Journal of Nutritional Biochemistry 12 (2001) 300–303

THE JOURNAL OF Nutritional Biochemistry

Transfer of iodine through the milk in protein-restricted lactating rats

Magna Cottini Fonseca Passos[a], Cristiane da Fonte Ramos[b], Sheila Cristina Potente Dutra[a], Egberto Gaspar de Moura[c,*]

[a]Department of Applied Nutrition, Nutrition Institute, State University of Rio de Janeiro Brazil
[b]Department of Anatomy, State University of Rio de Janeiro Brazil
[c]Department of Physiological Sciences, State University of Rio de Janeiro, 20550-030, Rio de Janeiro, RJ, Brazil

Received 27 July 2000; received in revised form 4 December 2000; accepted 21 December 2000

Abstract

Iodine supply is important to avoid neonatal hypothyroidism. This study evaluated whether protein restriction during lactation affects iodine transfer to the pups through the milk. We studied lactating rats fed an 8% protein-restricted diet (PR), a control 23% protein diet (C), and an energy-restricted diet group (ER). On days 4, 12 and 21, mothers were separated from their pups for 4 h, injected with ^{131}I IP, and put together with their pups. The animals were killed 2 h later. PR pups had a significant decrease in iodine uptake in the gastric content and duodenal mucosa on the 4th day. On the contrary, at 12 and 21 days radioiodine was increased in the gastric content and in the duodenal mucosa. ER pups had an increase in iodine uptake in the gastric content and in the duodenal mucosa only at the end of lactation. The thyroid iodine uptake in PR pups was significantly decreased on the 4th day and significantly increased on the 21st day compared to control. When injected IP with an equivalent amount of ^{131}I, the PR pups had a decrease in thyroid iodine uptake on the 4th and 12th day, while ER pups had no significant changes. So, these data suggest that protein restriction during lactation was associated with lower iodine secretion into the milk in the beginning of lactation. However, at the end of lactation, an adaptation process seems to occur leading to a higher transfer of iodine through the milk that compensates the impairment of thyroid iodine uptake in these pups.

Keywords: Iodine; Protein restriction; Milk; Rats; Lactation

1. Introduction

There are some reports showing an increase of total and free T_3 serum concentration [1,2] in post-weaned rats fed protein-deficient diets. However there are scanty data about the effects of protein restriction during lactation on thyroid function or iodine metabolism of mothers and their offspring.

Changes in serum T_3 concentration [3], in neural development [4], insulin secretion [5] and body weight [6] were reported in models of protein malnutrition only during lactation in the post-weaned rats. These studies reinforce the metabolic imprinting concept, intended to describe the basic biological phenomena that putatively underlie relations among nutritional experiences of early life and later diseases [7].

Iodine accumulated in lactating mammary gland and secreted into milk is used by the newborn for thyroid hormone biosynthesis. An adequate supply of iodine for sufficient production of thyroid hormones is essential for proper development of the newborn's nervous system [8]. Iodine deficiency at this early stage of life results in severe mental retardation [8].

Previously we showed that the dams submitted to a protein-restricted diet during lactation had, at the end of lactation, a significant decrease in the ^{131}I thyroid uptake and increase in the ^{131}I uptake by the mammary gland and milk when compared to the control dams [9]. It seems that the iodine metabolism in protein or energy-restricted rats during lactation could be modified and can reflect an adaptive mechanism of dams to increase the supply of iodine to the offspring through the milk. This adaptation could be of

This work was supported by a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and by funds from Pós-Graduação em Biologia (PGBN-UERJ).

* Corresponding author. Tel.: +1-021-5876434; fax: +1-021-5876129.

E-mail address: egmoura@uerj.br (E.G. de Moura).

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PII: S0955-2863(01)00142-5

Table 1
Composition of the control and low-protein diets

	Control[§]	Protein-Restricted*
Ingredients (g/Kg)		
Soybean + Wheat	230.0	80.0
Corn starch	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)	17038.7	17038.7

[§] 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 corn starch. 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 diets [11].

some importance in the prevention of the neonatal hypothyroidism.

So, this study was designed to evaluated whether protein restriction during lactation affects iodine transfer to the pups through the milk during three different periods of lactation.

2. Material and methods

Wistar rats were kept in a room with controlled temperature (25 ± 1°C) and with artificial dark-light cycles (lights on from 7:00 A.M. to 7:00 P.M.). 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 and handling of experimental animals followed the principles described in the Guide for the Care and Use of Laboratory Animals [10].

Mothers were randomly assigned to one of the following groups: (C) control group, with free access to a standard laboratory diet containing 23% protein; (PR) protein-restricted group, with free access to an isoenergy and protein-restricted diet containing 8% protein; and, (ER) energy-restricted group, 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 in both ER and PR groups were about the same. Table 1 shows the composition of the diets, which follows recommended standards [11].

The protein-restricted diet was prepared in our laboratory using the control diet and replacing part of its protein with cornstarch. The amount of starch was calculated so as to make up for the decrease in energy content due to protein reduction.

Within 24 hours of birth, excess pups were removed, so that only 6 pups were kept per dam because it has been shown that this procedure maximizes lactation performance [12]. Malnutrition was started at birth, which was defined as day 0 (d0) of lactation, and continued to the day the animal was sacrificed.

To study iodine transfer to the pups through the milk during three different periods of lactation we used three dams on day 4, two dams on day 12, and four dams on day 21 of lactation. In the day of sacrifice the dams were separated from their pups for 4 h. After this time the dams received a single intraperitoneal (IP) injection containing 2.22×10^4 Bq of ^{131}I (IPEN, São Paulo, Brazil) and the pups were allowed to nurse for 2h. Then, only the male pups were killed with a lethal dose of pentobarbital and blood was obtained by cardiac puncture. The thyroid gland and duodenum were excised and weighed. The stomach was excised and rinsed in normal saline. The stomach contents were obtained through an incision made in the fundal area of the stomach. This content was carefully weighed. Gastric ^{131}I content was considered as ^{131}I content in milk.

In another set of experiments, we evaluate thyroid ^{131}I uptake in pups, in which we used three dams on day 4, two dams on day 12, and three dams on day 21 of lactation. Each male pup received an IP injection containing 2.22×10^4 Bq of ^{131}I (IPEN, São Paulo, Brazil).

The ^{131}I uptake in all tissues was individually evaluated in a gammacounter (Cobra Auto-gamma, Packard Instrument Co., Downers Grove, Illinois, USA).

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

3. Results

The thyroid iodine uptake was significantly ($p < 0.001$) increased at the end of lactation in the three experimental groups. The thyroid iodine uptake in PR pups was significantly decreased on the 4th day (66%), normal on the 12th day and significantly increased on the 21st day (56%) compared to the control group. The ER pups demonstrated no significant changes in the thyroid iodine uptake on the 4th and 12th days, however, they demonstrated a significant increase (98%) at the end of lactation (Fig. 1A). When injected IP with an equivalent amount of ^{131}I, the PR pups had a significant decrease in thyroid iodine uptake on the 4th (91%) and 12th day (85%), while ER pups had no significant changes (Fig. 1B).

Fig. 2 depicts the ^{131}I gastric content and duodenum iodine uptake of pups on days 4, 12 and 21. The control and ER groups had a significant ($p < 0.05$) decrease in the radioiodine gastric content from the beginning to the end of the lactation. Pups of PR mothers had a significant decrease in iodine uptake in the gastric content (50%) and duodenum

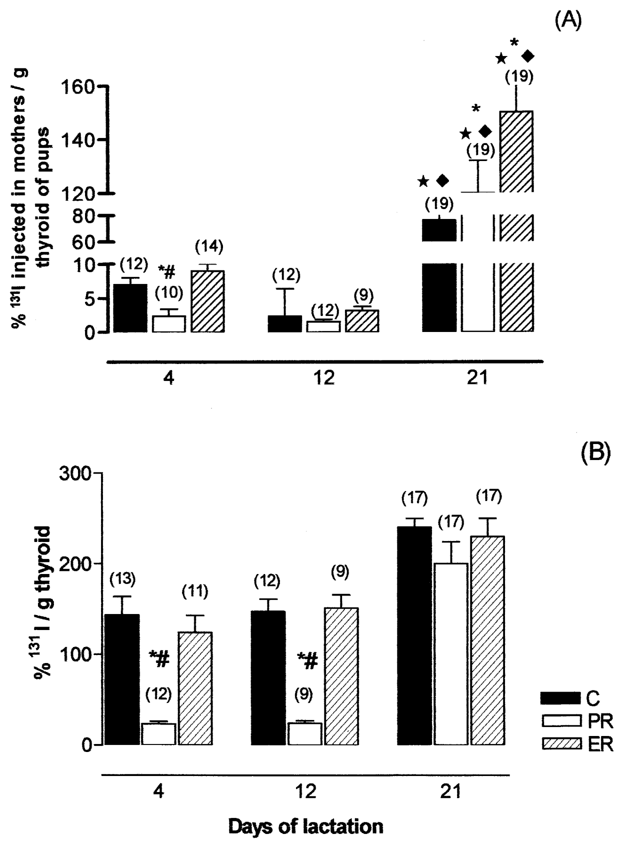

Fig. 1. Thyroid uptake in pups nursing mothers injected with ^{131}I (A) and thyroid uptake in pups injected with ^{131}I (B). The mothers were submitted to the control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diets during lactation. Values are given as the mean ± SEM. Significant differences between the diet-restricted groups and controls (*), between the two diet-restricted groups (#), between the day 4 and 21 (◆) or between the day 12 and 21 (★) were determined by a multiple comparison of means test with the level of significance set at $p < 0.05$ (see Material and methods). The numbers of animals studied are shown in parentheses.

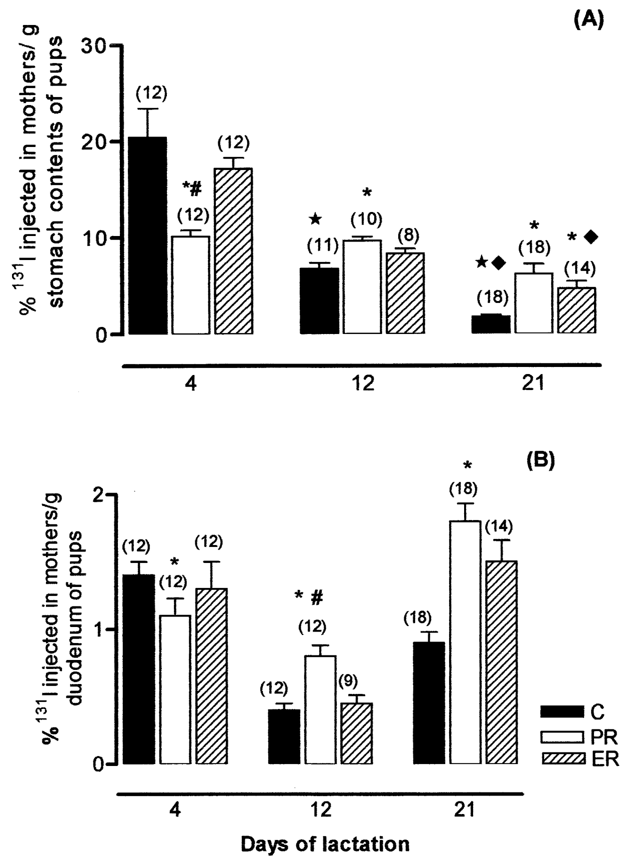

Fig. 2. ^{131}I stomach content (A) and duodenum uptake (B) in pups nursing mothers fed control (black bars), protein-restricted (white bars), and energy-restricted (hatched bars) diets groups during lactation. Values are given as the mean ± SEM. Significant differences between the diet-restricted groups and controls (*), between the two diet-restricted groups (#), between days 4, 12 and 21 (◆) or between day 12 and 21 (★) were determined by a multiple comparison of means test with the level of significance set at $p < 0.05$ (see Material and methods). The numbers of animals studied are shown in parentheses.

(24%) on the 4th day. On the contrary, at 12 and 21 days radioiodine was increased in the gastric content (42% and 200%, respectively), and duodenum (81% and 102%, respectively). ER pups only had an increase in iodine content in the stomach and duodenum at day 21.

4. Discussion

It is the first time that iodine metabolism is evaluated in protein malnutrition during lactation. Despite the impairment of thyroid iodine uptake in the offspring of protein-malnourished dams during the first half of lactation, which was shown by the direct IP injection of ^{131}I in pups, the higher transfer of iodine, which was detected by the 12th day of lactation surpass the iodine deficient transport in the thyroid. This compensation was shown by the increase in the uptake at the end of lactation when the radioiodine was injected in the mother. Besides the higher supply, it is probable that this compensation is due also to an increase in iodine absorption by the gastrointestinal tract.

In the beginning of lactation, the lower duodenal uptake can be caused by morphological alterations due to protein deficiency. Shrader et al. [13] studying the effect of protein restriction on intestinal development during different stages of gestation, showed that pups at 4 and 8 days of life had a decrease in the number of absorptive cells and in the number and length of villi; these differences disappeared at 12 days. In adult rats, Qu et al. [14] demonstrated that protein restriction was associated with a decrease in intestinal mRNA levels of IGF-1, a regulatory hormone of gut proliferation, and changes in mucosal histology.

The data of normal thyroid ^{131}I uptake in ER injected pups are not in agreement with those shown in adult male rats after five days of fasting [15]. Its possible that the amount of energy intake by the ER pups was insufficient to impair the iodine thyroid uptake. We showed recently [6] that the milk of ER mothers had a low total energy only at the beginning of lactation, from the middle of lactation we observed normal values of total milk energy due to an increase lipid concentration in the milk of these animals. On the other hand, milk total energy was lower in PR group, consequent to a lower protein concentration. Despite the normal thyroid ^{131}I uptake in ER injected pups, there was also a higher transfer of iodine at the end of lactation. We do not know what could be the effect of this higher supply of iodine on thyroid function in these animals.

We speculate that the sodium iodine symporter (NIS) transcriptional activity in the mammary gland could be regulated by protein malnutrition. Recently, Spitzweg et al. [16] showed that the nucleotide sequences of hNIS cDNA derived from mammary gland, gastric mucosa and salivary 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. Therefore, we cannot discard the possibility that, as in the mammary gland, the expression of NIS in the gastric mucosa characterized by Kotani et al. [17] can be regulated by nutritional factors.

In the small intestine data about the presence of NIS is still controversial. Vayre et al. [18] did not find by immunohistochemical methods positive staining in the human small intestine, while Mitsuma et al. [19] found positive immunostaining in the rat small intestine mucosa.

It has long been shown that there is a competition for iodine between the mammary glands and the thyroid in lactating rats fed a normal diet [20]. It has also been demonstrated that NIS is not detectable in the mammary gland of pregnant or no pregnant rats, its present only in mammary glands of lactating rats [21]. These data reinforce the concept that specific adaptive responses occur during lactation which are important to adequately supply iodine for sufficient production of thyroid hormones, which are essential for proper development of the newborn's nervous system [8]. However, the consequences of protein deficiency on this adaptive physiological mechanism are not known.

So, our data suggest that protein restriction during lactation is associated with lower iodine secretion into the milk in the beginning of lactation. However, at the end of lactation, an adaptation process seems to occur leading to a higher transfer of iodine into the milk that compensates the impairment of thyroid iodine uptake in these pups. Iodine metabolism is differentially affected according to the kind of malnutrition.

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