Increased 5'-iodothyronine deiodinase activity is a maternal adaptive mechanism in response to protein restriction during lactation

P C Lisboa, M C F Passos¹, S C P Dutra, R S Santos, I T Bonomo, A P Cabanelas², C C Pazos-Moura² and E G Moura

Departamento de Ciências Fisiológicas, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil
¹Departamento de Nutrição Aplicada, Instituto de Nutrição, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil
²Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil

Abstract

We have shown that protein restriction during lactation is associated with higher levels of serum and milk tri-iodothyronine (T₃) with lower serum thyroxine (T₄), suggesting an increased T₄ to T₃ conversion. To investigate this hypothesis, the activity of type 1 (D1) and/or type 2 (D2) iodothyronine deiodinases was evaluated on days 4, 12 and 21 of lactation in several tissues of dams fed an 8% protein-restricted (PR) diet and controls fed a 23% protein diet. Serum TSH, T₃ and T₄ were measured by radioimmunoassay. Deiodinase activity was determined by the release of ¹²⁵I from ¹²⁵I-reverse T₃, under specific conditions for D1 or D2. PR dams had a transitory reduction in liver D1 activity (P<0·05) on day 12, and a small increase in thyroid D1 on day 12 followed by a small decrease on day 21. However, thyroid D2 activity was higher than controls (P<0·05) during the whole of the lactation period. Mammary gland D1 and D2 activities were lower on day 4 of lactation in PR dams (P<0·05), and D2 was higher on day 21 (P<0·05). Potentially, a lower conversion of T₃ to di-iodothyronine in the mammary glands of PR dams at the beginning of lactation may serve to provide more T₃ through the milk. Brown adipose tissue (BAT) D2 activity was higher (P<0·05) in PR dams during all periods of lactation. PR dams showed higher skeletal muscle D1 activity only at the end of lactation, but no changes in D2 activity. Higher pituitary D1 and D2 activities in the PR group (P<0·05) at the end of lactation could have contributed to the lower serum TSH. These data suggest that the higher thyroid and BAT D2 activity during the whole of lactation and skeletal muscle D1 activity at the end of lactation may contribute to the higher serum T₃ in PR dams.

Journal of Endocrinology (2003) 177, 261–267

Introduction

More than a third of the world’s children are affected by protein-energy malnutrition. For all the indicators (wasting, stunting and underweight), the most favourable situation of low or moderate prevalence occurs in Latin America, in Asia most countries have a high or very high prevalence, and in Africa a combination of these circumstances is found. A total 80% of the children affected live in Asia, 15% in Africa and 5% in Latin America (de Onis et al. 1993).

The prevalence of stunting has fallen in developing countries from 47% in 1980 to 33% in 2000, although progress has been uneven according to the region. Stunting has increased in Eastern Africa, but decreased in Asia and South America, Northern Africa and the Caribbean show modest improvement, and Western Africa and Central America show very little progress. Despite an overall decrease in stunting in developing countries, child malnutrition still remains a major public health problem in these countries (de Onis et al. 2000).

The effect of malnutrition upon thyroid function in humans (LoPresti et al. 1991) and other adult animals is well documented (Harris et al. 1978, Moura et al. 1987). Our previous studies showed short- and long-term effects of malnutrition during lactation on the thyroid function of mothers and their offspring (Ramos et al. 2000, Passos et al. 2001a,k, 2002). Dams submitted to a protein-restricted (PR) diet during lactation had, at weaning, a significant decrease in ¹³¹I thyroid uptake and an increase in ¹³¹I uptake by the mammary gland and a higher transfer of ¹³¹I to the milk when compared with controls (Ramos et al. 2000). These animals had higher tri-iodothyronine (T₃) levels in serum and milk, and lower serum levels of...
thyroxine ($T_4$) during lactation (Passos et al. 2001a). In addition, these dams show a higher transfer of iodine through the milk to the pups’ thyroid (Passos et al. 2001b), despite the impairment of thyroid iodine uptake observed in the offspring during the first half of lactation when they received i.p. injection of radiiodine (Passos et al. 2001b). Serum $T_3$ concentrations in these pups are significantly increased at the beginning of lactation, despite lower serum $T_4$ concentrations (Passos et al. 2001a). The transfer of $T_4$ to the milk could be very important for the thyroid function of pups for the first 4 days of life, which are critical for central nervous system (CNS) development. As malnutrition continued during lactation, this adaptive mechanism did not persist up to day 12, when the rise in serum $T_3$ in the pups was not present. However, this stage does not seem to be so critical for the neural development of the animal. Hence, these changes observed in malnourished lactating mothers could be important in the prevention of neonatal hypothyroidism.

The mechanism by which $T_3$ is increased in the serum and the milk of PR lactating rats has not yet been elucidated. Our hypothesis is that it could be due to a higher production by conversion of $T_4$ to $T_3$.

The enzyme 5'-iodothyronine deiodinase is responsible for converting $T_4$ to $T_3$, the bioactive hormone. Based on several functional criteria, tissue distribution and on different protein sequences, 5'-deiodinases are classified into two isoenzymes: type 1 (D1) and type 2 (D2). In murines, D1 is predominantly found in the thyroid, liver and kidney and is responsible for generating most of the circulating $T_3$. D2 is predominantly expressed in brain, pituitary and brown adipose tissue (BAT), where it appears to catalyse the local $T_4$ to $T_3$ conversion (Bianco et al. 2002). However, it has been shown that the contribution of D2 to serum $T_3$ may also be significant (Salvatore et al. 1996a, Nguyen et al. 1998, Sabatino et al. 2000).

We therefore analysed the effects of a PR diet during lactation on D1 and D2 activities in several tissues of rats during lactation, aiming to identify the role of iodothyronine deiodinases in the higher $T_3$ production in protein restriction during lactation.

### Materials and Methods

Wistar rats were kept in a room with controlled temperature (25 ± 1 °C) and with artificial light:darkness cycles (lights on from 0700 to 1900 h). Three-month-old, nubile female rats were housed with a male rat and, after mating, each female was placed in an individual cage with free access to water and food until parturition. The use of the animals in our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of State University of Rio de Janeiro, based on the principles described in the Guide for the Care and Use of Laboratory Animals (Bayne 1996).

The dams were randomly assigned to one of two groups: a control group, with free access to a standard laboratory diet containing 23% protein and a PR group, with free access to an isonenergy and PR diet containing 8% protein. Table 1 shows the composition of the diets.

The PR diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amounts of starch, mineral and vitamin mixture were calculated so as to make up for the decrease in energy content due to protein reduction. Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diet and contain the recommended amount of iodine (Reeves et al. 1993).

Excess pups were removed within 24 h of birth, so that only six pups were kept per dam because it has been shown that this procedure maximizes lactation performance (Fishbeck & Rasmussen 1987). Malnutrition was started at birth, which was defined as day 0 of lactation, and continued to the day on which the animal was killed.

The lactating dams received 0·6 µCi $^{125}$I i.p. on days 4, 12 and 21 of lactation, and 2 h later they were killed with a lethal dose of ether (Ramos et al. 1997). Thyroid glands were excised and weighed and thyroidal $^{125}$I uptake was individually determined with a gamma-counter (Cobra Auto-gamma; Packard Instrument Co., Downers Grove, IL, USA).

To study D1 and D2 deiodinases activities during three different periods of lactation we used four to six dams in each period. On days 4, 12 and 21 of lactation the dams from each group were killed with a lethal dose of

### Table 1 Composition of the control and low-protein diets

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Control</th>
<th>Low protein†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean + wheat</td>
<td>23·0</td>
<td>80·0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>676·0</td>
<td>826·0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>50·0</td>
<td>50·0</td>
</tr>
<tr>
<td>Vitamin mix‡</td>
<td>4·0</td>
<td>4·0</td>
</tr>
<tr>
<td>Mineral mix‡</td>
<td>40·0</td>
<td>40·0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Macronutrient composition (%)</th>
<th>Control</th>
<th>Low protein†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>23·0</td>
<td>8·0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>66·0</td>
<td>81·0</td>
</tr>
<tr>
<td>Fat</td>
<td>11·0</td>
<td>11·0</td>
</tr>
<tr>
<td>Total energy (kJ/kg)</td>
<td>17 038·7</td>
<td>17 038·7</td>
</tr>
</tbody>
</table>

*Standard diet for rats (Nuvihab-NUVITAL, Nutrientes LTDA, Parana, Brazil).
†The low-protein diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of the latter was calculated so as to make up for the decrease in energy content due to protein reduction.
‡Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diet and contain the recommended amount of iodine (Reeves et al. 1993).
pentobarbital and blood was obtained by cardiac puncture. The thyroid, liver, pituitary, mammary gland, skeletal muscle and BAT were excised and they were kept individually at −70 °C until assay. Blood samples were centrifuged to obtain serum, which was kept at −20 °C until assay.

**Deiodinase activity measurement**

Thyroid, pituitary, liver, muscle and BAT deiodinase activities were measured based on methods previously described (Pazos-Moura et al. 1991, Curty et al. 2000, Lisboa et al. 2001) and, for mammary deiodinase activities, we used protocols described previously (Aceves & Valverde 1989, Song et al. 2000), with slight modifications. D1 and D2 assays were performed in phosphate buffer containing 1 mM EDTA, pH 6·9. To evaluate D1 activity in thyroidal and hepatic microsomal fractions, we added 1·5 µM reverse T3 (rT3) and 10 mM dithiothreitol (DTT). In other tissues, deiodinases were accessed using 2 nM rT3 and 40 mM DTT. In order to distinguish D1 and D2 activity, assays were carried out in the presence of 1 mM propylthiouracil (PTU) (to inhibit D1) or 100 nM T4 (to suppress D2). Equal aliquots of 125I-rT3 (1·07 mCi/µg; New England Nuclear-Dupont, Boston, MA, USA), which was purified by paper electrophoresis, were placed in each assay tube. The reaction was started by the addition of the samples, corresponding to the following amount of protein (µg): 4–18 thyroid, 15–30 pituitary, 25–117 liver, 10–30 BAT, 110–230 skeletal muscle and 100–660 mammary gland. A blank tube was run in parallel with each set of assays; this contained 50 µl of the substrate solution and 50 µl of buffer, and its value was subtracted from that of enzyme samples. Reactions were performed in a shaking bath at 37 °C, and stopped after 30 min (thyroid and liver D1), 1 h (thyroid D2; pituitary), 2 h (BAT D2; muscle) or 4 h (mammary gland) by the addition of a mixture of 8% bovine serum albumin and 10 mM PTU, followed by cold 20% trichloroacetic acid. Samples were centrifuged (2000 g, 4 °C, 5 min) and 200 µl of the supernatants were applied to Dowex 50 W-X2 columns (100–200 mesh hydrogen form; BioRad, Richmond, CA, USA). Free 125I, eluted from the column with 10% acetic acid, was measured in a gamma-counter. Deiodination percentage in the presence of the enzyme was around 10–20%, except in the mammary gland which was 2–4% of the total released iodide. The amount of free 125I in the blank was generally less than 1–2% of the total radioactivity in the reaction mixture. The specific enzyme activity was expressed in fmol, pmol or nmol rT3 deiodinated/h per mg protein. Protein was measured by the method described by Bradford (1976).

![Figure 1](https://www.endocrinology.org)

*Figure 1* Liver D1(A), thyroid D1 (B) and thyroid D2 (C) activities in lactating rats fed a control (solid bars) and PR diet (open bars). Values represent the means ± S.E.M. of four to six rats per group. *P*<0·05, significant difference between groups.
Serum thyrotropin (TSH), T₃ and T₄ quantification

Serum TSH was determined by specific radioimmunoassay (RIA), using a kit for rat TSH supplied by the NIDDKD (Bethesda, MD, USA) and data are expressed in terms of the reference preparation provided (RP-3).

Total serum T₃ (TT₃) and T₄ (TT₄) were measured by RIA, using commercial kits (Coat-A-Coat; DPC, Los Angeles, CA, USA), in which we used control standard curves diluted in iodothyronine-free rat serum (charcoal-treated).

Statistical analysis

Values are given as means ± s.e.m. Statistical significance of experimental observations was determined by two-way ANOVA followed by Student’s t-test (non-paired). The level of significance was set at P<0·05.

Results

The effects of a PR diet on hepatic D1 activity, and thyroid D1 and D2 activities of lactating rats at different stages of lactation are demonstrated in Fig. 1. PR dams had a lower liver D1 activity on day 12 compared with controls (74%, P<0·05), but not at days 4 and 21. The PR group had a significantly higher thyroid D2 activity on days 4 (sevenfold), 12 (+55%) and 21 (+83%) compared with controls. With regard to thyroid D1, the PR group had a higher activity on day 12 (+50%, P<0·05) and a lower activity on day 21 of lactation (−20%, P<0·05), as shown in Fig. 1.

We detected lower mammary gland D1 (−58%, P<0·05) and D2 (−67%, P<0·05) activities on day 4 in PR dams, compared with controls. At weaning, there was a higher mammary D2 activity (+48%) in PR animals compared with controls (Fig. 2).

BAT D2 and skeletal muscle D2 and D1 activities are presented in Fig. 3. BAT D2 activity was higher in the PR group at all periods of lactation investigated; however, it was significant only on days 4 and 12. The PR group did not show any alteration in skeletal muscle D2 activity, although they showed higher skeletal muscle D1 activity at the end of lactation (2·5-fold, P<0·05).

As verified in Fig. 4, the PR group showed an important increase in both pituitary D1 (five times) and D2 (3·8 times) activity at the end of lactation.

Table 2 shows serum TSH, T₃ and T₄ concentrations and 2-h thyroid radioiodine uptake during lactation. From the middle to the end of the lactation period, the PR group had lower serum TSH compared with controls. Serum T₃ was higher in PR mothers during all stages of lactation (46%, 75% and 46%, days 4, 12 and 21 respectively, P<0·01). On the contrary, serum T₄ was lower in PR mothers at the beginning (day 4, 19%, P<0·05) and at the end (day 21, 34%, P<0·05) of lactation. Thyroid radioiodine uptake was lower in PR mothers during all stages of lactation compared with the controls (85%, 79% and 67%, days 4, 12 and 21 respectively, P<0·001).

Discussion

The higher serum T₃ of dams fed a low-protein diet cannot be explained by alterations in liver D1 activity, which showed a transitory decrease at mid lactation. Also, thyroid D1 seems not to be contributing to the rise in the circulating T₃ of PR dams observed during all lactation periods, since the increase in the enzyme activity at mid lactation was followed by a decrease in late lactation. On the other hand, thyroid D2 activity was higher during all periods of lactation investigated, suggesting that thyroidal T₄-to-T₃ conversion may be contributing to the higher serum T₃ in PR dams. In favour of this possibility is the fact that in rats, contrary to humans, the thyroid contributes to a significant proportion of total body T₃ production, around 50% (Chanoine et al. 1993).
Until recently, the D1 pathway was considered to be the major one for extrathyroidal T3 production in euthyroid rats. The D2 pathway was considered to contribute to extrathyroidal T3 production only when its activity was sufficiently elevated and/or liver and kidney D1 activity was depressed (Silva & Larsen 1985). However, in both rats and humans, evidence has been presented that D2 contributes significantly to the generation of the serum T3 pool. Nguyen et al. (1998) showed that, in rats, the D2 activity is responsible for the generation of approximately 50% of T3 from T4. D2 is present in BAT, brain, pituitary and recently it has been detected in skeletal muscle (Salvatore et al. 1996a) and in human and rat thyroid glands (Salvatore et al. 1996b, Bates et al. 1999, Dutra et al. 2003).

The D2 activity of BAT was increased in PR dams during all periods of lactation, especially from the beginning to mid lactation and, therefore, BAT exportation of T3 is another important mechanism that may explain the rise in serum T3 in the PR dams. Evidence that BAT is a source of serum T3 was produced by studies on cold-exposed rats, whose BAT 5'-D2 activities were elevated (Silva & Larsen 1985).

Despite the relevance of D2 activity in human skeletal and cardiac muscle for T3 peripheral production (Salvatore et al. 1996a, Hosoi et al. 1999, Sabatino et al. 2000), we did not find important changes in skeletal muscle D2 activity at any time evaluated. On the other hand, the rise in skeletal muscle D1 activity at the end of lactation potentially contributes to higher serum T3 in PR dams (Fig. 3C), but the large mass of this tissue in the body must be considered.

Our initial hypothesis was that one of the important sites for T3 production for serum and milk in PR dams was the mammary gland, since some authors (Valverde & Aceves 1989, Jack et al. 1994) reported increased mammary D1 activity in lactating rats. However, contrary to this initial hypothesis, we observed lower mammary D1 and D2 activities at the beginning of lactation. It is possible that this change could work as an adaptive mechanism, aiming to prevent T3 inactivation, since those deiodinases are also capable of catalysing T3 deiodination to di-iodothyronine (Körhle 1999). Probably, the T3 that reaches the mammary tissue in this critical period must be further transferred to the pups through the milk, in order to minimize the effects of neonatal hypothyroidism, especially during CNS development. However, at the end of lactation, the high mammary D2 activity present in the PR group may potentially contribute to elevate serum T3 concentrations.

At the end of the lactation period, there was an increment in both pituitary D1 and D2 activities in PR dams. These changes could contribute to the TSH suppression observed in these rats. On the other hand, the low serum T4 may be the cause of the increase in thyroid,
pituitary and BAT D2 activity. A possible mechanism is an increase in the enzyme half-life, since T4 promotes higher rates of D2 proteolysis (Steinsapir et al. 1998, 2000).

Another interesting observation from the present study is the evidence that in special situations, such as malnutrition, other factors may be more important than TSH in stimulating thyroid deiodinases (Erickson et al. 1982, Wu et al. 1985, Salvatore et al. 1996b, Murakami et al. 2001), since the D2 activity was increased despite the lower serum TSH in PR dams.

It is unlikely that the higher T3 serum concentration is caused by an increase in T3/T4 production by the thyroid, since serum TSH and thyroid radioiodine uptake are lower (Table 2). The fact that thyroid iodine uptake was not higher makes it unlikely that an iodine deficiency caused by the reduced diet or protein malnutrition, affecting either ingestion or iodine intestinal absorption, could be responsible for the changes observed here. On the other hand, the content of iodine in the low protein diet was compensated by the addition of a mineral mix with an adequate amount of iodine.

In conclusion, the present study has shown that nutritional factors modulate both 5′-deiodinase isoforms differently in several tissues. Moreover, this study has suggested that the higher thyroid and BAT D2 activities are the major contributors for the higher serum T3 in PR dams, although skeletal muscle D1 may be important at late stages of lactation. Therefore, alterations in 5′-deiodinase represent a maternal protective mechanism against the drop in serum T4 induced by protein restriction during lactation.

Acknowledgements

This work was supported by a grant from Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico and by funds from Pós-Graduação em Biologia. P C Lisboa is a recipient of a fellowship from FAPERJ. We also thank Mr Nelcir Rodrigues and Ms Lauciene Andrade for technical assistance.

References

Bates JM, St Germain DL & Galton VA 1999 Expression profiles of the three iodothyronine deiodinases, D1, D2, D3 and developing rat. Endocrinology 140 844–851.

Table 2 Serum TT3, TT4, TSH and 2-h 131I thyroid uptake in lactating rats fed a normal (C) and PR diet. Values represent means ± S.E.M. of five mothers for each group.

<table>
<thead>
<tr>
<th>Days of lactation</th>
<th>Group</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT3 (nmol/l)</td>
<td>4</td>
<td>47·60 ± 3·80 69·40 ± 5·20*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>45·40 ± 2·20 79·60 ± 3·10*</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>66·80 ± 5·40 97·20 ± 6·9*</td>
</tr>
<tr>
<td>CT4 (nmol/l)</td>
<td>4</td>
<td>1·65 ± 0·13 1·33 ± 0·12*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1·75 ± 0·13 1·59 ± 0·12</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1·82 ± 0·08 1·20 ± 0·09*</td>
</tr>
<tr>
<td>TSH (ng/ml)</td>
<td>4</td>
<td>1·69 ± 0·24 1·50 ± 0·24</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1·68 ± 0·18 1·17 ± 0·13*</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3·24 ± 0·48 2·52 ± 0·23*</td>
</tr>
<tr>
<td>2-h 131I thyroid uptake (%/g tissue)</td>
<td>4</td>
<td>132·70 ± 21·70 19·30 ± 2·20*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>100·80 ± 8·80 21·03 ± 5·30*</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>199·60 ± 33·39 66·43 ± 7·40*</td>
</tr>
</tbody>
</table>

*P<0·05, significant difference between groups.


Steinsapir J, Bianco AC, Buettnner C, Harney J & Larsen PR 2000 Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. *Endocrinology* **141** 1127–1135.


Received in final form 30 January 2003
Accepted 4 February 2003