Low-Protein Diet Changes Thyroid Function in Lactating Rats (44539)

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Abstract. **Lactating rats were fed with free access to an 8% protein-restricted diet (PR); the control group was fed a 23% protein diet (C). An energy-restricted (pair-fed) group was given the same food as the animals in the control group, but the amounts of food consumed by both PF and PR were about the same. The body weight and serum albumin concentration of PR and PF dams were significantly ($P < 0.05$) lower than that of the controls. The PR group had a significant increase in serum-free triiodothyronine (FT_3) concentration, 24-hr mammary gland and milk radioiodine (I^{131}) uptake (67%, 278%, and 200%, respectively) as compared with the controls. On the other hand, those animals had a significantly lower serum-free thyroxine (FT_4) concentration and 2- and 24-hr thyroid I^{131} uptake (67%, 64%, and 74%, respectively). Protein malnutrition during lactation did not alter thyroid or liver 5′-deiodinase activity significantly. However, PF dams had a significantly lower (25%) thyroid 5′-deiodinase activity. These data suggest that protein-restricted lactating dams had an adaptive change in the thyroid function, which could be important to increase the transference of iodine or triiodothyronine through the milk to their pups and prevent sequelae of neonatal hypothyroidism.** [P.S.E.B.M. 2000, Vol 224:256–263]

Malnutrition is the most prevalent form of nutritional disorder among children in developing countries. In 1993, Onis *et al.* (1) reported that, based on OMS data, about 43% of children in developing countries suffer from malnutrition during some period in their lives. Protein malnutrition often occurs during gestation, lactation, and the first 2 years of life (2).

Thyroid hormones play an important role in mammary gland development (3) and in the increasing synthesis and secretion of important milk proteins, such as α-lactalbumin (4). However, lactation is accompanied by a paradoxical decrease in circulating levels of triiodothyronine (T_3) and thyroxine (T_4), as well as an increase in 3-3′-5′-reverse triiodothyronine (rT_3) concentration (5). Lorscheider and Reineke (6) showed that as the number of pups per litter increased from 0 to 12, serum T_4 had a corresponding reduction. That particular rearrangement in circulating thyronines was associated with a significant reduction of T_4-5′-monodeiodinase activity in the kidney (7) and liver (7, 8) and the concurrent appearance of that enzymatic activity in the lactating mammary gland (8).

Adult, food-restricted rats have several changes in thyroid function, such as a decrease in circulating levels of T_3, as well as an increase in rT_3 values (9–11) with reduced (11–13) or normal (14, 15) serum TSH. In addition, food restriction has been associated with a reduction in ^{131}I uptake by the thyroid, in thyroid peroxidase activity (12, 16), and in hepatic T_4 to T_3 conversion caused by a decrease in

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5′-monodeiodinase activity (10). Contrary to what was observed in total energy restriction, in postweaned rats fed protein-deficient diets there are some reports showing an increase in total and free T_3 serum concentration (17, 18), which has not been confirmed by others (19).

Several studies suggest that malnutrition in growing animals causes changes in thyroid economy that are different from those described in adult animals. Several experimental models were designed to evaluate thyroid function in young malnourished animals, but no model was restricted to evaluate malnutrition only during the lactation period. Most authors studied malnutrition over the gestational period or both the gestational and lactation periods. Coleoni *et al.* (20) showed that adult rats whose mothers had been fed a low-protein diet during gestation and lactation and up to 50 days after weaning presented higher T_3 serum concentration. Changes in neural development (21) and insulin secretion (22, 23) were reported in models of malnutrition only during lactation in the malnourished weaning rat. We have shown recently, for the first time, that in postweaned rats whose dams were fed a low-protein diet during suckling, the total serum T_3 concentration was lower than that of the control group (24).

During gestation, protein malnutrition causes a delay in thyroid follicle formation and a reduction in gland area, follicle number, colloid space, and cell size in the thyroid tissue of fetal and neonatal pups from protein-deprived dams (25). Some authors have shown that fetuses or newborns from protein-deprived dams had a decrease in total serum T_3 concentration with normal total serum T_4 and thyroid stimulating hormone (TSH) concentration (26, 27). Oberkotter and Rasmussen (28) reported that the offspring of chronically food-restricted rat dams had lower total serum T_3 (TT_3) and higher serum rT_3 levels in 7-,14-, or 21-day-old animals and lower total T_4 (TT_4) only in 14-day-old animals.

Chronically food-restricted female rats have alterations in milk composition and volume during lactation. Total milk fat, protein, and energy available to the pups are reduced (29, 30). There are scanty data about the thyroid function of protein-malnourished dams. It had been reported that female rats submitted to energy-restricted diet since 42 days of age and continuing during gestational and lactational period, showed persistent decline in circulating T_3 levels at lactaton (28). There is considerable doubt about the extent to which nutritional status influences lactation performance and its implications for infant growth and nutritional status, since changes in iodine metabolism could have consequences for the thyroid function of the neonate, with possible impairment in central nervous system development. We hypothesized that the thyroid status of lactating, food-restricted dams can suffer adaptive changes that could be of importance in maintaining thyroid status in the offspring. Conversely, protein malnutrition during lactation can cause changes in the thyroid function of the offspring. It is not known if these changes are reversible after the reestablishment of a normal protein diet.

No study to our knowledge has been designed specifically to test the hypothesis that protein malnutrition during only the lactation period causes alterations in the thyroid function of the dams. Therefore, this paper is the first to report the effect of protein malnutrition during lactation on the thyroid function of the dams.

Materials and Methods

Wistar rats were kept in a room with controlled temperature (25° ± 1°C) and with artificial dark-light cycle (lights on from 0700 hr to 1900 hr). Three-month-old, virgin female rats were caged with one male rat at a proportion of 2:1. After mating, each female was placed in an individual cage with free access to water and food until delivery. The use and handling of experimental animals followed the principles described in the *Guide for the Care and Use of Laboratory Animals* (31).

Dams were assigned randomly to one of the following groups: (i) control group, with free access to a standard laboratory diet containing 23% protein; (ii) protein-restricted (PR) group, with free access to an isoenergy and protein-restricted diet containing 8% protein; and (iii) an energy-restricted group (pair-fed, PF), receiving a standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PR group. In this way, the amounts of food consumed by both PF and PR groups were about the same. Table I shows the composition of the diets, which follows recommend standards (32).

Within 24 hr of birth, excess pups were removed, so that only six male pups were kept per dam because it has been shown that this procedure maximizes lactation perfor-

Table I. Composition of the Diets

	Control[a]	Low-protein[b]	PF
Ingredients (g/kg)			
Soybean + wheat	230.0	80.0	230.0
Corn starch	676.0	826.0	676.0
Soybean oil	50.0	50.0	50.0
Vitamin mix[c]	4.0	4.0	4.0
Mineral mix[c]	40.0	40.0	40.0
Macronutrient composition (%)			
Protein	23.0	8.0	23.0
Carbohydrate	66.0	81.0	66.0
Fat	11.0	11.0	11.0
Total energy (KJ/kg)	17038.7	17038.7	17038.7
Mean energy intake (KJ/day)	512.2	311.8	311.8

[a] Standard diet for rats (Nuvilab-NUVITAL Nutrientes LTDA, Paraná, Brazil).

[b] The low-protein diet was prepared in our laboratory using the control diet and replacing part of its protein with corn starch. The amount of the latter was calculated to make up for the decrease in energy content due to protein reduction.

[c] Vitamin and mineral mixture were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (32).

mance (33). Malnutrition was started at birth, which was defined as Day 0 (d0) of lactation and was ended at weaning (d21).

To determine 2-hr or 24-hr radioactive iodine uptake by the thyroid and extra thyroidal tissues, dams received a single intraperitoneal injection containing 2.22 × 10^4 Bq of ^{131}I (IPEN, São Paulo, Brazil). Females were separated from their litters 2 hr before sacrifice, being allowed access to the appropriate diet during the separation period; after 90 min they were injected subcutaneously with 1 ml oxytocin (Eurofarma, 5 UI oxytocin/ml) (29, 30). Thirty minutes after oxytocin administration, milk samples were obtained by gently stripping the thoracic and abdominal teats. Then, the animals were sacrificed with a lethal dose of pentobarbital, and blood was obtained by cardiac puncture. The thyroid and mammary gland of dams were excised and weighed. The ^{131}I uptake in all tissues and milk was individually evaluated in a gamma counter (Cobra Auto-gamma, Packard Instrument Co., Downers Grove, IL).

Blood samples were centrifuged to obtain serum, which was individually kept at −20°C until assay. T_4 and T_3 free serum concentrations were measured by radioimmunoassay (RIA) using commercial kits (Coat-A-Coat, DPC, Los Angeles, CA), in which we used control standard curves diluted in iodothyronine-free rat serum (charcoal-treated). (The assays were validated since the values for these controls were almost superimposed on the kit standard curve.) Serum TSH was measured by RIA (34) using a specific rat TSH kit supplied by the NIDDK (Bethesda, MD, rp-3). Serum albumin was used as an index of protein malnutrition and was determined by a colorimetric method (Bioclin, Belo Horizonte, MG, Brazil).

Liver and thyroid were dissected out and processed for type I 5′-deiodinase (5′-DI) activity determinations as previously described (35, 36). Briefly, the tissues were homogenized in 50 m*M* Tris-HCl buffer, pH 6.8, centrifuged at 1500*g* (thyroid) or 15,000*g* (liver), at 4°C for 30 min (thyroid) or 20 min (liver). Supernatants of both tissues were centrifuged at 190,000*g* for 90 min, and the pellets, containing the microsomal fractions, were resuspended and stored at −70°C until assayed. Hepatic (190–356 µg of protein) and thyroid (42–270 µg of protein) microsomal fractions were assayed for type I 5′-DI activity in phosphate buffer containing 1 m*M* EDTA (Reagen, Brazil), pH.6.9, in the presence of 1.5 µ*M* rT_3 and 100 m*M* DTT. Equal volumes of the ^{125}I-rT_3 (Amersham, England), purified before each set of assays by paper electrophoresis, were added to each tube assay. Incubations, in a shaking water bath at 37°C, were stopped after 30 min by the addition of a mixture of 8% BSA (Sigma, St. Louis, MO) and 10 m*M* PTU, followed by cold 20% TCA (Reagen, Brazil). Samples were then centrifuged (2000 rpm, 4°C, 5 min), and 200 µl of the supernatant were applied to Dowex 50 W-X2 (BioRad, Hercules, CA) columns. ^{125}I, eluted from the column with 10% acetic acid, was measured in a gamma counter. The specific enzyme activity was expressed by picomoles of rT_3 deiodinated/hr × mg of protein. Protein was measured by the method described by Bradford (37).

Statistical Analysis. The data are reported as mean ± SEM. The statistical significance of experimental observations was determined by the one-way analysis of variance followed by Newman Keuls test with the level of significance set at $P < 0.05$.

Results

Lactating rats given free access to the low-protein diet consumed only 60% of diet compared with the amount eaten by rats receiving the normal diet. The PR and PF dams had a significantly ($P < 0.05$) lower serum albumin concentration. The body weights of PR and PF dams were significantly ($P < 0.05$) lower than the controls from 6 to 21 days after the beginning of lactation. Thyroid weight at the end of lactation did not differ among the groups (Table II).

Figures 1, 2, and 3 depict the radioactivity in the thyroid gland, mammary gland, and milk after 2 hr and 24 hr from a single injection of ^{131}iodine. Comparing the values of PR, PF, and control dams, the former showed lower ($P < 0.001$) ^{131}I thyroid uptake, both at 2 hr and 24 hr. The PF group had values intermediate between the PR and control groups (Fig. 1A).The PF group had a significantly ($P < 0.05$) lower thyroid uptake than that of the control group when the uptake was measured after 24 hr of ^{131}I injection (Fig. 1B).

In contrast to thyroid uptake, the mammary gland of PR dams had a significant ($P < 0.01$) increase at 24 hr as com-

Table II. Body Weight, Serum Albumin Concentration, and Thyroid Weight in Dams Submitted to a Control (C), a Protein-Restricted (PR), or an Energy-Restricted (PF) Diet During Lactation

	End of lactation (21d)		
	C	PR	PF
Body weight (g)	205.1 ± 4.4 (10)	141.9 ± 4.0^a (9)	139 ± 4.5^a (9)
Serum albumin (g/l)	0.33 ± 0.02 (7)	0.21 ± 0.02^a (9)	0.27 ± 0.01^{a,b} (6)
Thyroid weight (mg)	13.0 ± 0.7 (10)	10.8 ± 1.0 (9)	16.8 ± 2.4 (6)

A control (C) group had free access to a standard laboratory diet containing 23% protein, and a protein-restricted (PR) group had free access to an isoenergy and protein-restricted diet containing 8% protein. An energy-restricted (pair-fed) group received the same food as the animals in the control group, but the amounts of food consumed by both PF and PR were about the same. Values are given as the mean ± SEM. Significant differences between either the diet-restricted groups and controls (^{a}), or between the two diet-restricted groups (^{b}), were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

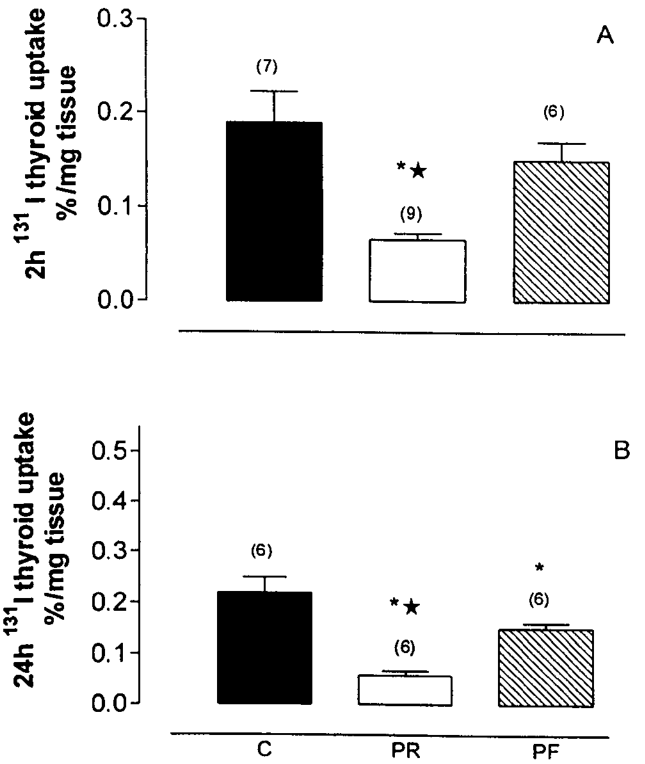

Figure 1. (A) Two-hr and (B) 24-hr ^{131}I thyroid uptake in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between either the diet-restricted groups and controls (*), or between the two diet-restricted groups (★), were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

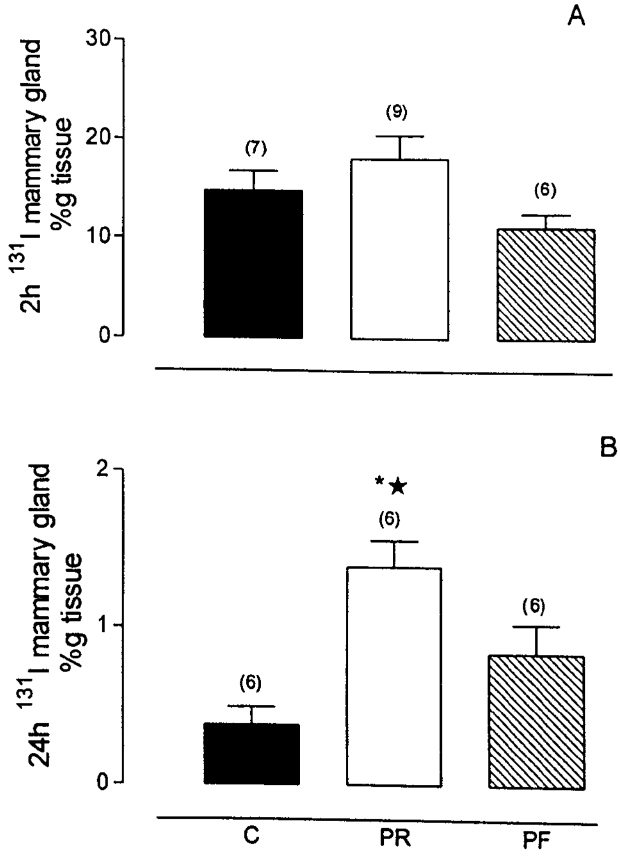

Figure 2. (A) Two-hr and (B) 24-hr ^{131}I mammary gland uptake in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between either the diet-restricted groups and controls (*) or between the two diet-restricted groups (★) were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

pared with the control and PF groups (Fig. 2B). In the PF group, mammary gland uptake was not significantly altered compared with controls (Figs. 2A & 2B). Those changes in mammary gland uptake of PR dams were parallel to the changes in radioactivity present in the milk (Fig. 3). However, in the milk, the radioactivity was significantly ($P < 0.05$) increased only at 24 hr in both malnourished groups (Fig. 3B).

Figure 4 shows the effects of dietary treatment on serum FT_3 and FT_4 concentrations. Protein restriction during lactation was associated with a significant ($P < 0.05$) increase of serum FT_3 concentration (Fig. 4A) and lower ($P < 0.001$) serum FT_4 concentration as compared with controls (Fig. 4B). When compared with the PF group, the increase of serum FT_3 in the PR group was even greater ($P < 0.001$). In contrast, PF group had a decrease ($P < 0.05$) in serum FT_3 concentration as compared with controls (Fig. 4A). Serum FT_4 was also decreased ($P < 0.05$) in the PF group (Fig. 4B).

Serum TSH in PR dams did not differ significantly from controls; however, the PF group showed significantly lower ($P < 0.001$) serum TSH as compared with the control and PR groups (Fig. 5).

Thyroid or liver 5′-deiodinase activity was not significantly affected by protein restriction during lactation. However, PF dams had a significantly ($P < 0.05$) lower thyroid 5′-deiodinase activity (Fig. 6A).

Discussion

According to the results described in the literature (24, 29; 38), we showed that reductions in the protein/energy ratio of diets given to lactating rats can markedly impair food intake. However, since our experimental design included the pair-fed group, we were able to identify protein restriction–specific changes. The alterations observed in the pair-fed group were different from those observed in the PR group, and were similar to those often reported by other researchers for adult animals subjected to total energy restriction.

Protein restriction in lactating dams was associated with a state characterized by a significant reduction in body weight and serum albumin concentration, an indication of malnutrition.

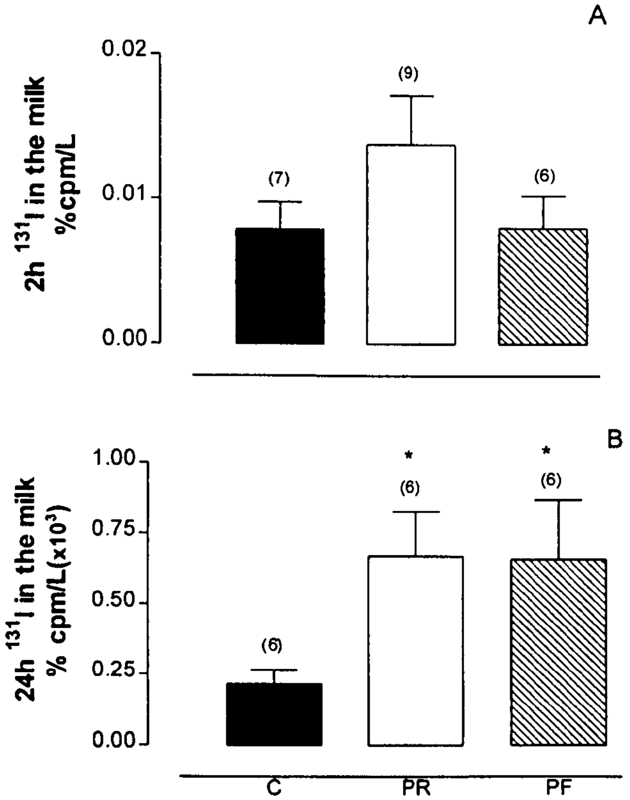

Figure 3. (A) Two-hr and (B) 24-hr ^{131}I milk radioiodine content in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between the diet-restricted groups and controls (*) were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

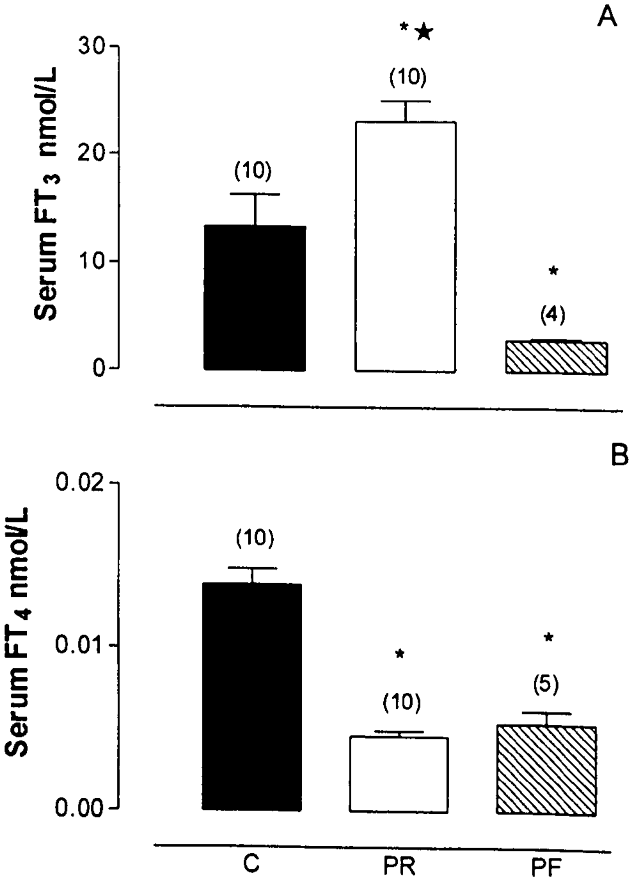

Figure 4. (A) Free T_3 and (B) Free T_4 serum concentrations in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between either the diet-restricted groups and controls (*) or between the two diet-restricted groups (★) were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

During lactation, protein-restricted dams had a significant reduction in the 2 hr and 24 hr ^{131}I thyroid uptake when compared with the control group. Concomitantly ^{131}I uptake by the mammary gland was increased as was the iodine concentration in the milk at 24 hr in the PR group. Although those parameters also showed a parallel change in pair-fed dams, the alterations occurred only at 24 hr in the milk, suggesting a deviation of iodine from the thyroid to the milk in both groups. To the best of our knowledge, there are no published data about the amount of iodine in the milk under conditions of protein or energy restriction during lactation.

Since the decrease in thyroid and increase in mammary gland radioiodine uptake are not exactly time or magnitude correlated, it is not likely that the higher supply of serum iodine caused by the blunted thyroid uptake would explain the increment in mammary gland uptake and iodine content in the milk. We speculate that the sodium iodine symporter (NIS) transcriptional activity in the mammary gland could be regulated by protein malnutrition. Recently, Spitzweg *et al.* (39) showed that the nucleotide sequences of hNIS cDNA derived from mammary gland, gastric mucosa, and parotid glands revealed full identity with the human thyroid–derived NIS cDNA sequence. It is possible that the variability of NIS gene expression levels in that situation may be caused by differences in NIS gene transcriptional activity. Our data suggest that the regulation of NIS in the mammary gland could have some physiological importance.

Another mechanism to explain the increase in iodine content in the milk could be the higher serum prolactin concentration in dams submitted to protein or energy restriction. Rillema and Rowady (40) related that the prolactin stimulates iodine mammary gland uptake in rats, probably by regulating NIS activity and expression on the mammary gland (41). Serum prolactin concentration has been found to be increased in mothers with energy restriction during lactation (42). We did not evaluate prolactin in our lactating rats, but according to those previous data in humans, we can suggest that an increase in prolactin could stimulate iodine uptake into the mammary gland.

In malnutrition, serum TSH concentration can be reduced (11–13) or normal (14, 15). Our data showed increased serum TSH concentration in protein-restricted, lactating rats and decreased serum TSH concentration in calo-

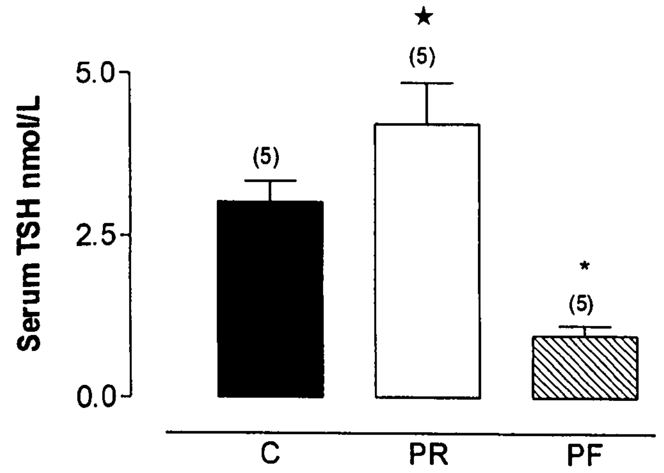

Figure 5. TSH serum concentration in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between the diet-restricted groups and controls (*) were determined by a multiple comparison of means test with the level of significance set at $P < 0.05$ (see Materials and Methods). The numbers of animals studied are shown in parentheses.

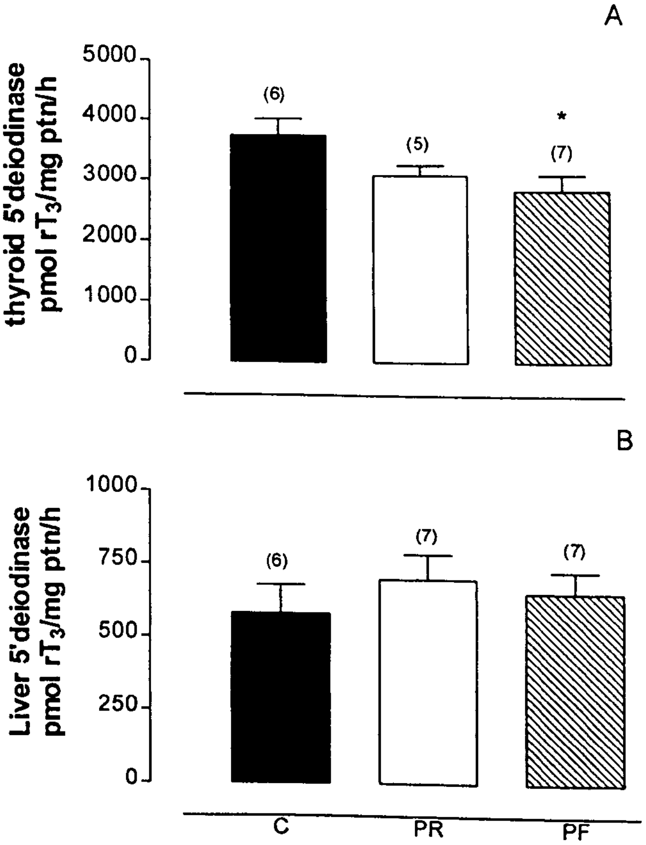

Figure 6. (A) Thyroid and (B) liver 5′-deiodinase activity in the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diet groups during lactation. Values are given as the mean ± SEM. Significant differences between the diet-restricted groups and controls (*) were determined by a multiple comparison of means test with the level of significance set at $P <$ 0.05 (see Materials and Methods). The numbers of animals studied are shown in parentheses.

rie-restricted, lactating rats. Decreased thyroid radioiodine uptake in the PF group can be explained by suppressed serum TSH concentration. Decreased thyroid radioiodine uptake in the PR group, despite increased serum TSH concentration, probably represents one of the adaptation mechanisms of protein malnutrition. Since the iodine uptake by the thyroid is an active process dependent on a protein carrier (43), generalized deficits in the protein synthesis or in the enzymatic machinery could be responsible for those alterations. Conversely, the supply of iodine or thyroid hormone levels could regulate the symporter in the mammary gland in the opposite direction. We are now beginning to evaluate the control of these symporters by dietary manipulation.

The pituitary feedback system appears to be functional in protein-restricted, lactating rats, since T_4 and not T_3 is the main regulator of TSH secretion (44). However, this feedback system was impaired in calorie-restricted, lactating rats as low serum FT_4 did not increase serum TSH concentration.

Reduced free T_4 with increased free T_3 in the serum of protein-restricted dams suggests increased T_3 production by the thyroid or by peripheral T_4 to T_3 conversion. That is a specific consequence of protein deficiency since total energy restriction was followed by decreases in both T_4 and T_3 serum concentrations. However, previous studies have demonstrated that the increase in serum T_3 also occurs in nonpregnant protein-restricted rats (17, 18), leading to specific metabolic changes (18, 45). The metabolic consequences of higher serum T_3 in states of protein deficiency are not known. If that change in thyroid hormone production has some adaptive advantage, it has not been verified for pregnant and nonpregnant animals. Despite similar results for FT_4, the PF group showed FT_3 serum concentrations lower than the ones in the control group and similar to the findings of other reports on nonlactating animals. This again reinforces the concept that specific adaptive responses occur when the energy restriction is associated with a lower protein proportion in the diet.

To investigate the mechanism by which serum T_3 is increased in the presence of decreased serum T_4, we evaluated the activity of the enzyme responsible for production of serum T_3, the type I 5′-deiodinase in the liver and thyroid, which are major contributors to serum T_3 in the rat (46).

Decreased thyroid 5′-DI activity correlates well with decreased serum T_3 in pair-fed animals. Thus, it is likely that the reduction in serum TSH in those dams is responsible for the lower 5′-DI activity in the thyroid gland. However, neither the thyroid nor the liver deiodinase changed in PR animals. The fact that we did not find changes in thyroid or liver deiodinase did not exclude the increase in other tissues as mammary gland, kidney, muscle, or brown adipose tissue. Some authors (8, 47) have reported that type I 5′-deiodinase is decreased in the liver and increased in the mammary gland of lactating rats, suggesting that 5′-DI activity in mammary gland could change according to different nutritional conditions.

Our data suggest that the iodine metabolism in protein or energy-restricted rats during lactation could be modified and might reflect an adaptive mechanism of dams to increase the supply of iodine to the offspring through the milk. Thyroid hormone production is different in both dietary treatments. The increase of serum T_3 concentrations in protein-restricted dams suggests an adaptation designed to provide more T_3 to the offspring. That adaptation could be of some importance in the prevention of the neonatal hypothyroidism.

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