro addition of sulfhydryl donors. Other studies have suggested an important role of reduced circulating thyroid hormone levels in sustaining a reduction in hepatic deiodination of iodothyronines in experimental diabetes mellitus (Chopra et al. 1981). By analogy, one must consider a role of reduced circulating thyroid hormone levels in the observed reduction in nuclear binding of T\textsubscript{3}. However, previous studies have not indicated a reduction in hepatic nuclear binding of T\textsubscript{3} in hypothyroidism (Oppenheimer et al. 1975; Spindler et al. 1975; DeGroot et al. 1976; Bernal et al. 1978). It appears, therefore, that other, non-thyroidal factors may be of major importance in effecting a reduction in nuclear binding of T\textsubscript{3} in diabetes mellitus.

Dillmann et al. (1978a) and Dillmann & Oppenheimer (1979) have reported that glucagon infusion in euthyroid rats is associated with a decrease in MBC of T\textsubscript{3}. Decreased nuclear T\textsubscript{3} binding capacity has also been found after partial hepatectomy (Dillmann et al. 1978b), which is associated with hyperglucagonaemia. Similarly, fasting is associated with diminished nuclear T\textsubscript{3} receptors in liver (vide supra) and increased glucagon levels in the circulation. We found a striking negative correlation between plasma glucagon and MBC of T\textsubscript{3} binding by hepatic nuclei (Fig. 4). These various considerations suggest an important influence of circulating glucagon on abnormalities in nuclear binding of T\textsubscript{3} in experimental diabetes mellitus.

However, nuclear binding of T\textsubscript{3} also correlated significantly with the serum glucose concentration, though not as well as with serum glucagon concentration. This observation makes us wonder whether an alteration in glucose metabolism per se, an associated metabolic disturbance, e.g. hyperglucagonaemia, or general ill-health due to a systemic disease or a combination of these factors is responsible for the reduction of receptor binding of T\textsubscript{3} in diabetes mellitus. The issue can be resolved only after study of disease states that do not manifest hyperglucagonaemia.

The multiplicity of changes in thyroid hormone physiology in diabetes mellitus may have significance. The combination of (i) inhibition of extra-thyroidal conversion of T\textsubscript{4} to T\textsubscript{3} (ii) reduction in nuclear T\textsubscript{3} receptor binding capacity, and (iii) putative mitigation of some thyroid hormone effects at the post-receptor level independent of receptor occupancy (Ruegamer et al. 1965; Dillmann & Oppenheimer 1979) suggests a concerted natural attempt to minimize the effects of T\textsubscript{3} on the tissues. This may signify a process of adaptation postulated previously (Chopra et al. 1978; Gardner et al. 1979) to operate when catabolism is overactive or life-threatening and anabolism is of survival value to the organism.

References


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