Generalized deficiency of 3,5,3'-triiodo-L-thyronine (T₃) in tissues from rats on a low iodine intake, despite normal circulating T₃ levels

Francisco Escobar del Rey, Carmen Ruiz de Oña, Juan Bernal, Maria Jesus Obregón and Gabriella Morreale de Escobar

Unidad de Endocrinología Experimental, Instituto de Investigaciones Biomédicas, C.S.I.C. & Facultad de Medicina, U.A.M., Madrid, Spain

Abstract. Rats fed a low iodine diet have decreased total and nuclear T₃ concentrations in the liver and brain, as compared with rats supplemented with iodine, possibly because of the very low plasma and tissue T₄ pools in low-iodine diet rats, leading to decreased intracellular generation of T₃ in those tissues. If so, T₃ levels should not decrease in heart and skeletal muscle, as plasma T₃ is normal in low-iodine diet rats and these two tissues derive their intracellular T₃ directly from plasma T₃. We have studied this point in male rats fed a low-iodine diet, a low-iodine diet + iodine, and the stock diet. As in previous studies, low-iodine rats had very low plasma T₄ and high plasma TSH levels, plasma T₃ levels being normal. Liver T₃ decreased, and so did the brain T₃ levels despite a compensatory increase in type II 5' iodothyronine deiodinase activity. Contrary to expectations, T₃ concentrations were lower in the heart and skeletal muscle of low-iodine diet rats. Attempts to clarify the possible mechanism(s) involved have been unsuccessful so far. The present results show that, despite normal plasma T₄, a deficiency of T₃ occurs in more tissues of rats on a low iodine intake than previously assumed. If the present results are pertinent to inhabitants from areas with severe iodine deficiency, it would appear that they might suffer from a generalized tissue T₃ deficiency (and hypothyroidism?), even if overt clinical signs are not usually present.

We have shown (Santisteban et al. 1982; Obregón et al. 1984) that rats fed chronically with a diet deficient enough in iodine to result in very low circulating levels of T₄, have low nuclear T₃ concentrations in the liver and brain despite normal plasma T₃ levels. End points of thyroid hormone action in the brain, liver and pituitary also decrease, supporting the conclusion that such tissues are not only thyroid hormone-deficient, but also hypothyroid. The nuclear to plasma ratio of plasma-derived ¹²⁵I-labelled T₃ in the liver and brain was the same for the rats on the low-iodine diet (LID) and for their iodine-supplemented controls (LID + I), indicating that the low nuclear T₃ concentrations found in LID rats were not due to decreased uptake of circulating T₃ by these tissues. We concluded that the low nuclear T₃ concentrations were due to the marked decrease in the intracellular T₄ needed for the generation of T₃. Even if the intracellular T₃ derived from plasma T₃ were normal, nuclear T₃ levels would decrease in tissues, such as the brain and anterior pituitary, which derive an important fraction of their intracellular T₃ from T₄ (Obregón et al. 1979; Larsen et al. 1981; Crantz & Larsen 1980; van Doorn et al. 1983, 1985).

The heart and skeletal (thigh) muscle of euthyroid rats, on the contrary, are believed (van Doorn et al. 1983, 1985) to derive only a very minor fraction of their intracellular T₃ locally from T₄, the major fraction being obtained from plasma T₃. Thus, heart and muscle T₃ should be normal in rats with normal serum T₃, even if intracellular T₄ pools were markedly reduced. It seemed that rats...
on LID might be used to test this hypothesis, as they have normal circulating levels of T₃ and markedly reduced serum and tissue T₄.

As will be seen, however, the concentrations of T₃ decreased in the heart and skeletal muscle of LID rats, and not only in their brain and liver. Thus, T₃ concentrations were reduced in all the tissues we have so far studied, whether or not they are believed to derive most of their intracellular T₃ from local generation from T₄, or from plasma T₃.

Materials and Methods

Experiment A

Female rats were fed a Remington-type LID (0.02–0.06 μg I/g) or the same diet supplemented with 7–10 μg I per rat per day (LID + I), as previously described (Escobar del Rey et al. 1986, 1987). Males born from these rats were weaned to the respective LID or LID + I diet until they were used for the present study, at 3.5–4 months of age. Age-paired rats born and bred on the laboratory stock diet (C), containing 1–2 μg I/g, were studied concomitantly.

The rats (8 per group) were anesthetized with ether, bled from the abdominal aorta and perfused with PBS containing 0.1 mmol/L PTU (6-propyl-2-thiouracil), as previously described (Santisteban et al. 1982; Obregon et al. 1984; Escobar del Rey et al. 1987). The thyroid, whole brain, liver, heart and thigh muscle were dissected out and kept at −20°C. The plasma was spun off and kept frozen.

The concentrations of T₄ and T₃ were determined in extracted plasma and tissues as previously described in detail (Escobar del Rey et al. 1986, 1987; Morreale de Escobar et al. 1985). In the case of the heart and thigh muscle, the whole organ (heart) or 1.5–2.0 g of hindleg muscle was finely minced with a stainless steel surgical blade while still frozen, and then transferred to the tissue grinder for homogenization in methanol. All results were corrected for individual recoveries of both iodothyronines, using high specific activity [¹³¹I]T₄ and [¹²³I]T₃, synthesized as described (Obregon et al. 1980). The methods used for determination of total thyroidal iodine content (Benotti & Benotti 1963) and plasma TSH levels (Obregon et al. 1980) have been reported previously.

Experiment B

Male rats, weighing 100–110 g, born and bred on the stock diet, were given 1% KClO₃ in the drinking water for 4 days, after which they were either placed on LID, LID + I, or the stock diet (C). Three months later 5 rats per group were injected under light ether anesthesia into the femoral vein with 0.2 ml PBS containing 4 μCi of high specific activity [¹³¹I]T₄, obtained as described (Obregon et al. 1980, 1984). One hour later, which is the equilibrium time for the heart (Oppenheimer et al. 1974), the rats were anesthetized with ether, bled and perfused as indicated for those of Experiment A, except that PTU was not used to avoid later interferences in the measure of iodothyronine deiodinase activities. The plasma, most of the heart and 1-g aliquots of liver were extracted with methanol, after which [¹²⁵I]T₃ was added as internal recovery tracer. After accurate counting of both isotopes and thorough extraction, the pooled supernatants were evaporated to dryness at 40°C and the dry extracts dissolved in 100 μl of methanol ammonia (99:1) containing carrier T₄, T₃ and KI. They were submitted to thin layer chromatography as described by van Doorn et al. (1983).

After staining, the [¹³¹I] to [¹²⁵I] ratio was determined accurately in the T₃ spot, and the initial concentrations of [¹³¹I] present as T₃ in plasma, heart and liver 1 h after injection of [¹³¹I]T₄ were calculated. From these data the concentration of [¹²³I]T₃ was determined for the heart and liver (in % dose/g tissue) and plasma (in % dose/ml) and the tissue to plasma [¹³¹I]T₃ ratio was calculated for each animal.

Small pieces of heart muscle (ca. 50 mg), and the cerebral cortex were kept frozen for the determination of iodothyronine deiodinase activities.

T₃ binding to plasma proteins

To 20 μl of plasma was added 6 μl containing 0.1 μCi [¹²⁵I]T₃ (290 μCi/ml and 370 nmol/l from New England Nuclear, Boston, Mass) 0.2 mol/l Tris-glycine buffer, pH 8.6. After 1 h at room temperature, 7.5 μl of this mixture were submitted to electrophoresis for 90 min on 7.5% polyacrylamide gel slabs, in 0.05 mol/l Tris-glycine buffer, bromophenol blue being added to an empty lane to determine the electrophoretic front. The gel slabs were dried and autoradiographed, after which the radioactive spots were cut out and counted. The percentage of total recovered radioactivity in each well-defined spot was then calculated. The 3 main radioactive spots were identified on the basis of mobilities described by Davis et al. (1970) and Young et al. (1982) as non-bound [¹²³I]T₃ migrating with the electrophoretic front, and as [¹²³I]T₃ migrating with prealbumin (r-TBPA) and with albumin (r-Alb). We did not identify r-TBG as a spot distinct from r-Alb, probably because the amount of [¹²³I]T₃ added raised the plasma T₃ to about 5 μg/l, and this 10-fold increase over endogenous levels is likely to result in saturation of r-TBG, with displacement of the iodothyronine from r-TBPA to r-Alb, and with an increased fraction in non-bound form (Davis et al. 1970; Young et al. 1982). It is likely that the binding capacity of plasma proteins was being assessed, not the endogenous T₃ distribution.

Plasma dialyzable T₃

This was carried out by equilibrium dialysis (Oppenheimer et al. 1963) as follows: To 0.25 ml of plasma was added 1 μl containing 12 nCi and 7 fmol T₃ of the high specific activity [¹²⁵I]T₃ prepared as described (Obregon et al. 1980). After 30 min at room temperature, the
plasma was diluted to 6 ml in PBS containing 0.02% sodium azide. From this 5 ml was transferred to a dialysis bag and dialyzed for 20 h at 37°C against 25 ml of the above-mentioned PBS. TCA precipitable radioactivity was then determined in appropriate aliquots from the dialyzate and the content of the dialysis bag. The percentage of added labelled T₄, which was dialyzable was then calculated for individual plasmas.

**Determination of iodothyronine 5’ deiodinase (5’ D) activity**

This was assayed in homogenates from the cerebral cortex and heart muscle as described by Visser et al. (1982) measuring the release of ¹²⁵I⁻ from [¹²⁵I]T₄ (100 000 cpm/vial) during 1 h, in the presence of 2 nmol/l T₄, 1 µmol/l T₃, 20 mmol/l DTT (dithiotheitol), in the presence (5’D-II) or absence (total 5’ D) of 1 mmol/l PTU. The ¹²⁵I⁻ was separated by ion-exchange chromatography on Dowex-50W-X2 columns equilibrated in 10% acetic acid (Leonard & Rosenberg 1980). Protein content was 150–250 µg/vial for cerebral cortex and 200–300 µg/vial for the heart homogenate. When heart homogenates were used, formation of ¹²⁵I⁻ and [¹²⁵I]T₂ was also measured using [¹²⁵I]T₃ as substrate, and the same conditions as described above.

**Statistical analysis**

Mean values (±SEM) were calculated. The presence of significant differences associated with different experimental groups was assessed by one-way analysis of variance, after testing for homogeneity of variances by Bartlett's test. Square root or logarithmic transformations were usually adequate to ensure this homogeneity, when not found with the raw data. If the value of the F ratio indicated the presence of significant differences associated with treatments, these were identified using the protected least significant difference (LSD) test. All these analyses were performed as described by Snedecor & Cochran (1980).

### Table 1.

Body weight, thyroid weight and thyroidal total iodine content of adult male rats on LID, LID + I and on the stock diet (C), corresponding to Experiment A. Mean values (±SEM) are given.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Thyroid weight (mg)</th>
<th>Thyroidal total iodine (nanoatoms/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LID</td>
<td>8</td>
<td>263 ± 30</td>
<td>84.9 ± 6.2</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>LID + I</td>
<td>8</td>
<td>289 ± 20 SIGN</td>
<td>25.5 ± 1.6 SIGN</td>
<td>182 ± 28 SIGN</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>413 ± 28 SIGN</td>
<td>26.5 ± 1.0 SIGN</td>
<td>273 ± 27 SIGN</td>
</tr>
</tbody>
</table>

SIGN: Indicates that the difference with respect to the LID group is statistically significant (P < 0.05).

**Results**

**Experiment A**

The rats bred on LID weighed less than those on LID + I. Rats from both groups weighed less than those on the stock diet, as shown in Table 1. The rats on LID were markedly iodine-deficient: the iodine content in the thyroid was only 1.9% of that found for LID + I rats, and the thyroid weight was increased 4-fold (Table 1).

Fig. 1 shows the T₄, T₃ and TSH levels found in plasma of LID rats. As expected using this diet (Santisteban et al. 1982; Obregón et al. 1984; Escobar del Rey et al. 1986, 1987), the plasma T₄ decreased markedly in comparison with both LID + I and C animals, whereas plasma TSH increased. Plasma T₃ levels were the same.

![Fig. 1.](image-url)

Concentrations of T₄, T₃ and TSH in the plasma of LID, LID + I, and C rats from Experiment A. TSH concentrations are expressed in weight equivalents of the r-TSH RP 2 reference preparation. Data are from the same animals as in Table 1. The asterisks, placed midway above data bars, identify those differences between mean values which were statistically significant. The plasma T₄ of LID rats decreased to 15% of the level found for LID + I animals, plasma T₃ was the same, and TSH increased more than 10-fold.

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Concentrations of T₄, T₃, and TSH in the liver, brain, muscle and heart of the same rats as in Table 1 and Fig. 1. The meaning of the asterisks is the same as indicated in the legend to Fig. 1. The T₃ concentration in LID rats decreased to 8.0, 16.9, 6.9 and 8.1% of the LID + I levels in the liver, brain, heart and muscle, respectively. The corresponding decreases in T₃ concentrations of the LID rat were 66.9, 60.6, 67.9 and 48.7% of LID + I values.

Fig. 2 shows the concentrations of T₄ and T₃ in the liver and brain of LID rats. As expected (Santisteban et al. 1982; Obregon et al. 1984), T₃ concentrations in both tissues were very low in LID rats, and T₃ levels also decreased, despite normal plasma T₃.

Fig. 2 also shows the T₄ and T₃ concentrations in the heart and thigh muscle. The concentration of T₄ in both tissues was markedly decreased. The concentration of T₃ was also lower for LID than for LID + I rats. This decrease was at least as marked as that found in brain and liver T₃.

In general there were no statistically significant differences between data from rats on iodine-supplemented LID and rats on the stock diet (LID + I vs C), though mean values were often higher for the former, with some differences being statistically significant: the plasma, muscle, and brain T₄ concentrations and the muscle T₃ levels were higher for LID + I than for C animals.

Table 2 shows the changes observed in the distribution of [¹²⁵I]T₃ among plasma proteins. The [¹²⁵I]T₃ migrating with r-TBPA was the same for the three experimental groups; [¹²⁵I]T₃ migrating with r-Alb was clearly increased in both LID and LID + I rats as compared with C animals. Conversely, the proportion of [¹²⁵I]T₃ moving with the electrophoretic front was markedly reduced in the LID and LID + I rats as compared with C animals. These results indicate an increased binding capacity of the plasma from LID and LID + I animals as compared with that of rats on the stock diet, but no difference in binding capacity between LID and LID + I rats.

**Experiment B**

The thyroid weight of the rats on LID for 3 months was significantly increased (86.3 ± 5.7 mg) as compared with that of rats on LID + I (31.4 ± 2.0 mg) or
Fig. 3. 

\[^{[131I]}T_3\] tissue to plasma ratios (in % dose/g to % dose/ml) of LID, LID + I and C rats from Experiment B, 1 h after injection of \[^{[131I]}T_3\]. Meaning of asterisks is the same as in the legend to Fig. 1. The heart to plasma ratios of stable \[^{[131I]}T_3\] in the same animals were 1.39, 2.01 and 2.05 for the LID, LID + I and C groups.

C rats (34.8 ± 3.4 mg). Fig. 3 shows the heart to plasma \[^{[131I]}T_3\] ratios 1 h after injection of \[^{[131I]}T_3\]. This corresponds to the equilibrium time point for the heart (Oppenheimer et al. 1974). The heart to plasma \[^{[131I]}T_3\] ratio in LID rats was 62% that of LID + I animals; the latter was 78% that of C rats. As may be seen from Table 3, the reduction in the heart to plasma \[^{[131I]}T_3\] ratio observed in LID rats was due to changes both in the heart and the plasma \[^{[131I]}T_3\] levels; the former decreased and the latter increased in LID rats, as compared with the concentrations in LID + I and C rats. This was only evident when \[^{[131I]}T_3\] was measured, as there were no differences between groups in the concentrations of total radioactivity (not shown). Even within the 60-min period of study, thyroidal recycling of \(^{131}I\) released by deiodination of \[^{[131I]}T_3\] might be more rapid and quantitatively important in the case of the iodine-deficient LID rats, as compared with LID + I and C animals. Thus, it is possible that the proportion of \[^{[131I]}T_3\] found as \[^{[131I]}T_3\] is overestimated in the plasma of the LID rats. But if appreciable recycling of \(^{131}I\) occurred in the LID animals, the proportion of \(^{131}I\) found as \[^{[131I]}T_3\] in the heart of the LID rats would actually be even lower than reported in Table 3. As also shown in Table 3, the proportion of circulating \(^{131}I\) as \(^{131}T_3\) increased in the plasma from LID rats, and decreased in the heart. No such differences were observed between LID + I and C samples.

Fig. 3 also shows the liver to plasma \[^{[131I]}T_3\] ratios, which were the same for LID and LID + I rats, but lower than in C animals. In contrast to the heart, the proportion of the total liver \(^{131}I\) found as \[^{[131I]}T_3\] (Table 3) was the same for the three experimental groups.

Changes in plasma total \(^T_4\) and \(^T_3\) (data not shown) were similar to those described for Experiment A: the \(^T_4\) of the rats on LID + I was higher (143%) than that of C rats, and that of LID animals was markedly decreased, to 26% of the value found for LID + I rats. The plasma \(^T_3\) of LID rats was higher in LID + I than in C rats, and the same in LID and LID + I animals.

The proportion of dialyzable \(^T_3\) as determined by equilibrium dialysis was the same for LID and LID + I rats (0.43 ± 0.02 and 0.43 ± 0.01%, respectively), both being decreased as compared with C animals (0.45 ± 0.01%, \(P<0.05\)). The concentration of free plasma \(^T_3\) was the same for the LID (2.74 ± 0.53 pmol/l), LID + I (2.99 ± 0.66 pmol/l) and C (3.47 ± 0.30 pmol/l) rats.

The proportions of \[^{[125I]}T_3\] bound to plasma proteins are shown in Table 2. As for Experiment A, the proportion of \[^{[125I]}T_3\] bound to r-TBPA was the same for all three experimental groups; that of \[^{[125I]}T_3\] bound to r-Alb was higher for LID and LID + I animals than for C rats, that migrating with the

Table 3.

Percentage of total \(^{131}I\) as \(^T_3\), and concentration of \[^{[125I]}T_3\] in plasma, heart and liver, 1 h after the injection of the labelled iodothyronine. Data are means ± SEM, and correspond to rats from LID, LID + I and C groups of Experiment B.

<table>
<thead>
<tr>
<th>Group</th>
<th>% of total (^{131}I) found as [^{[125I]}T_3] in</th>
<th>[^{[125I]}T_3] concentration (% dose/ml or g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Heart</td>
</tr>
<tr>
<td>LID</td>
<td>54.3 ± 0.5</td>
<td>57.9 ± 2.9</td>
</tr>
<tr>
<td>LID + I</td>
<td>46.2 ± 1.9</td>
<td>76.9 ± 3.8</td>
</tr>
<tr>
<td>C</td>
<td>48.3 ± 1.1</td>
<td>85.4 ± 2.3</td>
</tr>
</tbody>
</table>

a and b have the same meaning as in Table 1.
front lower. These results are consistent with those found by equilibrium dialysis, inasmuch as they indicate an increased binding capacity of the plasma from LID and LID + I rats, as compared with that from C animals.

5'-D II activity in cerebral cortex was the same for LID + I (13.4 ± 1.7 fmol I⁻¹ · h⁻¹ · (mg protein)⁻¹) and C (18.6 ± 4.9 fmol I⁻¹ · h⁻¹ · (mg protein)⁻¹) rats, and markedly increased in the LID animals (64.0 ± 9.2 fmol I⁻¹ · h⁻¹ · (mg protein)⁻¹), the difference vs LID + I and C rats being statistically significant (P < 0.001). 5’D activities in heart muscle were very low (3.0 ± 0.25 fmol I⁻¹ · h⁻¹ · (mg protein)⁻¹) for C rats, near the detection limit, no difference being found with tissue from LID or LID + I animals. Using labelled T₃ as substrate, we did not observe any appreciable deiodination either in the 5 or 5’ position with homogenates of heart from any of the three experimental groups.

Discussion
The male LID rats used for the present study had lower concentrations of T₃ in the liver and brain, despite normal serum levels of T₃, in agreement with previous results in females (Santisteban et al. 1982; Obregón et al. 1984; Escobar del Rey et al. 1986, 1987). As shown here, the 5’D-II activity in the cerebral cortex of the LID rats increases in response to the lower availability of T₃, suggesting that the decrease in cerebral T₃ would have been even greater than actually found, if such a compensatory mechanism had not been operative.

The unexpected finding reported here is that T₄ levels are also lower in heart and skeletal muscle, two tissues considered to derive most of their intracellular T₃ from plasma T₃, not from local deiodination of T₄ (Obregón et al. 1979; van Doorn et al. 1983, 1985). It had been assumed that the marked reduction of the T₄ pool in these animals should scarcely affect T₃ levels in these two tissues, considering that plasma T₃ in LID rats is normal.

Though we have not clarified the mechanism(s) involved, several possibilities ought to be taken in consideration in an attempt to identify them.

1) Changes in circulating T₄ and T₃ and in tissue uptake of T₃
The LID diet itself appears to be nutritionally inadequate, even when supplemented with iodine to correct for its low iodine content. This conclusion is based on the fact that young rats weaned on LID + I grew poorly (Escobar del Rey et al. 1987), and that rats fed LID + I present alterations in thyroid hormone economy associated with nutritional imbalances: In Experiment A and B, either plasma T₄ or T₃ were higher in LID + I than in C rats. Increased levels of T₄, T₃, or both, have been found in rats on a nutritionally deficient iodine-supplemented Remington-type LID (Okamura et al. 1981a,b), on another diet also containing wheat gluten as protein source (Cree & Schalch 1985), on a valine-deficient diet (Rabolli & Martin 1977; Edozien et al. 1978; Glass et al. 1978; Tulp et al. 1979; Young et al. 1980, 1982, 1983; Smallridge et al. 1982), on vitamin A-deficient diets (Morley et al. 1978; Garcin & Higuerrt 1980), and in obese Zucker rats fasted for several weeks (Young et al. 1985). In these studies the proportions of dialyzable iodothyronines were often, but not always, decreased when the total hormone level increased, indicating increased binding by the plasma proteins. In some of the studies this was confirmed, as changes were found in the distribution of labelled T₄ or T₃ indicative of increased binding capacity of the plasma proteins. Thus, the present results, as well as those of several previous studies (Young et al. 1980, 1982, 1983) strongly suggest that the increased total plasma T₃ and/or T₄ levels are due to an increased iodothyronine-binding capacity of the plasma proteins. Such changes would explain the decreased tissue to plasma [¹³¹I]T₃ ratio found for the heart and liver in LID + I as compared with C rats in Experiment B. Though 60 min after the injection of [¹³¹I]T₃ is past the equilibrium time point for the liver (Oppenheimer et al. 1974), previous experiments carried out at the equilibrium time point for this tissue (Obregón et al. 1984) also showed that there is no difference between the liver to plasma labelled T₃ ratios of LID and LID + I rats. A decrease of the tissue to plasma [¹³¹I]T₃ ratio had already been reported previously with iodine-supplemented LID diets in liver and liver nuclei (Okamura et al. 1981a,b; Obregón et al. 1984) and in brain and brain nuclei (Obregón et al. 1984) studied at their respective equilibrium time points. Okamura et al. (1981a) found that the T₃ metabolic clearance rate decreases in rats fed iodine-supplemented LID, and this is consistent with the higher concentration of [¹³¹I]T₃ in plasma of the LID + I rats in Experiment B, as compared with C
animals. The decreased tissue uptake, however, appears to be balanced by the increased circulating hormone levels, and availability of $T_3$ and $T_4$ to tissues is not impaired: the concentrations of $T_3$ and $T_4$ in the tissues studied so far (liver, brain, heart and skeletal muscle), were the same, or actually somewhat higher (i.e., muscle $T_3$ and brain $T_3$) in LID + I as compared with C rats.

Iodine deficiency, superimposed on other nutritional deficiencies of the diet, did not further increase the plasma $T_3$-binding capacity, a finding which would explain why there was also no further decrease in the liver to plasma $^{[35]}T_3$ ratio. But it does not explain how in the same LID animals in Experiment B in which the liver to plasma $^{[35]}T_3$ ratio remained unchanged, the heart to plasma $^{[35]}T_3$ ratio decreased.

Thus, changes in binding to plasma proteins could explain the changes in tissue uptake caused by nutritional deficiencies of the diet itself, but do not afford an easy explanation for the decreased concentrations of $T_3$ in the heart, skeletal muscle, brain and liver, caused by iodine deficiency. On the other hand, it is possible that changes in binding to plasma proteins might have tissue-specific effects on the intracellular delivery of the iodothyronines, a problem fraught with considerable theoretical controversy (Pardridge 1981; Robbins & Bartalena 1986; Ekins 1986):

2) Changes in local metabolism and/or tissue exit rate of $T_3$ and $T_4$

The idea that most muscle and heart $T_3$ is derived from plasma $T_3$, and only in a minor proportion from local $T_4$ deiodination, is based on results from steady-state experiments in which the tissue ratio of $T_3$ derived from $T_4$ to the plasma-derived $T_3$ is compared with the ratio found in plasma. When the tissue ratio is similar to that of the plasma, it is concluded that very little intracellular $T_3$ is derived from $T_4$ compared with the amount derived directly from plasma. However, it cannot be excluded that $T_3$ is generated locally from $T_4$, but is being rapidly metabolized, or rapidly exported into the plasma (van Doorn et al. 1983, 1985), thus lowering the ratio measured for that tissue. If this were the case for the heart and skeletal muscle, local generation of $T_3$ from $T_4$ might be higher than previously believed, and changes in the size of the $T_4$ pool, the activity of the deiodinating enzymes, the $T_3$ exit rate, etc., might play a role in determining the decreased concentrations of $T_3$ in heart and muscle of LID animals.

van der Heide et al. (1987) recently reported a marked increase in locally generated $T_3$ in the heart of rats on amiodarone, suggesting that conversion of $T_4$ to $T_3$ in the heart might indeed contribute to the intracellular $T_3$ levels to a greater extent than assessed from earlier experiments (van Doorn et al. 1983, 1985). Active conversion of $T_4$ to $T_3$ has been reported (Rabinowitz & Hercker 1971) for rat heart perfused in vitro. Our preliminary attempts to measure $5'D$ using heart homogenates have, however, disclosed much lower activities than in other tissues, such as the brain, no differences being detectable between the experimental groups.

Neither did we find any significant $T_3$ degradation in vitro using heart homogenates, which might have explained the finding (Table 3) that 60 min after the injection of $^{[35]}T_3$, the proportion of tissue radioactivity still found as $T_3$ was smaller in the heart (but not in the liver or plasma) of LID rats. An increased $T_3$ exit rate from the heart of LID rats, or a decreased $T_3$ uptake, could also account for these findings, as well as for the decreased heart to plasma $^{[35]}T_3$ ratio, and decreased $T_3$ concentration. In vitro studies which might clarify these points have not yet been performed.

General comments and clinical implications

Although elucidation of the mechanism(s) involved requires further experiments, all tissues obtained so far from rats on a diet deficient enough in iodine to result in markedly decreased plasma $T_4$ levels, are deficient in $T_3$. In the case of the liver (Santisteban et al. 1982) and brain (Obregon et al. 1984), this decreased concentration of total and of nuclear $T_3$ is accompanied by a decrease in thyroid hormone activity, as measured by biological end points appropriate for the tissue. The same was found for thyroid hormone activity at the anterior pituitary level (Santisteban et al. 1982). The liver, brain and anterior pituitary of LID rats are hypothyroid, despite normal plasma $T_3$, although not to the same degree as animals in which plasma $T_4$ and $T_3$ are both low. The present data show that heart and skeletal muscle are also moderately $T_3$-deficient, and markedly deficient in $T_4$. Although nuclear $T_3$ concentrations and end points of thyroid hormone action were not measured in heart and
skeletal muscle, it appears reasonable to assume that nuclear T₃ concentrations are lower, and that both tissues might also suffer from some degree of hypothyroidism. If the present results obtained in LID rats are pertinent to man, inhabitants from areas with very severe iodine deficiency might suffer from a more generalized tissue T₃ deficiency (and hypothyroidism?) than was previously recognized. Normal plasma T₃ levels do not ensure tissue euthyroidism when plasma T₄ is very low, even if overt clinical hypothyroidism only becomes evident when plasma T₃ also decreases (Goslings et al. 1977). Such a generalized tissue deficiency of T₄ and T₃ is a potential candidate for inclusion among the many iodine deficiency disorders (Hetzelf 1983) described in areas of severe iodine deficiency. A situation of prolonged T₄ and T₃ deficiency may well have adverse effects in many tissues, especially during growth and development, even if it is not accompanied by the overt clinical signs of hypothyroidism. The present results stress once more the importance of an adequate iodine intake, and of iodine prophylactic policies in those areas where iodine intake is chronically reduced.

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References


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Dr F. Escobar del Rey,
Unidad de Endocrinología Experimental, Instituto de Investigaciones Biomédicas, Facultad Autónoma de Medicina, Arzobispo Morcillo 4, E-28029 Madrid, Spain.