Nuclear Thyroxine and Triiodothyronine Receptors in Human Mononuclear Cells in Diabetes Mellitus

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Summary. The number of nuclear thyroxine (T_4) or triiodothyronine (T_3) receptors and the serum values of thyroxine, triiodothyronine, reverse triiodothyronine and TSH were investigated in 13 patients with Type 1 (insulin-dependent) diabetes (group 1), in 10 patients with Type 2 (non-insulin-dependent) diabetes (group 2) and in age and weight matched non-diabetic subjects. All patients were clinically euthyroid, although serum T_3 was low and reverse T_3 high in group 1 compared with the non-diabetic subjects. The Type 1 diabetic patients had evidence of poor metabolic control because of weight loss and high glycosylated haemoglobin levels. The maximal specific nuclear binding capacities for T_4 (1.8×10^{-16} mol T_4 /10 μ g DNA) and T_3 (1.4×10^{-16} mol T_3 /10 μ g DNA) were increased in group 1 compared with the normal subjects (T_4 : 1.1×10^{-16} mol T_4 /10 μ g DNA, $p < 0.010$, T_3 : 0.9×10^{-16} mol T_3 /10 μ g DNA, $p < 0.05$), whereas the nu-

clear binding affinity for T_4 ($K_a = 1.5 \times 10^9$ l/mol) and T_3 (5.1×10^9 l/mol) was similar to that of the normal subjects (T_4 , $K_a = 2.0 \times 10^9$ l/mol; T_3 , $K_a = 5.6 \times 10^9$ l/mol). In contrast, neither the nuclear binding of T_4 (1.0×10^{-16} mol T_4 /10 μ g DNA) and T_3 (1.1×10^{-16} mol T_3 /10 μ g DNA) nor the binding affinity for T_4 ($K_a = 3.3 \times 10^9$ l/mol) and T_3 ($K_a = 3.9 \times 10^9$ l/mol) in group 2 differed from those of the non-diabetic subjects. In conclusion, nuclear T_4 and T_3 receptor number in cells from patients with poorly controlled Type 1 diabetes was raised compared with normal subjects. This increase may have been responsible for the euthyroidism in these patients in whom the serum T_3 was low. In contrast, cellular binding of T_4 and T_3 appeared normal in the Type 2 diabetic subjects.

Key words: T_4 receptors, T_3 receptors, Type 1 and Type 2 diabetes.

Various abnormalities of thyroid function have been described in diabetes mellitus, including the depression of total serum triiodothyronine (T_3) and thyroxine (T_4) concentrations in untreated patients [1]. However, decreased circulating concentrations of T_4 and T_3 do not necessarily reflect decreased hormone concentrations at the receptor site. As the magnitude of effect of thyroid hormones is directly correlated with the quantity of iodothyronine bound specifically to cell nuclei in various organs, we have determined the relationship between the concentrations of thyroid hormones in the blood and nuclear T_4 and T_3 receptor concentration in patients with diabetes mellitus.

Materials and Methods

Patients

Two groups of diabetic patients and two groups of non-diabetic subjects were studied. Group 1 comprised 13 patients with Type 1 diabetes, who were all judged to require insulin on clinical grounds. The duration of their diabetes ranged from 4 weeks to 10 years. At the time of investigation all patients were underweight and had elevated levels

of glycosylated haemoglobin, suggesting poor metabolic control. On the day of investigation and the two previous days, ketone bodies were present in the urine, although none of the patients was acidotic as judged by serum bicarbonate levels. Group 2 consisted of ten patients with Type 2 diabetes of duration from 1 to 10 years (Table 1). No patient had a history of thyroid disease. The control groups comprised eight subjects matched with the patients in group 1 and five subjects matched with the patients in group 2 with regard to weight and age (Table 1). All the control subjects were without any signs of diabetes or thyroid disease. Blood samples were drawn for the cellular experiments, total serum thyroxine (T_4), total serum triiodothyronine (T_3), T_3 uptake ratio, serum reverse triiodothyronine (rT_3), serum thyrotropin (TSH), serum thyroglobulin antibodies and blood glycosylated haemoglobin A_1 after informed consent. A TRH test was performed in ten patients with diabetes (five with Type 1 and five with Type 2) and 10 control subjects (five in each group).

Methods

Commercially available methods were used for the determinations of serum T_4 (Clinical Assays Gammacoat T_4 kit, Travenol Lab., Cambridge, UK), serum T_3 (Amerlex T_3 RIA kit, Amersham International, Amersham, UK), T_3 uptake (Clinical Assays Gammacoat T_3 uptake kit, Travenol Lab., Cambridge, UK), TSH (Normal Human Serum TSH RIA kit, Diagnostic Products Cooperation, Los Angeles, USA), reverse T_3 (Medilab, Copenhagen, Denmark), thyroglobulin antibod-

Table 1. Clinical and biochemical data from the subjects studied

	Age range (years)	Weight (kg) (% Ideal body weight) ^a	Glycosylated haemoglobin (%)	Free thyroxine index ^d	Serum triiodothyronine (nmol/l)	Serum reverse triiodothyronine (nmol/l)
Type 1 diabetes (n=13)	17–30	70.8 ± 6.2 (–9 ± 1%)	12.2 ± 2.5	95.8 ± 9.8	1.24 ± 0.33 ^b	0.57 ± 0.03 ^c
Control group 1 (n=13)	20–30	75.3 ± 2.7 (+1 ± 1%)	5.0 ± 1.0	103.0 ± 16.8	1.80 ± 0.30	0.43 ± 0.10
Type 2 diabetes (n=10)	50–72	87.3 ± 2.2 (+3 ± 1%)	8.1 ± 3.0	90.2 ± 19.8	1.34 ± 0.28	0.41 ± 0.04
Control group 2 (n=10)	52–80	85.5 ± 5.2 (+2 ± 2%)	5.4 ± 0.4	100.0 ± 15.3	1.30 ± 0.42	0.35 ± 0.05

Results expressed as mean ± SD. ^a Percentage over or underweight compared with ideal weight (Natvig 1956, Oslo) is shown. ^b $p < 0.05$; ^c $p < 0.010$ compared with the respective control groups; ^d Free thyroxine index = serum T₄ × T₃ uptake ratio

Fig. 1. Specific nuclear binding of ¹²⁵I-T₄ in nuclei from patients with Type 1 diabetes. Left panel: percentage ¹²⁵I-T₄ specific bound to nuclei from eight patients with Type 1 diabetes (x—x) and to nuclei from eight control subjects (●—●) with increasing concentrations of ¹²⁵I-T₄ (mean ± SD). Right panel: representative Scatchard plots of specific nuclear binding of ¹²⁵I-T₄ to nuclei from a patient with Type 1 diabetes (x—x) and to nuclei from a control subject (●—●).

Fig. 2. Specific nuclear binding of ¹²⁵I-T₃ in nuclei from patients with Type 1 diabetes. Left panel: percentage ¹²⁵I-T₃ specific bound to nuclei from five patients with Type 1 diabetes (x—x) and to nuclei from five control subjects (●—●) with increasing concentrations of ¹²⁵I-T₃ (mean ± SD). Right panel: representative Scatchard plots of specific nuclear binding of ¹²⁵I-T₃ to nuclei from a patient with Type 1 diabetes (x—x) and to nuclei from a control subject (●—●).

ies (Thymune-T, Wellcome, Beckenham, Kent, UK) and glycosylated haemoglobin A₁ (HgA₁ column test kit, Bio-Rad, Richmond, California, USA).

Isolation of Mononuclear Cells from Blood

Mononuclear cells were isolated from 100 ml of blood by a modification of the method of Böyum [2]. Blood was diluted with 0.9% NaCl and centrifuged at 800 *g* for 15 min in Lymphoprep (NycMed, Copenhagen, Denmark). The interphase was harvested and washed twice in Hanks solution. This yielded lymphocytes (80%–90%) and

monocytes (10%–20%) but no erythrocytes or platelets as judged by light microscopy. Judged by the trypan blue exclusion test, 98% cells were viable after isolation and incubation for 120 min. The number of cells was determined in a Bürker-Türk chamber (Scherf, Marburg, FRG).

Uptake of ¹²⁵I-T₄ or ¹²⁵I-T₃ in Mononuclear Cells

Mononuclear cells and ¹²⁵I-T₄ or ¹²⁵I-T₃ were added to tubes containing 1 ml phosphate buffered Hanks medium (pH 7.4). The number of cells in each tube was adjusted to 15 × 10⁶ cells in a final volume

Fig. 3. Specific nuclear binding of ¹²⁵I-T₄ in nuclei from patients with Type 2 diabetes. Left panel: percentage ¹²⁵I-T₄ specific bound to nuclei from five patients with Type 2 diabetes (○—○) and to nuclei from five control subjects (▲—▲) with increasing concentrations of ¹²⁵I-T₄ (mean ± SD). Right panel: representative Scatchard plots of specific nuclear binding of ¹²⁵I-T₄ to nuclei from a patient with Type 2 diabetes (○—○) and to nuclei from a control subject (▲—▲)

Fig. 4. Specific nuclear binding of ¹²⁵I-T₃ in nuclei from patients with Type 2 diabetes. Left panel: percentage ¹²⁵I-T₃ specific bound to nuclei from five patients with Type 2 diabetes (○—○) and to nuclei from five control subjects (▲—▲) with increasing concentrations of ¹²⁵I-T₃ (mean ± SD). Right panel: representative Scatchard plots of specific nuclear binding of ¹²⁵I-T₃ to nuclei from a patient with Type 2 diabetes (○—○) and to nuclei from a control subject (▲—▲)

Table 2. Increase of serum TSH after injection of TRH in the diabetic and non-diabetic subjects

	TSH (mU/l)		
	0 min	20 min	60 min
Group 1 (Type 1 diabetes, n = 5)	1.1 ± 0.5	17.9 ± 5.2 ^a	9.8 ± 2.6
Control group 1 (n = 5)	1.4 ± 0.3	8.6 ± 1.9	7.5 ± 2.7
Group 2 (Type 2 diabetes, n = 5)	1.1 ± 0.6	6.2 ± 2.1	5.3 ± 1.3
Control group 2 (n = 5)	1.0 ± 0.4	5.8 ± 3.9	5.0 ± 2.0

Results expressed as mean ± SD, ^a *p* < 0.10 compared with the non-diabetic group. Reference: serum TSH in euthyroid subjects < 3.0 mU/l

of 1 ml. The concentration of ¹²⁵I-T₄ or ¹²⁵I-T₃ varied from 1.5×10^{-10} mol/l to 5.0×10^{-9} mol/l. The specific activity of the preparation was 50 µCi/µg (Amersham International, Amersham, UK) and the preparation contained < 3% free ¹²⁵I and < 1% ¹²⁵I-T₃ in the ¹²⁵I-T₄ preparation and < 1% ¹²⁵I-T₂ in the ¹²⁵I-T₃ preparation. No other iodothyronines could be detected by column chromatography. The cells were incubated at 37 °C for 90 min. The incubation was terminated by cooling the tubes to 3 °C, as previous experiments had demonstrated that binding at 3 °C was negligible for up to 18 h.

Isolation of Nuclei

After incubation, the cells were washed five times in cold Hanks solution and resuspended in 5 ml of cold buffer (0.25 mol sucrose, 1.1 mmol MgCl₂ and 20 mol TRIS/l), pH 7.85, containing 0.5% triton x-100. This suspension was homogenized for 30 s in a Potter-Elvehjem homogenizer (Braun, Melsungen, FRG) to enhance the disruption of the cell membrane, and then centrifuged at 1000 g for 10 min. All procedures were carried out at 0–3 °C. Washing was repeated once with 5 ml buffer. This procedure yielded nuclei that under phase contrast microscopy appeared intact and clean. More than 90% of the cellular DNA was confined to the nuclear fraction. Finally, 1 ml 95% ethanol was added to extract the iodothyronines. The radioactivity in the nuclei and the supernatant was counted in a gamma scintillation counter (Selektrotronik, Copenhagen, Denmark). DNA was determined by the method of Burton [3].

Determination of Deiodination and Deiodination Products

Column chromatography was performed on acrylic columns (K 15/30, Pharmacia, Uppsala, Sweden) filled with Sephadex G25 medium (Pharmacia), equilibrated with phosphate buffer (0.02 mol/l) (pH 7.4) and NaOH (0.1 mol/l), NaCl (0.005 mol/l) at a rate of 32 ml/h. Eighteen fractions (5 ml/fraction) were eluted with phosphate buffer and 32 fractions (5 ml/fraction) were eluted with NaOH/NaCl. For reference, chromatography was carried out with ¹²⁵I, ¹²⁵I-T₃, ¹²⁵I-3,3', 5'-T₃ and ¹²⁵I-3,3'-T₂.

Table 3. Maximal specific nuclear binding capacities and equilibrium association constants (K_a) for nuclear binding of T₄ and T₃ in the diabetic and non-diabetic groups

		Type 1 diabetes (n=8)	Control group 1 (n=8)	Type 2 diabetes (n=5)	Control group 2 (n=5)
T ₄ binding	Maximal specific binding capacity (mol × 10 ⁻¹⁶ /10 µg DNA)	1.8 ± 0.4 ^a	1.1 ± 0.2	1.0 ± 0.1	1.1 ± 0.3
	K_a (X 10 ⁹ l/mol)	1.5 ± 0.2	2.0 ± 0.6	3.3 ± 1.0	2.9 ± 0.8
T ₃ binding	Maximal specific binding capacity (mol × 10 ⁻¹⁶ /10 µg DNA)	1.4 ± 0.3 ^b	0.9 ± 0.2	1.1 ± 0.3	1.2 ± 0.3
	K_a (X 10 ⁹ l/mol)	5.1 ± 2.5	5.6 ± 2.2	3.9 ± 1.2	4.3 ± 2.9

Results expressed as mean + SD, ^a $p < 0.010$, ^b $p < 0.05$ compared with the respective control groups

Fig. 5. Total cellular binding of T₄ and T₃ in cells from patients with diabetes. Percentage ¹²⁵I-T₄ (top panel) and ¹²⁵I-T₃ (bottom panel) bound to cells from patients with Type 1 diabetes (x—x, n=8) and Type 2 diabetes (○—○, n=5) and the respective control groups (●—●, n=8), (▲—▲, n=5), with increasing concentrations of ¹²⁵I-T₄ or ¹²⁵I-T₃. Each point represents mean of eight and five observations respectively, SD for each mean being < 0.5%

Analysis of Binding and Deiodination

Experimental correction for non-specific binding was accomplished by incubation of duplicate samples, one with radioactive T₄ or T₃ alone and one with radioactive T₄ or T₃ plus a 10⁶-fold excess of non-radioactive T₄ or T₃ added 1 h previously. Specific binding was determined as total binding minus non-specific binding. Degradation of T₄ during incubation was determined by column chromatography and all results concerning T₄ binding characteristics were corrected for non-T₄ radioactivity (endogenous T₄ to T₃ conversion).

The method of Scatchard [4] was used to analyze the binding affinity of T₄ and T₃ and to estimate the maximal capacity of the high affinity binding sites by means of linear regression analysis. The results of the determinations of maximal specific binding capacity and k_a presented, are the means of two separate determinations, the coefficient of variation being < 12%.

Wilcoxon's non-parametric rank sum test was used for statistical analysis.

Results

Clinical examination of all diabetic patients showed them to be euthyroid. The results of the measurements of the blood hormone levels are shown in Table 1, and the results of the TRH tests in Table 2. In all subjects, the levels of thyroglobulin antibodies were normal.

Cellular Experiments

Nuclear uptake of ¹²⁵I-T₄ and ¹²⁵I-T₃ reached a steady state after 90 min incubation. Recovery of the cellular radioactivity during the fractionation procedures was 91.7 ± 8.0% (mean ± SD). Deiodination of T₄ or T₃ during fractionation, extraction and chromatography was negligible, as control experiments showed deiodination of < 2% of the ¹²⁵I-T₄ after 10 h at 37 °C. Results from parallel incubation of cells from the same subject varied < 12%. Control experiments, in which intact cells were incubated with ¹²⁵I had shown that ¹²⁵I was not taken up in the cells. The DNA content in the nuclei was 10.7 ± 3.8 µg DNA/10⁶ nuclei (mean ± SD).

The results from the cellular experiments (Figs. 1 and 2) revealed an increased number of high affinity binding sites for T₄ and T₃ in group 1, whereas the binding affinity did not differ from that of the control groups (Table 3). Neither the number of high affinity binding sites for T₄ or T₃ (Figs. 3 and 4) nor the binding affinity for the respective hormones in group 2 differed from those of the control group (Table 3).

The total cellular concentration of both T₄ and T₃ was identical in all three groups and constant in the tested concentration range (Fig. 5).

Discussion

The finding of subnormal total T₃ levels in both types of diabetic subjects has confirmed the findings of other investigators [1, 5, 6]. Apparently, the low serum levels of T₃ are due mainly to a reduction in extrathyroidal conversion of T₄ to T₃ [7]. However, in spite of the low serum levels of T₃, both groups were clinically euthyroid, and basal serum levels of TSH were normal.

The change in circulating thyroid hormone concentrations in diabetic patients clearly resembles those seen

in caloric deprivation [8], and recently these alterations in serum thyroid hormone indices occurring in diabetes mellitus have been suggested to be a response to the presence of a prolonged period of catabolism [5]. This observation corresponds well with the status of the patients in group 1, in whom weight loss, increased serum levels of rT₃ and the presence of urinary ketone bodies suggested a catabolic state. In contrast, there was no evidence of prolonged catabolism in group 2 as judged by the above parameters. The low serum T₃ levels in group 2 corresponded with the serum T₃ levels found in the age-matched control subjects suggesting a physiologically lower T₃ level in this older group.

The increased number of nuclear T₄ and T₃ receptors in group 1, as estimated by the maximal specific binding capacity for T₄ and T₃ in mononuclear blood cells, corresponds with the recent report of an increase of nuclear T₄ and T₃ receptor number in starved man [9]. In contrast with the present observation, a clear reduction in hepatic nuclear binding of T₃ in streptozotocin-treated rats was reported recently [10]. The explanation for this apparent discrepancy might be afforded by differences in the cellular metabolism of thyroid hormones in man and the rat [11].

The modest increase in the TSH response to TRH injection in group 1 corresponds well with the decreased serum level of T₃ observed in this group, but might also indicate a decrease of serum T₄, as an augmented TSH response to TRH indicates a decrease in serum T₄ or T₃ below the normal pituitary set point for feedback [12]. However, the normal basal TSH level could suggest this nuclear T₃ and/or T₄ deficiency in the pituitary to be partially compensated. The observed increase in nuclear T₄ and T₃ receptor number in group 1 might reflect a compensatory mechanism, which is probably evoked by the intracellular T₃ deficiency in order to maintain cellular homeostasis. This observation bears on the relationship between nuclear T₄ and T₃ receptors. The different binding characteristics between nuclear binding sites for T₄ and T₃ also observed in the present investigation suggest separate receptors for T₄ and T₃, although recent reports point to close relations between these sites [9]. It is, however, generally accepted that T₄ is capable of exerting biological effects without conversion to T₃ [13]. Thus, the increase in both T₃ and T₄ receptor number can be explained either by an immeasurable T₄ deficiency or, what seems more probable, by a parallel increase in both T₃ and T₄ receptors, caused by thyroid hormone deficiency.

The normal T₄ and T₃ receptor number in cells from group 2, and the normal TRH test results, indicate that the cellular metabolic state is unaltered and is normal.

Alterations in intracellular thyroid hormone concentration and the intracellular transport mechanism can be excluded as the intracellular concentration of T₄ or T₃ after 90 min incubation did not differ significantly between the groups.

Taken together, our results indicate a difference in nuclear T₄ and T₃ binding in patients with Type 1 and Type 2 diabetes. This difference probably reflects a compensated hypometabolic state in Type 1 diabetes related to a catabolic state, and may explain the clinical euthyroidism in group 1 in spite of their low serum T₃ levels.

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