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 Alcântara 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

(Requests for offprints should be addressed to E G Moura, Departamento de Ciências Fisiológicas-5o andar, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Av. 28 de setembro, 87-Rio de Janeiro, RJ 20551-030, Brazil; Email: egmoura@uerj.br)

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,b, 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 (T₄) 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 T₃ concentrations in these pups are significantly increased at the beginning of lactation, despite lower serum T₄ concentrations (Passos *et al.* 2001a). The transfer of T₃ 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 T₃ 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 T₃ 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 T₄ to T₃.

The enzyme 5'-iodothyronine deiodinase is responsible for converting T₄ to T₃, 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 T₃. D2 is predominantly expressed in brain, pituitary and brown adipose tissue (BAT), where it appears to catalyse the local T₄ to T₃ conversion (Bianco *et al.* 2002). However, it has been shown that the contribution of D2 to serum T₃ 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 T₃ 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).

Table 1 Composition of the control and low-protein diets

	Control*	Low protein†
Ingredients (g/kg)		
Soybean + wheat	230.0	80.0
Cornstarch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mix‡	4.0	4.0
Mineral mix‡	40.0	40.0
Macronutrient composition (%)		
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0
Total energy (kJ/kg)	17 038.7	17 038.7

*Standard diet for rats (Nuvilab-NUVITAL Nutrientes LTDA, Paraná, 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).

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 isoenergy 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, vitamin and mineral content due to protein reduction. Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93 G recommendation for rodent diet (Reeves *et al.* 1993). The mineral mix contained 0.01 g/kg potassium iodide, which is sufficient to equal the content of iodine in the standard diet.

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 ¹²⁵I 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 ¹²⁵I 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

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 $\pm$ S.E.M. of four to six rats per group. * $P < 0.05$, significant difference between groups.

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 $1.5\ \mu\text{M}$ reverse T_3 (rT_3) and 10 mM dithiothreitol (DTT). In other tissues, deiodinases were accessed using 2 nM rT_3 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 T_4 (to suppress D2). Equal aliquots of ^{125}I - rT_3 ($1.07\ \text{mCi}/\mu\text{g}$; 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 ^{125}I , 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 ^{125}I 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 rT_3 deiodinated/h per mg protein. Protein was measured by the method described by Bradford (1976).

Figure 2 Mammary gland D1 (A) and D2 (B) activities in lactating rats fed a control (solid bars) and PR diet (open bars). Values represent the means $\pm$ S.E.M. of four to six rats per group. * $P < 0.05$, significant difference between groups.

Serum thyrotropin (TSH), T_3 and T_4 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 T_3 (TT₃) and T_4 (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 $\pm$ 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, T_3 and T_4 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 T_3 was higher in PR mothers during all stages of lactation compared with controls (46%, 75% and 46%, days 4, 12 and 21 respectively, $P < 0.01$). On the contrary, serum T_4 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 T_3 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 T_3 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 T_4 -to- T_3 conversion may be contributing to the higher serum T_3 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 T_3 production, around 50% (Chanoine *et al.* 1993).

Figure 3 BAT D2 (A), and skeletal muscle D2 (B) and D1 (C) activities in lactating rats fed a control (solid bars) and PR diet (open bars). Values represent the means $\pm$ S.E.M. of four to six rats per group. * $P < 0.05$, significant difference between groups.

Until recently, the D1 pathway was considered to be the major one for extrathyroidal T_3 production in euthyroid rats. The D2 pathway was considered to contribute to extrathyroidal T_3 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 T_3 pool. Nguyen *et al.* (1998) showed that, in rats, the D2 activity is responsible for the generation of approximately 50% of T_3 from T_4 . 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 T_3 is another important mechanism that may explain the rise in serum T_3 in the PR dams. Evidence that BAT is a source of serum T_3 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 T_3 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 T_3 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 T_3 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 T_3 inactivation, since those deiodinases are also capable of catalysing T_3 deiodination to di-iodothyronine (Körhle 1999). Probably, the T_3 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 T_3 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 T_4 may be the cause of the increase in thyroid,

Figure 4 Pituitary D1 (A) and D2 (B) activities in lactating rats fed a control (solid bars) and PR diet (open bars). Values represent the means $\pm$ S.E.M. of four to six rats per group. * $P < 0.05$, significant difference between groups.

pituitary and BAT D2 activity. A possible mechanism is an increase in the enzyme half-life, since T_4 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 T_3 serum concentration is caused by an increase in T_3/T_4 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

Table 2 Serum TT_3 , TT_4 , TSH and 2-h ^{131}I thyroid uptake in lactating rats fed a normal (C) and PR diet. Values represent means $\pm$ S.E.M. of five mothers for each group

	Days of lactation	Group	
		C	PR
TT_3 (nmol/l)	4	47.60 $\pm$ 3.80	69.40 $\pm$ 5.20*
	12	45.40 $\pm$ 2.20	79.60 $\pm$ 3.10*
	21	66.80 $\pm$ 5.40	97.20 $\pm$ 6.9*
TT_4 (nmol/l)	4	1.65 $\pm$ 0.13	1.33 $\pm$ 0.12*
	12	1.75 $\pm$ 0.13	1.59 $\pm$ 0.12
	21	1.82 $\pm$ 0.08	1.20 $\pm$ 0.09*
TSH (ng/ml)	4	1.69 $\pm$ 0.24	1.50 $\pm$ 0.24
	12	1.68 $\pm$ 0.18	1.17 $\pm$ 0.13*
	21	3.24 $\pm$ 0.48	2.52 $\pm$ 0.23*
2-h ^{131}I thyroid uptake (%/g tissue)	4	132.70 $\pm$ 21.70	19.30 $\pm$ 2.20*
	12	100.80 $\pm$ 8.80	21.03 $\pm$ 5.30*
	21	199.60 $\pm$ 33.9	66.43 $\pm$ 7.40*

* $P < 0.05$, significant difference between groups.

suggested that the higher thyroid and BAT D2 activities are the major contributors for the higher serum T_3 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 T_4 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

Aceves C & Valverde C 1989 Type I, 5'-monodeiodinase activity in the lactating mammary gland. *Endocrinology* **124** 2818–2820.
Bayne K 1996 Revised guide for the care and use of laboratory animals. *Physiologist (American Physiology Society)* **39** 208–211.
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.
Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* **23** 38–89.
Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72** 248–252.

- Chanoine JP, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S & Leonard JL 1993 The thyroid gland is a major source of circulating T₃ in the rat. *Journal of Clinical Investigation* **91** 2709–2713.
- Cutry FH, Lisboa PC, Ortiga-Carvalho TM & Pazos-Moura CC 2000 The somatostatin analogue, octreotide, modulates iodothyronine deiodinase activity and pituitary neuromedin B. *Thyroid* **10** 647–652.
- Dutra SCP, Passos MCF, Lisboa PC, Santos R, Cabanelas AP, Pazos-Moura CC & Moura EG 2003 Liver deiodinase activity is increased in adult rats whose mothers were submitted to malnutrition during lactation. *Hormone and Metabolic Research* (In Press).
- Erickson VJ, Cavalieri RR & Rosenberg LL 1982 Thyroxine-5'-deiodinase of rat thyroid, but not that of liver, is dependent on thyrotropin. *Endocrinology* **111** 434–440.
- Fischbeck KL & Rasmussen KM 1987 Effect of repeated reproductive cycles on maternal nutritional status, lactational performance and litter growth in *ad libitum*-fed and chronically food-restricted rats. *Journal of Nutrition* **117** 1967–1975.
- Harris AR, Fang SL, Azizi F, Lipworth L, Vagenakis AG & Braverman LE 1978 Effect of starvation on hypothalamic-pituitary-thyroid function in rat. *Metabolism* **27** 1074–1083.
- Hosoi Y, Murakami M, Mizuma H, Ogiwara T, Imamura M & Mori M 1999 Expression and regulation of type II iodothyronine deiodinase in cultured human skeletal muscle cells. *Journal of Clinical Endocrinology and Metabolism* **84** 3293–3300.
- Jack LJ, Kahl S, St Germain DL & Capuco AV 1994 Tissue distribution and regulation of 5'-deiodinase process in lactating rats. *Journal of Endocrinology* **142** 205–215.
- Köhrle J 1999 Local activation and inactivation of thyroid hormones: the deiodinase family. *Molecular and Cellular Endocrinology* **151** 103–119.
- Lisboa PC, Cutry FH, Moreira RM, Oliveira KJ & Pazos-Moura CC 2001 Sex steroids modulate rat anterior pituitary and liver iodothyronine deiodinase activities. *Hormone and Metabolic Research* **33** 532–535.
- LoPresti JS, Gray D & Nicoloff JT 1991 Influence of fasting and refeeding on 3,3',5'-triiodothyronine metabolism in man. *Journal of Clinical Endocrinology and Metabolism* **72** 130–136.
- Moura EG, Ramos CF, Nascimento CCA, Rosenthal D & Breitenbach MMD 1987 Thyroid function in fasting rats: variations in ¹³¹I uptake and transient decrease in peroxidase activity. *Brazilian Journal of Medical and Biological Research* **20** 407–410.
- Murakami M, Araki O, Hosoi Y, Kamiya Y, Morimura T, Ogiwara T, Mizuma H & Mori M 2001 Expression and regulation of type II iodothyronine deiodinase in human thyroid gland. *Endocrinology* **142** 2961–2967.
- Nguyen TT, Chapa F & DiStephano JJ III 1998 Direct measurement of the contributions of type I and II 5'-deiodinase to whole body steady state 3,5,3'-triiodothyronine production from thyroxine in the rat. *Endocrinology* **139** 4626–4633.
- de Onis M, Monteiro C, Akre J & Glugston G 1993 The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. *Bulletin of the World Health Organization* **71** 703–712.
- de Onis M, Frongillo EA & Blossner M 2000 Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980. *Bulletin of the World Health Organization* **78** 1222–1233.
- Passos MC, Ramos CF, Mouço T & Moura EG 2001a Increase of T₃ secreted through the milk in protein restricted lactating rats. *Nutrition Research* **21** 917–924.
- Passos MC, Ramos CF, Dutra SCP & Moura EG 2001b Transfer of iodine through the milk in protein-restricted lactating rats. *Journal of Nutritional Biochemistry* **12** 300–303.
- Passos MC, Ramos CF, Dutra SCP, Mouço T & Moura EG 2002 Long-term effects of malnutrition during lactation on the thyroid function of offspring. *Hormone and Metabolic Research* **34** 40–43.
- Pazos-Moura CC, Moura EG, Dorris ML, Rehnmark S, Melendez L, Silva JE & Taurog A 1991 Effect of iodine deficiency and cold exposure on thyroxine 5'-deiodinase activity in various rat tissues. *American Journal of Physiology* **260** E175–E182.
- Ramos CF, Lima APS, Teixeira CV, Brito PD & Moura EG 1997 Thyroid function in post-weaning rats whose dams were fed a low-protein diet during suckling. *Brazilian Journal of Medical and Biological Research* **30** 133–137.
- Ramos CF, Teixeira CV, Passos MCF, Pazos-Moura CC, Lisboa PC, Cutry FH & Moura EG 2000 Low protein diet changes thyroid function in lactating rats. *Proceedings of the Society for Experimental Biology and Medicine* **224** 256–263.
- Reeves PG, Nielsen FH & Fahey GC 1993 AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. *Journal of Nutrition* **123** 1939–1951.
- Sabatino L, Iervasi G, Ferrazzi P, Francesconi D & Chopra IJ 2000 A study of iodothyronine 5'-monodeiodinase activities in normal and pathological tissues in man and their comparison with activities in rat tissues. *Life Sciences* **68** 191–202.
- Salvatore D, Bartha T, Harney JW & Larsen PR 1996a Molecular biological and biochemical characterization of the human type 2 selenodeiodinase. *Endocrinology* **137** 3308–3315.
- Salvatore D, Tu H, Harney JW & Larsen PR 1996b Type 2 iodothyronine deiodinase is highly expressed in human thyroid. *Journal of Clinical Investigation* **98** 962–968.
- Silva JE & Larsen PR 1985 Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. *Journal of Clinical Investigation* **76** 2296–2305.
- Song S, Sorimachi K, Adachi K & Oka T 2000 Biochemical and molecular biological evidence for the presence of type II iodothyronine deiodinase in mouse mammary gland. *Molecular and Cellular Endocrinology* **160** 173–181.
- Steinsapir J, Harney J & Larsen PR 1998 Type 2 iodothyronine deiodinase in rat pituitary tumor cells is inactivated in proteasomes. *Journal of Clinical Investigation* **102** 1895–1899.
- Steinsapir J, Bianco AC, Buettner 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.
- Valverde C & Aceves C 1989 Circulating thyronines and peripheral monodeiodination in lactating rats. *Endocrinology* **124** 1340–1344.
- Wu SY, Reggio R & Florsheim WH 1985 Characterization of thyrotropin-induced increase in iodothyronine monodeiodinating activity in mice. *Endocrinology* **116** 901–908.

Received in final form 30 January 2003
Accepted 4 February 2003