Adaptive changes in thyroid function of female rats fed a high-fat and low-protein diet during gestation and lactation

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Abstract

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The percent of lipids in the western diet has been continuously increasing in the last decades and is associated with a decrease in the proportion of protein intake. Recently, we demonstrated that protein malnutrition during lactation is associated with lower body weight and thyroid hypofunction in female rats and their offspring. Our objective in the present study was to determine if a high-fat and low-protein diet was associated with similar changes. Three-month-old female Wistar rats were randomly assigned to one of the following groups with 8 animals each: high-fat and low-protein (40% lipid, 5% protein, and 55% carbohydrate of the total energy content) from the 3rd week of gestation to the end of lactation; control group - standard diet (11% lipid, 23% protein, and 66% carbohydrate of the total energy content). Food consumption and body weight were monitored daily. Serum thyrotropin and thyroid hormone concentrations were determined by specific radioimmunoassay at the end of lactation. Animals receiving high-fat and low-protein diet had a significantly lower body weight (13.9% at weaning, $P < 0.05$) and serum albumin (25%, $P < 0.05$) and thyrotropin (26.2%, $P < 0.01$) concentrations, and a higher serum triiodothyronine concentration (74%, $P < 0.005$) and ^{131}I-thyroid uptake (77%, $P < 0.005$). These data show that a high-fat and low-protein diet can promote maternal thyroid hyperfunction that differs from the thyroid hypofunction observed in dams fed a low-protein diet, a phenomenon that can be of adaptive importance for pup nurturing.

Introduction

In recent years, attention has been focused on the effects of diet on cardiovascular and metabolic diseases. Many experimental and epidemiological studies have implicated specific dietary factors in the cause and prevention of conditions as diverse as cancer and coronary heart disease (1-3). Marked changes in food habits have been recently occurring in developing countries, characterized by a transition from energy and pro-

tein malnutrition to a gradual increase in dietary lipid content, also observed in the poorest populations (4,5). This dramatic increase in lipids in the westernized diet has not been properly studied regarding its physiological consequences during the perinatal period.

Some investigators have shown that the nutritional status of the mother during the gestational and lactational periods is essential to normal growth and development in humans (6-8) and in experimental animals (9-15).

Many experimental studies with high-fat diets have been used to study the effects of their consumption during gestation and lactation on nutrient intake, growth and survival of the pups. For the dams, the data are contradictory. The ingestion of a high-fat diet during gestation leads to a higher body weight gain by the mothers (14,16, 17). However, when this diet is given throughout gestation and lactation, the dams only maintain the body weight gained during pregnancy (14,17) or even suffer a large body weight loss (16). A high-fat diet seems to cause an increase in plasma triiodothyronine (T_3) levels in adult animals, suggesting that, through their actions on brown adipose tissue, thyroid hormones have an important thermogenic regulatory role that is essential for the maintenance of body weight (18-20).

We have recently shown that protein-restricted lactating dams present a loss of body weight gain (12), alterations in milk composition (11) and an important adaptive change in thyroid function by increasing the transfer of iodine and T_3 through the milk to their pups, probably preventing an eventual neonatal hypothyroidism (9,10,12).

In the present study, we determined if a high-fat and low-protein diet can cause changes in the body weight and thyroid function of lactating rats and if these changes are similar to those observed in animals submitted to protein and energy restriction.

Material and Methods

Wistar rats were kept in a room with controlled temperature (25 ± 1ºC) and with an artificial dark-light cycle (lights on from 7:00 am to 7:00 pm). Two 3-month-old virgin female rats were caged with one male rat. The use and handling of experimental animals followed the principles described in the Guide for the Care and Use of Laboratory Animals (21).

After mating, female rats were placed in individual cages and randomly assigned to one of the following groups with 8 animals each: high-fat and low-protein diet (HF/LP), with free access to a high-fat diet from the 3rd week of gestation to the end of lactation (40% lipid, 5% protein, and 55% carbohydrate of the total energy content); control, with free access to a stock diet (11% lipid, 23% protein, and 66% carbohydrate of the total energy content). The diets were prepared based on a stock commercial diet (Nuvilab, Nuvital Nutrients Ltda., Curitiba, PR, Brazil). The composition of the diets is shown in Table 1 and is based on recommended standards (22). The high-fat and low-protein diet was adapted from previous studies (14,16,17). The amounts of stock and high-fat diet eaten were measured daily.

Within 24 h of birth, excess pups were removed, so that only 6 male pups remained

Table 1. Macronutrient composition of the control and the high-fat, low-protein diets.

Macronutrient composition	Control diet	High-fat, low-protein diet
Protein	23.0	6.8
Carbohydrate	67.6	66.1
Fat	5.0	24.2
Saturated fatty acid	15.5%	52%
Polyunsaturated fatty acid	58%	3%
Monounsaturated fatty acid	23%	22%
Cholesterol	0%	0.1%
Total energy (kJ/100 g)	1711.1	2139.5

Data are reported as g/100 g diet and percent of total fat. Control diet is the standard diet for rats according to AIN-93G recommendations for rodent diets (22).

per dam because this procedure maximizes lactation performance (23).

Maternal body weight was monitored daily throughout the end of gestation and lactation. Serum albumin concentrations were measured at weaning (day 21).

On the day of sacrifice (day 21), the animals received a single intraperitoneal (*ip*) injection containing 2.22 x 10^4 Bq of ^{131}I (IPEN, São Paulo, SP, Brazil). After 2 h, they were killed with a lethal dose of pentobarbital and blood was obtained by cardiac puncture. The thyroid gland was excised and weighed and thyroid ^{131}I uptake was evaluated with a gamma counter (Cobra Autogamma, Packard Instrument Co., Downers Grove, IL, USA).

T$_3$ and tetraiodothyronine (T$_4$) were measured by commercial RIA adapted for rat serum, as described previously (24). Thyrotropin (TSH) was measured with a rat specific kit provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA) and results are reported in terms of the reference preparation (RP3). All assays were done with the same lot of reagents and at the same time, and the intra-assay coefficients of variation were: 4.5% for T3, 4.0% for T4 and 6.8% for TSH.

Statistical analysis

Two-way ANOVA was used to analyze the changes in food ingestion and body weight, and to test the effects of diet and time, followed by the Bonferroni post-test when P < 0.05. The Student *t*-test was applied to the data obtained on the 21st (last) day. The data are reported as means ± SEM and the level of significance was set at P < 0.05.

Results

Mothers on a high-fat, low-protein diet (group HF/LP) presented lower food consumption (40%, P < 0.001, Figure 1A), with a reduction of 82% in protein intake (P < 0.001, Figure 2B) and of 42% in carbohydrate (P < 0.001, Figure 2D) intake soon after the beginning of diet ingestion. Energy intake was lower (P < 0.05, Figure 2A) in this group on days 19 and 20, reaching a 23% lower level at parturition. However, fat intake was three times higher (P < 0.001, Figure 2C) during gestation. At the end of lactation, those differences were 46% lower for energy (P < 0.001, Figure 3A), 65% for protein (P < 0.001, Figure 3B) and 60% for carbohydrate intake (P < 0.001, Figure 3D) and 44% higher for fat intake (P < 0.01, Figure 3C) compared to control. The rate of carbohydrate/lipid consumed during lactation was lower for the HF/LP group (4.4 x 13.5).

The body weight gains during pregnancy and lactation are shown in Figure 4. At the end of pregnancy, HF/LP mothers showed a lower body weight gain, but at the beginning and in the middle of lactation, the mean body weights of HF/LP mothers did not differ
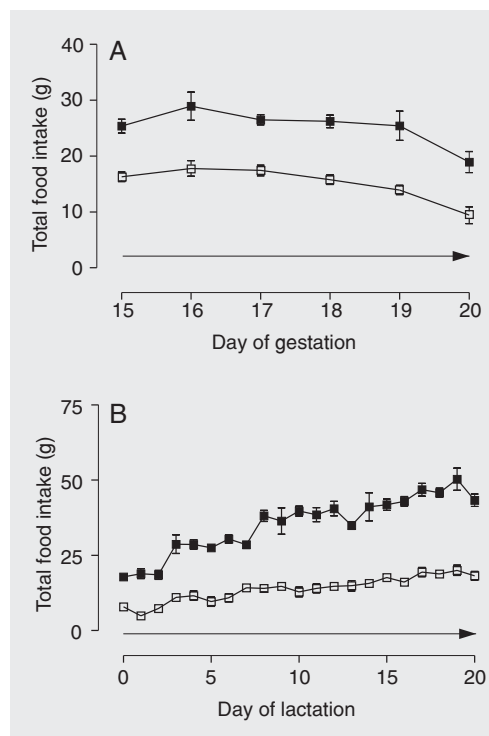

Figure 1. Total food intake by control dams (filled squares) and dams receiving a high-fat and low-protein diet (open squares) from day 15 of gestation to parturition (A) and from birth to weaning (B). The comparison was made for each day and the arrow indicates that the significance occurred during all the period. Data are reported as means ± SEM for 8 animals per group. *A*, F = 133 for diet and 8.8 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test). *B*, F = 1400 for diet and 26.2 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test).

Figure 2. Energy (A), protein (B), lipid (C), and carbohydrate (D) intake by control dams (filled squares) and dams receiving a high-fat and low-protein diet (open squares) during gestation. The comparison was made for each day and the arrow indicates that the significance occurred during all the period. Data are reported as means ± SEM for 8 animals per group. *A*, F = 32.3 for diet and 8.3 for time (P < 0.0001, two-way ANOVA; P < 0.05, Bonferroni post-test). *B*, F = 718.5 for diet (P < 0.0001) and 4.9 for time (P = 0.0006; two-way ANOVA; P < 0.001, Bonferroni post-test). *C*, F = 423.3 for diet and 10.3 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test). *D*, F = 146 for diet and 8.8 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test).
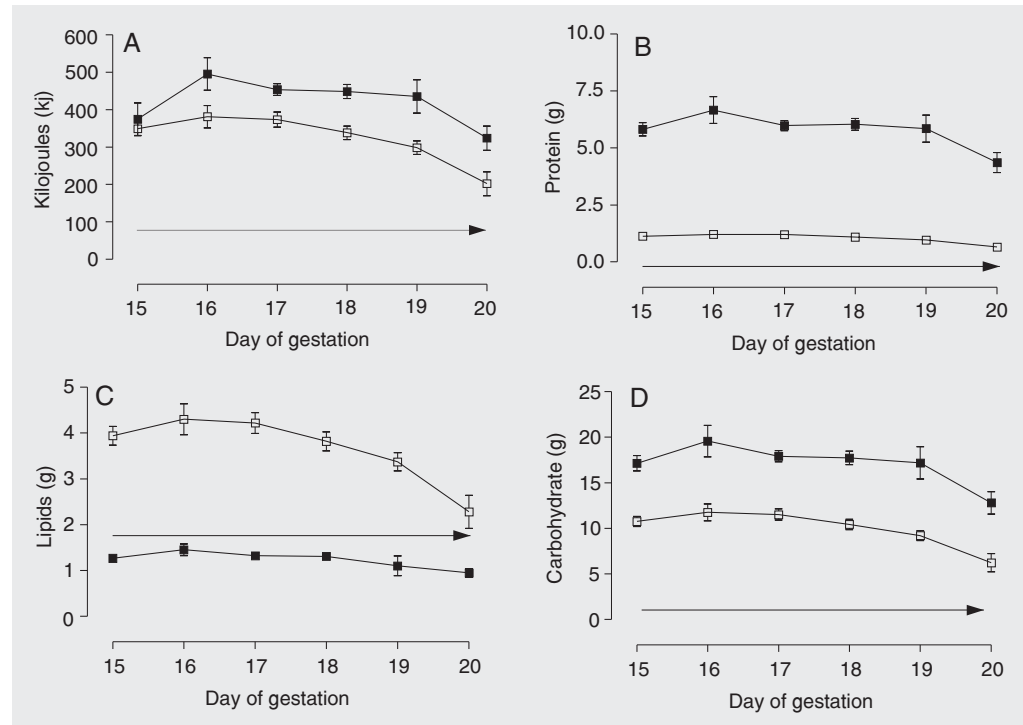

Figure 3. Energy (A), protein (B), lipid (C), and carbohydrate (D) intake of control dams (filled squares) and dams receiving a high-fat and low-protein diet (open squares), during lactation. The comparison was made for each day and the arrow indicates that the significance occurred during all the period. Data are reported as means ± SEM for 8 animals per group. *A*, F = 952.5 for diet and 36.7 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test). *B*, F = 1574 for diet and 23.7 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test). *C*, F = 126.5 for diet and 10.1 for time (P < 0.0001, two-way ANOVA; *P < 0.05 and +P < 0.01, Bonferroni post-test). *D*, F = 988.5 for diet and 24.1 for time (P < 0.0001, two-way ANOVA; P < 0.001, Bonferroni post-test).
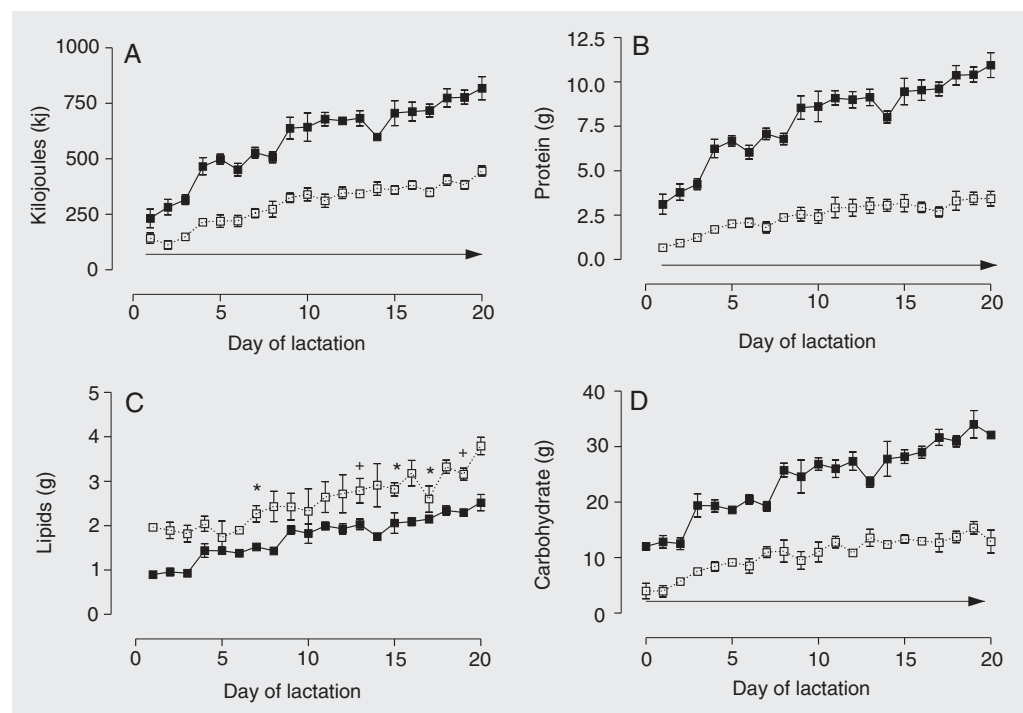
from controls. At the end of lactation, body weight was 14% lower (P < 0.05) compared to control.

At the end of lactation, HF/LP mothers had lower (25%, P < 0.05) serum albumin concentrations, confirming the low protein status of the dams.

Table 2 shows that thyroid radioiodine uptake was higher (77%, P < 0.005) in the HF/LP group. Serum TSH concentration was lower (26%, P < 0.01), serum T_3 was higher (74%, P < 0.005) but no changes were found in serum T_4 concentration.

Discussion

The high-fat and low-protein diet used in the present study was based on several models (14,16,25-27). In all of these studies, a low-protein, high-fat and high-calorie diet was used.

The dams that received the high-fat and low-protein diet presented a decrease in total food intake. The same alteration was also described in animals that received a cafeteria diet (14,16) or protein- and energy-restricted diets (12,28,29). Furthermore, since the high-fat diet is instituted abruptly during the third week of gestation, the novelty of the diet could create a food avoidance behavior that could explain the early reduction of food ingestion.

Some investigators have reported that the consumption of diets with high levels of carbohydrates could reduce the total food intake. This could be explained by the higher serotonin production with the ingestion of these diets (30,31). In protein-restricted diets, in which the protein is replaced with corn starch to maintain the energy content of the diet (12), the reduction in food intake could be explained by the higher proportion of carbohydrate in the diet. However, the diet used in the present experiment contained essentially the same amount of carbohydrate as the control diet, suggesting that other nutrients, perhaps protein, lipid, or

both, could be responsible for the reduction in food intake observed.

The reduction of energy intake may explain the lower body weight in the HF/LP group that was also found in another study during pregnancy (16). However, we cannot rule out the possibility that the higher lipid content of the diet might lead to a disabsorptive process, thus contributing to the lower body weight.

As also reported by others, a high-fat diet has a low-protein content (16,32), a fact that can explain why HF/LP dams had low serum albumin levels. Naismith et al. (15) have shown that low-protein diets cause somatic
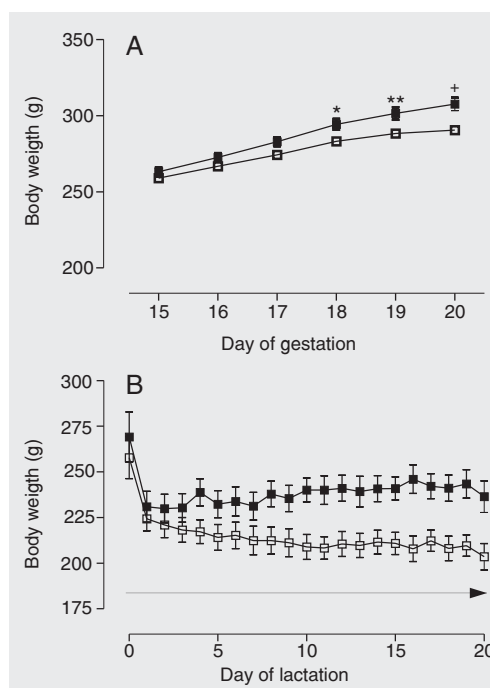

Figure 4. Body weight from day 15 of gestation to parturition (A) and from pup birth to weaning (B) of dams fed a normal (filled squares) and high-fat, low-protein diet (open squares) throughout this period. Data are reported as the means ± SEM for 8 animals per group. *A*, F = 37.6 for diet and 55.4 for time (P < 0.0001, two-way ANOVA; *P < 0.05, **P < 0.01 and +P < 0.001, Bonferroni post-test). *B*, F = 111.5 for diet and 2.6 for time (P < 0.0001, two-way ANOVA; P < 0.05, Bonferroni post-test). The arrow indicates that the significance occurred during all the period.

Table 2. Two-hour ^{131}I thyroid uptake and serum T_3, T_4 and TSH concentrations on day 21 of dams receiving the control and the high-fat, low-protein diets.

	Control diet group	High-fat, low-protein diet group
[131]I thyroid uptake	0.079 ± 0.0138	0.140 ± 0.0123**
Serum T_3 (ng/dL)	28.17 ± 3.82	49.05 ± 17.43**
Serum T_4 (ng/dL)	2.90 ± 0.13	2.54 ± 0.15
Serum TSH (µg/mL)	1.22 ± 0.1	0.90 ± 0.04*

Data are reported as means ± SEM for 8 animals in each group. *P < 0.01 and **P < 0.005 compared to control diet (Student *t*-test).

protein catabolism in the dams in the attempt to maintain adequate milk energy content. It seems that adaptive mechanisms play an important role by guaranteeing a normal milk supply to the pups at the expense of several maternal metabolic and hormonal changes.

We have shown that protein-restricted diets lead to a decrease in ^{131}I thyroid uptake and to higher serum TSH and lower T_4 concentrations, but surprisingly the animals in that study (12) had higher serum T_3 concentrations due in part to a higher thyroid T_4 to T_3 conversion (33). In contrast, energy-restricted mothers had no change in thyroid radioiodine uptake and lower serum TSH, T_4 and T_3 concentrations. In the protein-restricted mothers, this could be of adaptive relevance, since these animals secreted more T_3 in the milk for their progeny (10). In contrast, in the present study we observed a completely different pattern of thyroid dysfunction, with higher thyroid radioiodine uptake, higher serum T_3 concentration and lower serum TSH differing from both the protein- and energy-restricted diet. Thus, in the model used in the present study, the combination of energy and protein restriction with a high-fat diet causes thyroid hormone dysfunction that could provide more T_3 through the milk, without compromising thyroid function, as in the protein and energy restriction models, since there was higher thyroid uptake and serum T_4 concentration was normal. We do not know why the differences in diet composition preserve or impairs thyroid function.

The higher ^{131}I thyroid uptake could be consequent to a lower iodine supply caused by a decrease in its absorption. However, there is no report associating a high-fat diet with impairment of iodine absorption. Another important result that weakens the hypothesis of low iodine absorption was the lower serum TSH concentration, which in such situation was expected to be higher. Another possibility is a real increase in iodine uptake caused by changes in the sodium/iodide symporter (NIS) or in the iodolipids in the thyroid. Chazenbalk et al. (34) have demonstrated that the iodine thyroid uptake could be regulated by iodolipids formed in the thyroid cell. Thus, it is possible that the high lipid intake could change the synthesis of these putative iodolipids and be responsible for the increase in ^{131}I thyroid uptake observed in the HF/LP group, even with low TSH. Rats fed a cholesterol-rich diet showed higher ^{131}I thyroid uptake, but, contrary to our study, they showed also higher serum TSH and lower T_3 concentrations (35).

There are few studies about high-fat diets and thyroid regulation, and they also showed elevated plasma T_3 levels, normal T_4, and no report of TSH levels in adult non-pregnant and non-lactating animals (19,20). We also showed a higher serum T_3 concentration that could be the result of changes in iodine supply. In fact, iodine deficiency is associated with preferential T_3 production by the thyroid (36), but, as stated before, the occurrence of iodine deficiency was unlikely in this situation. Thus, our main hypothesis is that this diet induces thyroid hyperfunction, with stimulation of both iodine uptake and thyroid hormone synthesis. In this case, we would expect an increase in serum T_4 concentration, which, however, was not observed.

It is possible that this diet could increase T_4 deiodination; however, further studies are necessary to confirm this hypothesis. In fact, hyperthyroid states are associated with an increase in type 1 deiodinase activity (37). A high-fat diet can also increase the rate of T_4 uptake by target cells (38). It was demonstrated that fatty acids can regulate differently pituitary T_3 and T_4 uptake (39). Thus, it is possible that a higher T_4 uptake caused by the high-fat diet results in a normal serum T_4 concentration, but in higher serum T_3 levels and a higher thyroid hormone action.

The higher T_3 could suppress serum TSH levels even with normal T_4, since 50% of the

pituitary thyroid hormone receptor binding come from circulating T_3 (40). Another possibility is an increase in pituitary T_4 deiodination. The higher-lipid and/or low-protein diet could have a direct effect on TSH secretion or influence pituitary T_4 uptake or deiodination.

In the present paper, we showed for the first time that the lipid content of the diet can alter the thyroid function of lactating dams. This could be important for maternal adaptive changes and therefore for the pup's hormonal and nutritional status.

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