Effects of Protein and Energy Restricted Diet During Lactation Leads to Persistent Morphological Changes on Tibia Growth in the Weaned Pups

Efectos de las Restricciones de Proteínas y de Energía Durante la Lactancia Provocan Cambios Morfológicos Persistentes en el Crecimiento de la Tibia de Crías Destetadas

*Rodrigo M. P. Fernandes; **Antonio V. Abreu; **Alberto Schanaider; *Edivaldo R. Soares Jr.;
*Gustavo C.A. Peçanha; *Marcio A. Babinski & ***Cristiane F. Ramos

FERNANDES, R. M. P.; ABREU, A. V.; SCHANAIDER, A.; SOARES JR., E. R.; PEÇANHA, G. C. A.; BABINSKI, M. A. & RAMOS, C. F. Effect of protein and energy restricted diet during lactation leads to persistent morphological changes on tibia growth in the weaned pups. *Int. J. Morphol.*, 25(3):565-571, 2007.

SUMMARY: The purpose of this study was to evaluate the effects of maternal protein and energy restriction during lactation on the body weight and tibiae dimensions of pups at aging. At parturition, Wistar rat dams were randomly assigned to the following groups: 1) control group (C) - free access to a standard laboratory diet containing 23% protein, 2) protein-energy restricted group (PR) - free access to an isoenergetic, protein-restricted diet containing 8% protein, and 3) energy-restricted group (ER) – fed restricted amounts of a standard laboratory diet. At weaning, all pups were separated of dams and received free access to a standard laboratory diet containing 23% protein until 180 days when the rats were anesthetized and sacrificed. The dimensions of excised pup tibia were measured directly using pre-established anatomical points. Morphometrical analysis of the tibia showed that most of the measurements in the ER and PR groups were significantly lower than in the control group, with the greatest reductions occurring in the PR group. These results show that protein and energy restriction during lactation have an important influence on pup tibia development.

KEYWORDS: Growth and development; Morphometry; Rats; Tibia; Undernutrition.

INTRODUCTION

Malnutrition is the most prevalent form of nutritional disorder among children in developing countries (Seckler, 1985). Onís *et al.*, (1993), based on World Health Organization data, reported that child malnutrition remains a major public health problem in the world. The replacement of a balanced diet for another having more energy than proteins is a reality in these countries, once the foods with greater protein levels are often expensive and, frequently, unavailable. In this way, we can consider that the study of the energy-protein malnutrition is both important and contemporary (Araújo *et al.*, 2006).

In rapidly growing organisms malnutrition in early life is a serious challenge to which the system will try to adjust to survive. Protein malnutrition often occurs during gestation, lactation, and first 2 years of life (Desai *et*

al., 1980). Some authors showed that the nutritional status of the mother during gestational and lactational periods is essential to the normal growth and development in humans (Barker, 2000) or in experimental animals (Passos *et al.*, 2000).

The quantity or quality of nutrition at these critical periods has permanent consequences for later life. One of the mechanisms to adapt to inadequate supply of nutrients is slowing down the rate of cell division in tissues and organs, which may lead to an altered “programming” of the structure and function of the system (Lucas, 1998). In the human, malnutrition induced in early life is associated with an increased risk to develop type II diabetes, hypertension and cardiovascular disease at long term (Barker).

* Department of Morphology, Biomedical Center, Fluminense Federal University, Brazil.

** Department of Surgery, Orthopaedics and Traumatology, Federal University of Rio de Janeiro, Brazil.

*** Department of Anatomy, Biomedical Center, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

The rat has been used and considered a good model for nutritional research due to its wide variety of effects on endocrine systems (Console *et al.*, 2001; Teixeira *et al.*, 2002), which can reduce the body weight, reproductive behavior and adaptability to several diets (NRC, 1995; Natali *et al.*, 2000; Natali *et al.*, 2003; Mello *et al.*, 2004; Santos *et al.*, 2004; Faria *et al.*, 2004; Brasil *et al.*, 2005).

Regarding the skeletal system, several experimental models were designed to evaluate bone morphology remodeling in rats. It has been shown that food (Dickerson & McCance, 1961; Miller & Bowman, 1998; Morohashi *et al.*, 2000; Kawahara *et al.*, 2002; Allen & Bloomfield, 2003) and mineral restriction (Rodriguez *et al.*, 1998; Medeiros *et al.*, 2002; Chen *et al.*, 2002) can inhibit the growth and provide morphology remodeling.

Thus, we examined the effect of maternal protein and energy malnutrition during lactation on the growth and body size of the tibial growth of the offspring in the adulthood.

MATERIAL AND METHOD

Animal care. The study design and experimental protocols were approved by the Animal Care and Use Committee of the State University of Rio de Janeiro, which based its analysis on the Guide for the Care and Use of Laboratory Animals (Bayne, 1996). The experiments described here were done within the general guidelines of the Brazilian College for Animal Experimentation (COBEA).

Animals. Wistar rats obtained from Urogenital Research Unit, State University of Rio de Janeiro were housed at $25 \pm 1^\circ\text{C}$ and on a 12 h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) throughout the experiment. Three-month-old, virgin female rats were housed 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.

Experimental procedures and diets. Pregnant Wistar rats were separated at delivery into three groups: 1) control group (C) – with free access to a standard laboratory diet containing 23% protein, 68% carbohydrate, 5% lipid, 4% salts and 0.4% vitamins, 2) protein-energy-restricted group (PR) – with free access to an isoenergetic, protein-restricted diet containing 8% protein, and 3) energy-restricted group (ER) – fed a standard laboratory diet in restricted quantities that were calculated based on the mean ingestion of the PR group. We have previously shown that the PR group

consumes about 60% of the amount consumed by the control group, despite having free access to food (Passos *et al.*). Hence, the ER and PR groups ingested essentially the same amount of food.

The low-protein diet was prepared in our laboratory and its composition is shown in Table I. The vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves *et al.*, 1993). To evaluate the nutritional state, the food consumption and body weight (Fig.1) were monitored throughout the experiment. Within 24 h of birth, excess pups were removed to provide litters with only six male pups per dam since this procedure maximizes lactation (Fishbeck & Rasmussen, 1987). Malnutrition of the rats was initiated at birth, which was defined as day 0 of lactation (d0), and ended at weaning (d21) when the pups were separated of dams and received free access to a standard laboratory diet containing 23% protein until 180 days (d180). The rats were killed with an overdose of sodium pentobarbital (0.15 ml/100g body weight), always in the morning (d180).

Morphometrical parameters. The tibiae were excised and dissected, fixed in 4% formalin in 0.1 M phosphate buffer (pH 7.4) prior to being measured. The parameters (tibiae dimensions) used in the morphometrical analysis were: a) total length; b) proximal width; c) diaphysary width; d) distal width; e) proximal angle and distal angle. All of the macroscopical measurements were made to the nearest 0.01 mm using callipers.

The anatomical terminology was based on Greene (1963) as adapted for veterinary anatomy (Schaller, 1999).

Statistical analysis. The results were expressed as the mean $\pm$ standard deviation (SD). Statistical comparisons were done using one-way ANOVA followed by the Dunn multiple comparison test, with the level of significance set at $p < 0.05$. All statistical analyses were done using GraphPad Prism 4 statistical software (GraphPad Inc., CA, USA).

RESULTS

Figure 1 shows the body weight gain of pups in the three groups. The pups of dams fed a protein-restricted diet during lactation had a lower weight gain than the control group throughout the study (up to 21 days of age) ($p < 0.01$), with the difference between these two groups being ~58%. The pups in group ER had a lower weight

Table I. Composition of the control and protein-restricted diets.

	Control ^b	Protein-restricted ^c
Ingredients (g/kg)		
Total protein ^a	230.0	80.0
Corn starch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mixture ^d	4.0	4.0
Mineral mixture ^d	40.0	40.0
Macronutrient composition (%)		
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0

^aThe principal protein resources were soybean wheat, steak, fish and amino acids.

^bStandard diet for rats (Nuvilab-Nuvital Ltd., Curitiba, Paraná, Brazil).

^cThe protein-restricted diet was prepared in our laboratory by replacing part of the protein content of the control diet with cornstarch. The amount of the latter was calculated to replace the same energy content of the control diet.

^dVitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves *et al.*, 1993).

gain (~46% less) than the controls from day 6 onwards ($p < 0.01$). The PR group showed a lower weight gain than the ER group from day 1 until the end of the study ($p < 0.01$). Up to 180 days of age, the body weight gain of pups in the control, protein-restricted diet and energy-restricted diet groups are shown in Fig. 2.

All of the measurements for parameters; total length, proximal width, proximal and distal angle of tibiae in the ER and PR groups were significantly smaller than those of the control group, with the difference being greater in the PR group. There was no significant difference between groups ER and PR for all of the parameters analyzed and C vs ER for diaphysary and distal width (Table II).

Table. II. Body weight gain of pups in the control (C), protein-restricted diet (PR) and energy-restricted diet (ER) groups up to 180 days of age. The results are the mean $\pm$ SD of 12 pups per group. * and ** $p < 0,01$ vs C.

Parameter (mm)	C	ER	PR	C vs ER	P value C vs PR	ER vs PR
Total length	4.42 $\pm$ 0.14	4.18 $\pm$ 0.13	4.15 $\pm$ 0.12	< 0.01	< 0.001	> 0.05
Proximal width	0.79 $\pm$ 0.3	0.73 $\pm$ 0.3	0.71 $\pm$ 0.3	< 0.05	< 0.001	> 0.05
Diaphysary width	0.62 $\pm$ 0.03	0.57 $\pm$ 0.04	0.55 $\pm$ 0.04	> 0.05	< 0.01	> 0.05
Distal width	0.34 $\pm$ 0.03	0.31 $\pm$ 0.03	0.30 $\pm$ 0.02	> 0.05	< 0.05	> 0.05
Parameter (°)						
Proximal angle	89.0 $\pm$ 1.7	85.6 $\pm$ 3.0	84.5 $\pm$ 2.7	< 0.05	< 0.001	> 0.05
Distal angle	93.4 $\pm$ 2.7	90.0 $\pm$ 2.1	89.1 $\pm$ 2.7	< 0.05	< 0.01	> 0.05

Fig. 1. A rat tibia showing the measurements used in the morphometrical analysis. The results are the mean $\pm$ SD of 12 animals per group.

Fig. 2. Body weight gain of pups in the control (C), protein-restricted diet (PR) and energy-restricted diet (ER) groups up to 21 days of age. Group C – free access to water and a diet containing 23% protein; Group PR – free access to water and a diet containing 8% protein; Group ER – free access to water and limited access to a commercial diet containing 23% protein, which corresponded to the same amount ingested in the previous day by rats in group PR. The results are the mean $\pm$ SD of 12 pups per group.

DISCUSSION

The development of the skeleton is critically affected by malnutrition, and several studies have examined the effect of nutritional deficiencies on bone growth during gestation (Cameron & Eshelman, 1996), lactation (Herring, 1993; Miller & German, 1999), gestation and lactation (Riesenfeld, 1973), and the post-weaning period (Cameron & Eshelman; Ramos *et al.*, 1997; Brogan *et al.*, 1997). Different forms of retarded skeleton growth have been reported, depending on the type of malnutrition and/or its intensity, as well as the period in which the stress was applied. Additionally, growth of the tibia in rats may be influenced by sex, breed or strain, and nutritional status (Cameron & Eshelman; Miller & German). As there is no consensus regarding the morphometrical parameters that should be analyzed.

Tibiae underdevelopment was evident in weaned rats whose mothers were fed protein (PR) or energy (ER) restricted diets during lactation (Table II), and those changes were accompanied by quantitative alterations in the body weight (Figs. 1,2). These findings confirm previous observations (Herbert, 1980a; Engelbregt *et al.*, 2000; Teixeira *et al.*) that undernutrition (ER and PR groups) leads to a lower weight gain from the first day of lactation onwards (Fig.1).

The deficiency in body weight gain seen in malnourished offspring could result from a reduction or absence of growth hormone (GH) since food deprivation reduces the number of GH secretory cells, as shown by immunostaining of hypothalamic sections for GH releasing hormone (GHRH) and quantification of the mRNA levels for GHRH and GH (Herbert, 1980b; Faria *et al.*). Morphometrical and ultrastructural analyses of hypophyseal cells from adult monkeys fed a protein-restricted diet containing 10% protein have shown a decline in the number of somatotrophic, lactotrophic, gonadotrophic and tireotrophic cells. The volumetric density and frequency distribution of these cells were also significantly lower. (Heindel *et al.*, 1988; Rosenbaum & Leibel, 1998; Léonhardt *et al.*, 2003; Faria *et al.*).

Leptin, a circulating hormone secreted by adipous cells that controls the amount of food ingested and energy expenditure, plays a role key in the homeostasis of body weight (Keys *et al.*, 1950). Energy restriction during lactation causes a drastic reduction in the plasma leptin levels of offspring until weaning (Heilbronn & Ravussin, 2003). Consequently, low levels of leptin could alter the normal functioning of the hypothalamic-hypophyseal (GH) target organ (bone) axis.

Another hypothesis for the retardation in bone development seen in PR and ER rats may be related to inadequate maturation of the hypothalamic-hypophyseal (GH)-target organ (bone) axis in the offspring as a result of maternal malnutrition. In this case, low hormonal stimulation may be insufficient to stimulate normal development of the bones.

The loss of body weight and osseous tissue in the ER and PR groups may be caused by a reduction in the rate of metabolism. Part of this decline results from a reduced energy intake and a consequent decrease in the thermal effect of food, while part is attributable to the reduced size of the metabolizing mass. However, whether there is also a “metabolic adaptation,” defined here as a reduction in the metabolic rate that is disproportional to the decreased size of the respiring mass, is a subject of continued debate. In their investigation of the biology of semistarvation, Keys *et al.* defined metabolic adaptation as “a useful adjustment to altered circumstances” (Heilbronn & Ravussin).

Our results agree with reports showing that undernutrition during lactation delays offspring growth (Engelbregt *et al.*, 2002; Delemarre *et al.*, 2002) and skeleton development (Pucciarelli & Oyhenart, 1987; Miller & German).

Evidence from the present study supported the idea that the functional demands of the tibia are greater after birth and that, to reach functional adult proportions, growth in this bone occurred at a higher rate. Hence, there was an increased chance of being affected by an epigenetic factor such as dietary protein level (Miller & German).

According to Chavez & Martinez (1979), this disorder in bone mass produces biochemical alterations leading to poor growth. The growth retardation varies in accordance with the severity and duration of the nutritional deficiency. It may be associated with abnormalities in body size and composition in adulthood, as well as in bone length and skeletal mineral content (Dickerson & McCance; Le Roith & Pimston, 1973; Lalande *et al.*, 1998; Medeiros *et al.*; Allen & Bloomfield).

Our findings support the studies of Chavez & Martinez, since, the skeletal structure support of body and is used during movement, thus, its growth is continuously subject to muscular loading. We believe that in the present study, the nutritional influence was stronger than the biomechanical influence. Our results agree with Rozzi *et al.*, (2005) who showed that environmental stress during

development resulted in transitional growth perturbations.

In this paper, we shown, for the first time, that maternal PR and ER during lactation caused an offspring growth delay and morphological and quantitative tibia alterations that could not be restored by normalization of diet. Those results reinforce the concept of metabolic programming.

The present data should therefore provide important information for devising experiments and interpreting results when using the rat skeleton as a model for malnutrition, especially when making comparisons to human.

CONCLUSION

Our results indicate that the maternal nutritional state during lactation can affect the development of the tibia skeleton. Morphometrical analysis of the tibia revealed a significant reduction in most of the parameters in the two treated groups, particularly the PR group, when compared to the controls.

ACKNOWLEDGEMENTS. Supported by grants from the National Council of Scientific and Technological Development (CNPq), Foundation for Research Support of Rio de Janeiro (FAPERJ), Brazil.

FERNANDES, R. M. P.; ABREU, A. V.; SCHANAIDER, A.; SOARES JR., E. R.; PEÇANHA, G. C. A.; BABINSKI, M. A. & RAMOS, C. F. Efectos de las restricciones de proteínas y de energía durante la lactancia provocan cambios morfológicos persistentes en el crecimiento de la tibia de crías destetadas. *Int. J. Morphol.*, 25(3):565-571, 2007.

RESUMEN: Los objetivos de este estudio fueron evaluar los efectos de la restricción materna de proteínas y de energía durante la lactancia y sus efectos en el peso corporal y las dimensiones de las tibias de crías en ratas. En el parto, las crías de ratas Wistar fueron agrupadas aleatoriamente en los grupos siguientes: 1) grupo control (C) - con acceso libre a una dieta estándar del laboratorio, que contenía 23% de proteínas; 2) grupo con restricción de proteínas y energía (PR) - acceso libre a una dieta isoenergética, con restricción de proteínas, conteniendo un 8% de éstas y 3) grupo con restricción de energía, alimentado con restricción en la cantidad de alimento de la dieta estándar del laboratorio (ER). Al destete, todas las crías fueron separadas y recibieron la dieta estándar del laboratorio, conteniendo 23% de proteínas, hasta los 180 días, cuando fueron anestesiadas y sacrificadas. El análisis morfométrico de la tibia demostró que la mayoría de las mediciones en los grupos ER y PR, fueron significativamente menores que las del grupo control, con mayores reducciones en el grupo PR. Estos resultados muestran que las restricciones de energía y proteínas durante la lactancia tienen una influencia importante en el desarrollo de la tibia de las crías.

PALABRAS CLAVE: Crecimiento y Desarrollo; Morfometría; Ratas; Tibia; Desnutrición.

REFERENCES

- Allen, M. R. & Bloomfield, S. A. Hindlimb unloading has a greater effect on cortical compared with cancellous bone in mature female rats. *J. Appl. Physiol.*, 94(2):642-50, 2003.
- Araújo, E. J. A.; Sant'Ana, D. M. G.; Molinari, S. L. & Miranda-Neto, M. H. Quantitative Study of the Myenteric Plexus of the Descending Colon of Young Rats Subjected to Intense Protein Deficiency. *Int. J. Morphol.*, 24(4):591-7, 2006.
- Barker, D. J. P. In utero programming of cardiovascular disease. *Theriogenesis*, 53:555-74, 2000.
- Bayne, K. Revised Guide for the Care and Use of Laboratory Animals available. *Am. Phys. Soc. Physiol.*, 39:208-11, 1996.
- Brasil, F. B.; Faria, S. T.; Costa, W. S.; Sampaio, F. J. B. & Ramos, C.F. The pups' endometrium morphology is affected by maternal malnutrition during suckling. *Maturitas*, 51:405-12, 2005.
- Brogan, R. S.; Fife, S. K.; Conley, L. K.; Giustina, A. & Wehrenberg, W. B. Effects of food deprivation on the GH axis: immunocytochemical and molecular analysis. *Neuroendocrinol.*, 65:129-35, 1997.
- Cameron, G. N. & Eshelman, B. D. Growth and reproduction of hispid cotton rats (*Sigmodon hispidus*) in response to naturally occurring levels of dietary protein. *J. Mammal.*, 77:220-31, 1996.
- Chavez, A. & Martinez, C. *Nutrición y desarrollo infantil*. Mexico, Nueva Editorial Interamericana, 1979.
- Chen, H.; Hayakawa, D.; Emura, S.; Ozawa, Y.; Okumura, T. & Shoumura, S. Effect of low or high dietary calcium on the morphology of the rat femur. *Histol. Histopathol.*, 17(4):1129-135, 2002.

- Console, G. M.; Jurado, S. B.; Oyhenart, E.; Ferese, C.; Pucciarelli, H. & Gómez, D. C. L. A. Morphometric and ultrastructural analysis of different pituitary cell populations in undernourished. *Braz. Med. Biol. Res.*, 34:65-74, 2001.
- Delemarre, V. H. A.; Van-Coeverden, S. C. C. M. & Engelbregt, M. J. T. Factors affecting onset of puberty. *Horm. Res.*, 57:15-8, 2002.
- Desai, I. D.; Garcia-Tavares, M. L.; Dutra-de-Oliveira, B. S.; Douglas, A.; Duarte, F. A. & Dutra-de-Oliveira, J. E. Foods habits and nutritional status of agricultural migrant workers in Southern Brazil. *Am. J. Clin. Nutr.*, 33:702-4, 1980.
- Dickerson, J. W. T. & McCance, R. A. Severe undernutrition in growing and adult animals: The dimensions and chemistry of the long bones. *Br. J. Nutr.*, 15:567-76, 1961.
- Engelbregt, M. J. T.; Houdjik, M. E. C. A. M.; Popp-Snijders, C. & Delemarre, V. H. A. The effects of intra-uterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. *Pediatr. Res.*, 48:803-7, 2000.
- Engelbregt, M. J. T.; Van-Weissenbruch, M. M.; Popp-Snijders, C. & Delemarre, V. H. A. Delayed first cycle in intrauterine growth-retarded and postnatally undernourished female rats: follicular growth and ovulation after stimulation with pregnant mare serum gonadotropin at first cycle. *J. Endocrinol.*, 173:297-304, 2002.
- Faria, T. S.; Ramos, C. F. & Sampaio, F. J. Puberty onset in the female offspring of rats submitted to protein or energy restricted diet during lactation. *J. Nutr. Biochem.*, 15:123-7, 2004.
- Fischbeck, K. L.; & Rasmussen, K. M. Effect of repeated reproductive cycles on maternal nutritional status, lactational performance and litter growth in *ad libitum* fed and chronically food-restricted rats. *J. Nutr.*, 117:1967-75, 1987.
- Greene, E. C. *Anatomy of the Rat*. London, Hafner Publishing Company, 1963.
- Heilbronn, L. K. & Ravussin, E. Calorie restriction and aging: review of the literature and implications for studies in humans. *Am. J. Clin. Nutr.*, 78:361-9, 2003.
- Heindel, J. J.; Berkowitz, A. S.; Grotjan, H. E.; Herbert, D. C.; Klemckes, H. G. & Bartke, A. Pituitary and testicular function in the restricted rat. *Int. J. Androl.*, 11:313-26, 1988.
- Herbert, D. C. Morphology of the mammothrophs and gonadotrophs in the anterior pituitary gland of rats with protein-calorie malnutrition. *Am. J. Anat.*, 158: 521-531, 1980a.
- Herbert, D. C. Growth patterns and hormonal profile of male rats with protein-calorie malnutrition. *Anat. Rec.*, 197:339-54, 1980b.
- Herring, S.W. Formation of the vertebrate face: epigenetic and functional influences. *Am. Zool.*, 33:472-83, 1993.
- Kawahara, T.; Shimokawa, I.; Tomita, M.; Hirano, T. & Shindo, H. Effects of caloric restriction on development of the proximal growth plate and metaphysis of the caput femoris in spontaneously hypertensive rats: Microscopic and computer-assisted image analyses. *Microsc. Res. Tech.* 59(4):306-12, 2002.
- Keys, A.; Brosek, J.; Henschel, A.; Mickelsen, O. & Taylor, H.L. *The biology of human starvation*. University of Minnesota Press, Mineapolis, MN, 1950.
- Lalande, A.; Roux, C.; Graulet, A. M.; Schiavi, P. & De-Vernejoul, M. C. The diuretic indapamide on increases bone mass and decreases bone resorption in spontaneously hypertensive rats supplemented with sodium. *J. Bone. Miner. Res.*, 13(9):1444-50, 1998.
- Le Roith, D.; & Pimston, B.L. Bone metabolism and composition in the protein deprived rat. *Clin. Sci.*, 44:305-19, 1973.
- Léonhardt, M.; Lesage, J.; Croix, D.; Dutriez, C.I.; Beauvillain, J. C. & Dupouy, J. P. Effects of perinatal maternal food restriction on pituitary-gonadal axis and plasma leptin level in rat pup at birth and weaning and on timing of puberty. *Biol. Reprod.*, 68:390-400, 2003.
- Lucas, A. Programming by early nutrition: an experimental approach. *J. Nutr.*, 128:401S-406S, 1998.
- Medeiros, D.M.; Plattner, A.; Jennings, D. & Stoecker, B. Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. *J. Nutr.*, 132(10):3135-41, 2002.
- Mello, S. T. S.; Liberti, E. A.; Sant'Ana, D. M. G.; Molianri, S. L. & Miranda-Neto, M. H. Estudo morfoquantitativo do plexo mioentérico do duodeno de ratos submetidos a carência de proteínas e de vitaminas do complexo B. *Acta Scientiarum*, 26(2):251-6, 2004.

- Miller, S. C. & Bowman, B. M. Comparison of bone loss during normal lactation with estrogen deficiency osteopenia and immobilization osteopenia in the rat. *Anat. Rec.*, 251(2):265-274, 1998.
- Miller, J. P. & German, R. Z. Protein malnutrition affects the growth trajectories of the craniofacial skeleton in rats. *J. Nutr.*, 129:2061-9, 1999.
- Morohashi, T.; Ohta, A. & Yamada, S. Dietary fructooligosaccharides prevent a reduction of cortical and trabecular bone following total gastrectomy in rats. *Jpn. J. Pharmacol.*, 82(1):54-8, 2000.
- Natali, M. R. M.; Miranda-Neto, M. H. & Orsi, A. M. Effects of hypoproteic diet supply on adult Wistar rats (*Rattus norvegicus*). *Acta Scientiarum*, 22(2):567-71, 2000.
- Natali, M. R. M.; Miranda-Neto, M. H. & Orsi, A. M. Morphometry and quantification of the myenteric neurons of the duodenum of adult rats fed with hypoproteic chow. *Int. J. Morphol.*, 21(4):273-7, 2003.
- National Research Council (NRC). *Nutrient requirements of laboratory animal*. Washington, National Academic Press, 1995.
- Onís, M.; Monteiro, C. & Glugston, G. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global database on child growth. *Bull. W.H.O.*, 71:703-12, 1993.
- Passos, M. C. F.; Ramos, C. F. & Moura, E.G. Short and long term effects of malnutrition in rats during lactation on the body weight of offspring. *Nut. Res.*, 20:1603-12, 2000.
- Pucciarelli, H. M. & Oyhenart, E. E. Effects of maternal food restriction during lactation on craniofacial growth in weanling rats. *Am. J. Phys. Antropol.*, 72:67-75, 1987.
- Ramos, C. F.; Lima, A. P. S.; Teixeira, C. V.; Brito, P. D. & Moura, E.G. Thyroid function in post-weaning rats whose dams were fed a low-protein diet during suckling. *Braz. J. Med. Biol. Res.*, 30:133-7, 1997.
- Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. 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. *J. Nutr.*, 123:1939-51, 1993.
- Riesenfeld, A. The effect of extreme temperatures and starvation on the body proportions of the rat. *Am. J. Phys. Anthropol.*, 39: 427-60, 1973.
- Rodriguez, P. N.; Friedman, S. M.; Boyer, P. & Portela, M. L. Influence of dietary calcium concentration on body size and bone composition in rats during recovery from malnutrition. *J. Am. College. Nutr.*, 17(1):86-91, 1998.
- Rosenbaum, M. & Leibel, R. L. Leptin: a molecule integrating somatic energy stores, energy expenditure and fertility. *Trends. Endocrinol. Metabol.*, 9:117-23, 1998.
- Rozzi, F. V. R.; González, J. R.; Pucciarelli, H. M. Cranial growth in normal and low-protein-fed Saimiri. An environmental heterochrony. *J. Hum. Evol.*, 49:515-35, 2005.
- Santos, A. M. S.; Ferraz, M. R.; Teixeira, C. V.; Sampaio, F. J. B.; Ramos, C. F. Effects of undernutrition on serum and testicular testosterone levels and sexual function in adult rats. *Horm. Metab. Res.*, 36:27-33, 2004.
- Seckler, D. *Malnutrition: definition, incidence and effect on growth*. In *Growth: Genetics and Hormones*. New York, McGraw Hill, 1985.
- Schaller, O. *Nomenclatura anatômica veterinária ilustrada*. São Paulo, Manole, 1999.
- Teixeira, C. V.; Passos, M. C. F.; Ramos, C. F.; Dutra, S. P. & Moura, E.G. Leptin serum concentration, food intake and body weight in rats whose mothers were exposed to malnutrition during lactation. *J. Nutr. Biochem.*, 130:493-8, 2002.

Correspondence to:
Prof. Dr. Marcio Antonio Babinski
Department of Morphology, Biomedical Center,
Fluminense Federal University
Av. Hernani Mello 101
CEP 24.210-150
Niterói, Rio de Janeiro
BRAZIL

Fax : + 55 21 2587-6121
Phone: + 55 21 2587-6499

E-mail: mababinski@gmail.com
mababinski@vm.uff.br

Received: 06-06-2007
Accepted: 08-07-2007