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Original Article

# The prostate of weaned pups is altered by maternal malnutrition during lactation in rats

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**Abstract**

The aim of this study was to evaluate the effects of maternal malnutrition during lactation on prostate growth and estradiol serum concentration in the prostate of pups. At delivery, nine Wistar rats were separated into three groups: control group (C) with free access to a standard laboratory diet containing 22% protein; protein–energy-restricted group (PER) with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted group (ER) receiving standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PER group. All pups were killed at weaning. PER and ER groups presented a significant reduction in estradiol serum concentration (C = 73.8 ± 4.6, PER = 48.7 ± 3.2, ER = 59.7 ± 5.5 pg $mL^{-1}$, $P < 0.01$), total prostatic acini (C = 24 190.0 ± 716.5, PER = 20 290.0 ± 631.4, ER = 19 550.0 ± 759.1 $\mu m^2$; $P < 0.01$), lumen of the prostatic acini (C = 5 590.0 ± 165.4, PER = 3 776.0 ± 251.3, ER = 4 658.0 ± 198.1 $\mu m^2$; $P < 0.01$) and epithelial area of the prostate dorsal lobe (C = 18 120.0 ± 391.4, PER = 16 520.0 ± 799.2, ER = 14 890.0 ± 589.8 $\mu m^2$; $P < 0.01$). Testosterone concentration was significantly increased only in the PER group when compared with the C group (C = 0.09 ± 0.01, PER = 0.44 ± 0.04, ER = 0.15 ± 0.03 ng $mL^{-1}$, $P < 0.001$). An adequate nutritional state in early life is important for normal growth of the prostate gland, which seem to be related to serum levels of estradiol.

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## 1 Introduction

Malnutrition is known to have a wide variety of effects on the endocrine system [1, 2]. With regard to the reproductive system, it has been shown that food restriction can inhibit both the maintenance and onset of reproductive capability [3], and reduce the androgen receptor protein level in the testis of adult rats [4]. In addition, in adult rats, food restriction can reduce the body weight [4, 5], as well as testes, epididymis and prostate weights [4]. Serum concentration of luteinizing hormone, follicle-stimulating hormone and testosterone are reduced by undernutrition [4–7], which also leads to alterations in gonadotroph morphology that are typical of those found in cells whose secretory activities are suppressed [6].

Nutritional status of the mother during gestation and lactation is essential to the normal growth and development in humans and experimental animals [8]. Throughout life, normal development, growth

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and function of the prostate gland are dependent on androgens, which act in synergy with other modulating hormones such as estrogen and prolactin [9]. In rats, prostate development is initiated late in the fetal period and undergoes extensive branching morphogenesis and cellular differentiation during the neonatal period [10]. It is during this early developmental period that hormonal modulation can have a permanent and irreversible effect on the prostatic morphology, cellular organization and function.

Exposure of neonatal mice to the nonsteroidal estrogen, diethylstilbestrol, disturbs prostatic development, alters epithelial cell differentiation, and predisposes the animal to prostatic hyperplasia and dysplasia, analogous to human prostatic intraepithelial neoplasia [9, 11].

Despite the fact that malnutrition-related reproductive suppression in rats is a well-documented phenomenon, we do not have any previous knowledge to date on how maternal malnutrition during lactation affects the prostate growth of pups. Therefore, the present study aims to evaluate the effects of protein and energy maternal malnutrition during lactation on the prostate growth of the offspring and to correlate it with estradiol serum concentrations.

## 2 Materials and methods

### *2.1 Animals*

Wistar rats were kept in a room with controlled temperature (25 ± 1ºC) and with artificial dark-light cycle (lights on from 07:00 hours to 19:00 hours) all through the experiment. Virgin female rats (3-month-old) were caged with one male rat at a proportion of 2:1. After mating, each female rat was placed in an individual cage with free access to water and food until delivery. The handling of the animals was approved by the Animal Care and Use Committee of State University of Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals [12], and the study design was approved by the local Ethics Committee for the care and use of laboratory animals.

### *2.2 Experimental model*

Nine pregnant Wistar rats were separated at delivery into three groups: control group (C)—with free access to a standard laboratory diet; protein–energy-restricted group (PER)—with free access to an isoenergy and protein-restricted diet containing 8% protein; and energy-restricted group (ER)—receiving standard laboratory diet in restricted quantities, which were calculated according to the mean ingestion of the PER group. In spite of having free access to diet, the PER group consumed ~60% of that consumed by the control group. Therefore, the amount of food consumed in both ER and PER groups was almost the same.

The low-protein diet was prepared in our laboratory, and vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets [13]. The composition of both diets is shown in Table 1.

To evaluate the nutritional state, the food consumption and body weight were monitored throughout the experiment.

Within 24 h 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 performance. At weaning (21-day-old), seven pups from three different dams of each group were killed under thiopental anesthesia (0.10 mL per 100g body weight), always in the morning. Blood was collected by cardiac puncture and the serum was kept at –20ºC. The prostates were excised, dissected, weighed, fixed in formalin in phosphate buffer (4% in 0.1 mol $L^{-1}$; pH 7.4),

Table 1. Composition of control and protein–energy restricted diets.

| | Control[a] | Protein restricted[b] |
|---|---|---|
| Ingredients (g $kg^{-1}$) | | |
| Total protein[c] | 230.0 | 80.0 |
| Corn starch | 676.0 | 826.0 |
| Soybean oil | 50.0 | 50.0 |
| Vitamin mix[d] | 4.0 | 4.0 |
| Mineral mix[d] | 40.0 | 40.0 |
| Macronutrient composition (%) | | |
| Protein | 23.0 | 8.0 |
| Carbohydrate | 66.0 | 81.0 |
| Fat | 11.0 | 11.0 |
| Total energy (KJ $kg^{-1}$) | 17 038.7 | 17 038.7 |

[a]Standard diet for rats (Nuvilab-Nuvital Ltd, Paraná, Brazil).
[b]The protein-restricted diet was prepared in our laboratory using the rol diet, with replacement of part of its protein content with cornstarch. The amount of the latter was calculated to replace the same energy content of the control.
[c]The principal protein resources are soybean wheat, steak, fish and amino acids.
[d]Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves *et al*. [13]).

paraffin-embedded and sectioned at 5 μm and processed by routine histological methods .

*2.3 Hormonal evaluation*

The serum hormonal concentrations were determined by using specific radioimmunoassay (ICN Pharmaceuticals Inc, CA, USA). The intra- and inter-assay variation coefficients were 6.4% and 5.9% for estradiol, and 4.6% and 7.5% for testosterone, respectively.

*2.4 Histological and morphometric procedures*

For morphometric analysis, five samples from each prostate were randomly excised from the dorsal lobe, and from each sample, five different sections were obtained. The sections were then stained using hematoxylin-eosin. From each section, all acini of five different random fields were analyzed and quantified, totaling upto 25 test areas in each prostate and 125 fields in each group. The analyzed images were obtained at × 40 using a video camera coupled with a light microscope. The images were converted to digital signals and transferred to a computer.

The morphometric features assessed were the delineation of the luminal surface of the acini, which provided the total area of the lumen, and the basal limit of the acini, which provided the total acinar area. On the basis of these two measurements, the software Image Pro (Media Cybernetics, Bethesda, MD, USA) was used to estimate the total area of the epithelium ($\mu m^2$).

*2.5 Statistical analysis*

The data was reported as mean ± SD. The parameters were determined by the Kruskal–Wallis test followed by Dunn's multiple comparison test. The level of significance was set at $P < 0.05$.

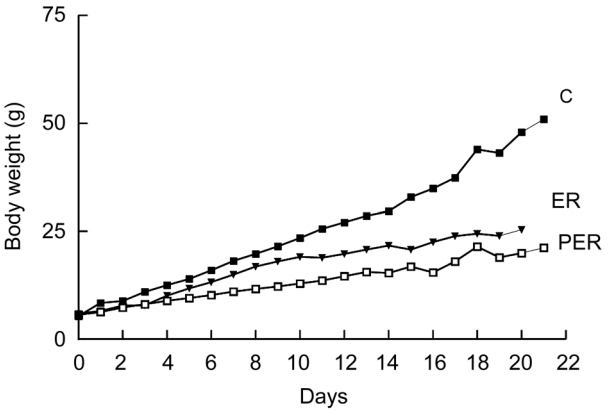


Figure 1. Body weight of 21-day-old rats whose dams were fed with a diet containing 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein but in restricted quantities—energy-restricted group (ER), during lactation period, $P < 0.01$, ER *vs.* C; $P < 0.01$, PER *vs.* C. Values are shown as mean ± SD of seven animals per group.

## 3 Results

When compared to controls, the mean body weight of pups whose mothers were submitted to PER or ER diet, was lower during the entire lactation period. The mean weight decrease reached 58% and 46% in the PER and ER groups, respectively, at the end of lactation ($P < 0.01$, Figure 1).

Table 2 shows the results of absolute and relative prostate weights and acinar morphometric analysis. All parameters, but epithelium and relative prostate weights, were reduced in both PER and ER groups ($P < 0.01$).

The serum estradiol concentrations were decreased in both PER and ER groups compared with controls (PER = 34%, ER = 9%; $P < 0.01$) (Figure 2A).

Table 2. Absolute and relative weights, areas of the acini, lumen and epithelium of the prostate in 21-day-old rats whose dams were fed a diet consisting of 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein but in restricted quantities—energy-restricted group (ER).

| Group | C | PER | ER |
|---|---|---|---|
| Prostate weight (g) | 0.060 ± 0.005 | 0.035 ± 0.003* | 0.042 ± 0.001* |
| Prostate weight/body weight (mg $g^{-1}$) | 1.250 ± 0.050 | 1.750 ± 0.090 | 1.650 ± 0.070 |
| Total acini area ($\mu m^2$) | 24 190.000 ± 716.500 | 20 290.000 ± 631.400* | 19 550.000 ± 759.1000* |
| Total lumen area ($\mu m^2$) | 5 590.000 ± 165.400 | 3 776.000 ± 251.300* | 4 658.000 ± 198.100* |
| Total epithelial area ($\mu m^2$) | 18 120.000 ± 391.400 | 16 520.000 ± 799.200 | 14 890.000 ± 589.800* |

Values are shown as mean ± SD of seven animals per group.
*$P < 0.01$, compared with C.

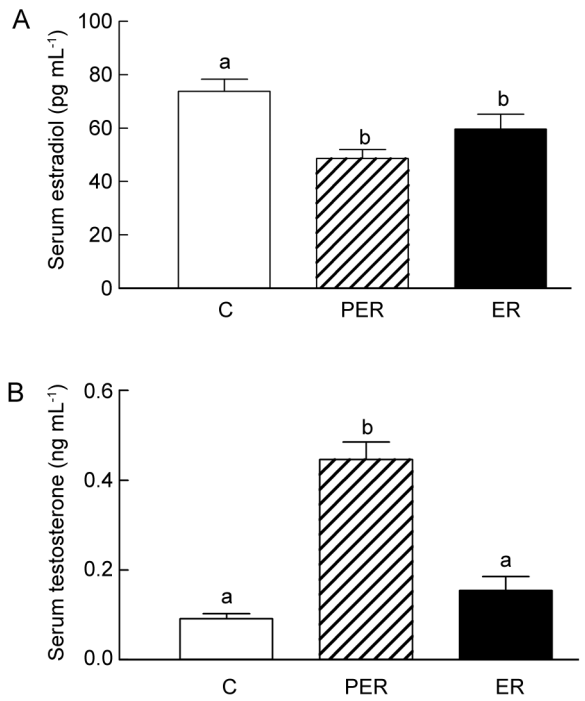


Figure 2. Serum estradiol (A) and testosterone (B) concentration of 21-day-old rats whose dams were fed with a diet consisting of 23% of protein—control group (C), a diet with 8% of protein—protein-restricted group (PER) or a diet with 23% of protein, but in restricted quantities—energy-restricted group (ER), during lactation period. Values are shown as mean ± SD of seven animals per group. Different letters mean statistical difference.

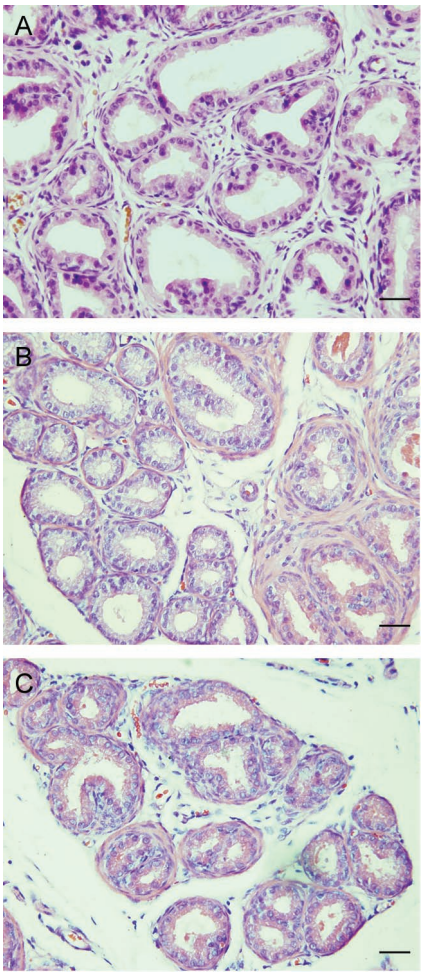


Figure 3. Histological sections of the dorsal lobe of the prostate of 21-day-old rats. (A): Control group; (B): protein–energy-restricted group; (C): energy-restricted group. There was a reduction in the total acini, total lumen and total epithelial areas in both malnourished groups (B and C). The sections were stained using hematoxylin-eosin. Bars = 50 μm.

Compared with the control group, the serum testosterone concentration was significantly increased in the PER group (390%; $P < 0.001$). Serum testosterone concentration in the ER group was slightly, but not significantly, higher than that in controls (Figure 2B).

The histological sections of the dorsal lobe of the prostate gland are shown in Figure 3. Both malnourished groups presented a reduction in the total acini, total lumen and total epithelial areas.

## 4 Discussion

The prenatal and early postnatal nutritional status has a critical role in postnatal growth and development. Early malnutrition may change the original programming of organs, especially those involved in development, which can result in long-term changes in metabolism [14, 15]. Recently, we have shown that maternal protein and energy malnutrition during lactation, in additional to growth retardation, leads to alteration in testicular morphology, testis steroid hormone genes expression, uterine endometrial glands number and folliculogenesis [16].

The maternal nutritional state during lactation is equivalent and possibly even more important than that during gestation, as evidenced by a study by Leonhardt *et al*. [17], which showed that the offspring whose dams were malnourished during lactation had more drastic consequences on gonadal development when compared with the offspring whose dams were malnourished only during pregnancy, or during pregnancy and lactation. Guzman *et al*. [18] showed similar results.

On the basis of these papers and the lack of studies

regarding the effects of malnutrition on the prostate gland, we decided to analyze the effects of maternal malnutrition during the lactation time on the prostate morphology of the weaned pups and its correlation with estradiol serum levels.

Some reported studies have shown that food deprivation decreased growth-hormone (GH)-releasing hormone and consequently the GH serum levels, which could easily explain the low weight gain of these animals [1]. We have also shown that pups whose mothers were subjected to protein or energy malnutrition during lactation present an alteration in the leptin serum concentration [2], which could also be related to the low weight gain of the animals.

The rodent prostate has a complex structure, consisting of a ventral prostate (VP), lateral prostate (LP), dorsal prostate (DP) and anterior prostate, or coagulating gland [19]. Although most studies have focused on the VP, this lobe is considered to have no counterpart in the primates, whereas the LP and the DP are histologically similar to the human prostate [20], and for this reason, we decided to evaluate the morphology of the dorsal lobe instead of others.

Our study shows, for the first time to our knowledge, that a protein and energy maternal malnutrition during lactation leads to a reduction in the total acini, lumen and epithelial areas of the dorsal prostate lobe. This result corroborates the reduction observed in the prostate weight, which is also in agreement with the literature [21].

After normalization of the body weight, the prostates sizes were not statistically different. We know that malnutrition does not have a direct effect on prostate size or on any other organ. It could possibly be a multifactorial disease, affecting several organs and functions, which may not be related to each other. Previous works have shown that malnutrition can affect several systems [1, 2].

Prolactin and GH belong to the same protein family sharing genomic, structural and biological features. The presence of GH and its receptor [22], as well as prolactin and prolactin receptor [23] in the prostate gland has been previously shown. There are some evidences correlating both prolactin [24] and GH [25] with prostate cancer, but there is clearly a need for more work to confirm this hypothesis. However, we cannot discard the fact that the effect of malnutrition on the prostate gland can also be mediated by GH and prolactin, although no data related to malnutrition and both hormones in the prostate have been reported to date.

Some of the effects of estrogen may be indirectly mediated through the hypothalamic–pituitary–testicular axis, although a direct response at the prostate level has also been documented [26]. In our study, maternal malnutrition resulted in low serum estradiol concentration in the pups of both malnourished groups, whereas testosterone concentration was higher only in the PER group. Hence, the prostate growth does not seem to be under testosterone control on the malnourished groups, as the testosterone alterations were different between these two groups while they showed the same growth retardation, a possibility related to the estrogen serum levels.

We can hypothesize that whether or not early maternal malnutrition causes a programming of the prostate, this offspring could develop some prostate alteration in adulthood as a result of the changes in serum levels of estradiol. Some reported results have supported this theory [28, 29]. Bianco *et al*. [27] showed that estradiol administration stimulates growth and expansion of the stromal, epithelial and luminal compartments of the mouse prostate lobes, showing characteristics of squamous metaplasia [28]. In the absence of estrogen, there is a lifelong elevation of androgens in male ArKO (aromatase knockout) mice and benign prostatic hyperplasia develops in maturity, suggesting that, in combination, androgens and estrogens can induce dysplasia, premalignant and malignant changes in the prostatic cells [29]. Both protein–energy restriction and energy restriction resulted in dorsal lobe prostate growth retardation, probably related to estradiol serum alteration. As there was no statistical difference between the results of the two malnourished groups, we can hypothesize that energy restriction, but not protein restriction, is the determinant factor in this study.

In conclusion, our results suggest that an adequate nutritional state in the early life is important for normal development of the prostate gland. We have shown that maternal malnutrition during lactation can affect the development of pups' prostate dorsal lobe by presumably altering the serum estradiol concentration. It is possible that malnutrition during lactation can program the prostate gland and could be responsible for some prostate alteration in the adulthood.

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