BIOMETRIC AND FOOD CONSUMPTION PARAMETERS OF RATS SUBJECTED TO HYPOPROTEIC AND HIPERCALORIC DIET

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ARAÚJO[1*], E.J.A.; SANT'ANA[1], D.M.G.; MOLINARI[2], S.L.; NETO[2], M.H.M. Biometric and food consumption parameters of rats subjected to hypoproteic and hipercaloric diet. *Arq. ciên. vet. zool. UNIPAR*, 8(2): p. 131-138, 2005.

ABSTRACT: The protein lack causing *Kwashiorkor* is the most prevalent kind of malnutrition, because the food sources of proteins are usually more expensive. For experimental investigations, the rat has provided the primary model to evaluate the consequences of the ingestion of diets with different protein levels; however, the degrees of severity of these diets for these species are still not clear. In this sense, we aimed at evaluating the severity of a 4%-hypoproteic diet on young rats. We used 30 Wistar rats (90 days old), which were divided in two groups: control (CG) and experimental (EG). CG rats were fed with normoprotein chow, while EG rats were fed with a chow having 4% protein, for 12 weeks. At the end of the experiment, we evaluated the weight, growth, and fat and lean masses of the animals. The rats from EG did not gain weight, they had growth retardation, and built less fat and muscle masses.
KEY WORDS: protein-energy malnutrition (PEM), biometric parameters, food consumption

PARÂMETROS BIOMÉTRICOS E DE CONSUMO ALIMENTAR DE RATOS SUBMETIDOS À DIETA HIPOPROTÉICA E HIPERCALÓRICA

ARAÚJO[1*], E.J.A.; SANT'ANA[1], D.M.G.; MOLINARI[2], S.L.; NETO[2], M.H.M. Parâmetros biométricos e de consumo alimentar de ratos submetidos à dieta hipoprotéica e hipercalórica. *Arq. ciên. vet. zool. UNIPAR*, 8(2): p. 131-138, 2005.

RESUMO: A carência de proteínas, causando *Kwashiorkor*, é o tipo de má-nutrição mais prevalente, pois fontes de alimentos protéicos, geralmente, são mais onerosas. Para estudos experimentais, o rato tem sido o principal modelo para avaliar as conseqüências de ingestão de dietas com diferentes teores protéicos, contudo ainda não estão claros os níveis de severidade dessas dietas para essa espécie. Nesse sentido, propõe-se avaliar a severidade de uma dieta hipoprotéica a 4% para ratos jovens. Para tanto, utilizaram-se 30 ratos Wistar (90 dias de idade), os quais foram divididos em dois grupos: controle (GC) e experimental (GC). O GC recebeu dieta normoprotéica, enquanto o GE recebeu dieta com 4% de teor de proteínas, ambos, durante 12 semanas. No final do experimento, avaliaram-se o peso, o crescimento, a massa
gorda e massa magra dos animais. Os animais do GE não ganharam peso, tiveram retardo no crescimento, formaram menos massa gorda e menos massa muscular.
PALAVRAS-CHAVE: má-nutrição protéico-energética (MPE); parâmetros biométicros; consumo alimentar

PARÁMETROS BIOMÉTRICOS Y DE CONSUMO ALIMENTAR DE RATONES SOMETIDOS A LA DIETA HIPOPROTÉICA E HIPERCALÓRICA

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RESUMEN: La falta de proteínas, causando Kwashiorkor, es el tipo de mala nutrición más predominante, pues fuentes de alimentos protéicos, generalmente, son más costosos. Para estudios experimentales, el ratón ha sido el principal modelo para evaluarse las consecuencias de ingestión de dietas con distintos teores protéicos, aún así todavía no están claros los niveles de severidad de esas dietas para esa especie. En ese sentido, proponemos evaluar la severidad de una dieta hipoprotéica al 4% para ratones jóvenes. Para tanto, utilizamos 30 ratones Wistar (90 días de edad), los caules fueron divididos en dos grupos: control (GC) y experimental (GE). El GC recibió dieta normoprotéica, mientras que el GE recibió dieta con el 4% de teor de proteínas, ambos durante 12 semanas. Al final del experimento, evaluamos el peso, el crecimiento, la masa gorda y masa

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delgada de los animales. Los animales del GE no ganaron peso, tuvieron retraso en el crecimiento, formaron menos masa gorda y menos masa muscular

PALABRAS CLAVE: mala nutrición proteica-energética (MPE); parámetros biométricos; consumo alimentar

Introduction

Malnutrition is a prominently social illness. It represents a real problem which since long time ago has demanded concern from the public health in many countries, although it is more prevalent in poorer nations. A diet containing insufficient amounts of proteins can trigger a serious illness in children, known as *Kwashiorkor*. A smaller ingestion of this compound leads to smaller availability of amino acids, interfering in the synthesis of many important proteins for the maintenance of homeostasis. Getting to know the several consequences of malnutrition in human beings has been the purpose of several works (ALLEYNE, 1972; GALICK *et al.*, 1980; STEFANINI *et al.*, 1995; SOLIMAN *et al.*, 2000; LESSA *et al.*, 2003; VALENZUELA *et al.*, 2003).

To understand the mechanisms of the alterations caused by malnutrition, many studies have been carried out using the rat to reproduce symptoms similar to human *Kwashiorkor* (ENWONWU & SREEBNY, 1970; PHILBRICK & HILL, 1974; ANTHONY & EDOZIEN, 1975; HEARD *et al.*, 1977; NATALI & MIRANDA-NETO, 1996; NATALI *et al.*, 2000; SANT´ANA *et al.*, 2001; ARAÚJO *et al.*, 2003; MELLO, 2004). The rat is considered a good model for nutritional research due to its reduced size, reproductive behavior and adaptability to several diets (NATIONAL RESEARCH COUNCIL, 1995).

The boundary between a moderate and a severe protein restriction is not yet clearly identified for the rat. In this sense, studies were made to evaluate the possible effects on the biometric and food consumption parameters of rats differing in strain and age. Different experimental diets, supplied for varied periods, were employed, such as protein (OBATOLU *et al.*, 2003) or with different protein levels: 0.4% (ANTHONY & EDOZIEN, 1975), 0.5% (ENWONWU & SREEBNY, 1970; PHILBRICK & HILL, 1974; ANTHONY & FALOONA, 1974), 0.8% (ANTHONY & EDOZIEN, 1975), 2% (EDOZIEN & SWITZER, 1978a,b), 5% (EDOZIEN & SWITZER, 1978a,b), 6% (AKINGBEMI *et al.*, 1995), 8% (AKINGBEMI & AIRE, 1994; NATALI *et al.*, 2000; SANT´ANA *et al.*, 2001; ARAÚJO *et al.*, 2003; MELLO, 2004), 10% (EDOZIEN & SWITZER, 1978a,b), 12% (HEARD *et al.*, 1977; OBATOLU *et al.*, 2003) and 15% (ANTHONY & FALOONA, 1974; EDOZIEN & SWITZER, 1978a,b). The commercial chows for rodents establish a minimal protein level of 22%, but it should be taken into account that some studies (NATIONAL RESEARCH COUNCIL, 1995) demonstrated that diets with 12% protein do not cause metabolic changes in rats during the growth period.

Based on this parameter, our research group has analyzed the effect of a diet with protein level of 8% on rats during gestation and the prenatal period (NATALI & MIRANDA-NETO, 1996; NATALI *et al.*, 2000), as well as in young rats (SANT´ANA *et al.*, 2001; ARAÚJO *et al.*, 2003; NATALI *et al.*, 2003; MELLO, 2004). In both periods, it was observed a significant growth retardation of these animals. In this study, we evaluate the severity of a 4%-protein diet, in the long run, on young rats, concerning biometric and food consumption parameters.

Material and Methods

We used 30 male Wistar rats (*Rattus norvegicus*) aging 90 days (291.52±38.24g), which were kept in individual metabolic cages in a biotery with controlled temperature (± 25ºC) and light/dark cycles (12/12 hrs). During the whole experiment, water and chow were supplied *ad libitum*.

The Committee on Ethics in Research Involving Animal Experimentation of Paranaense University (UNIPAR) approved the protocols for animal handling and killing.

The animals were randomly allotted to two groups: control (n=15) and experimental (n=15). The control group (CG) received NUVILAB® commercial chow for rats. This chow was bromatologically analyzed and from the results the components were calculated to be added so that it could decrease the protein level to 4% and keep the vitamin and mineral balance. Thus, for each kg of the experimental chow, it was used 153.85g of ground commercial chow, 53.10g of a mixture of mineral salts, 172.87g of sucrose as table sugar, 391.41g of commercial corn starch, 140.7g of vegetal oil as lipid source, and 18g of ground pills of vitamins B complex. The other vitamins were in excess in the initial formulation and the supplementation was unnecessary. Water was added to allow mixture of the components and their pelletization. The pellets were dried in stove at 55 degrees and then they had their percent composition assessed (Table 1).

Both groups, CG and EG, were kept for 12 weeks. Each animal's well being, weight and food and water

TABLE 1 - Percent composition of the chow offered to the Control Group (NUVILAB®) and of the hypoproteic chow prepared for the Experimental Group

Component	Control Chow - NUVILAB® (%)	Experimental Chow (%)
Humidity	8.58	8.9
Proteins (% N x 6.25)	26.02	4.07
Lipids	6.45	14.8
Ashes	9.05	8.97
Fibers	7.24	2.34
Carbohydrates (by ≠)	42.66	60.92
Energy Value	332.77 Kcal/100g	402.60 Kcal/100g

Source: Percent composition carried out by the Laboratory of Physic-Chemistries Analyses of the Paranaense University.

ingestion were monitored daily. At the end of the experiment, the rats were fasted for about 12 hours and anesthetized with the following mixture, applied intramuscularly: Acepran (1.26 ml/Kg) + 10% Ketamine (1.26 ml/Kg) + 2% Xilazine (0.42 ml/Kg) and 1% Atropine (0.22 ml/Kg) (PACHALY *et al.*, 2003). We laparotomized the animals and collected 4 ml of blood of five animals of each group through cardiac puncture. This blood was used for the dosage of creatinine by the Express-Plus Automatized System.

To follow-up the growth and variations of the body composition of the rats during the experiment, we evaluated the body weight (weekly); the length from the nose to the tail tip (at the beginning and at the end of the experiment); the retroperitoneal and periepididymal fats (collected from killing) as indicative of fat mass; and the muscle mass, indirectly assessed through blood creatinine level (GUYTON & HALL, 2002).

To identify significant differences between CG and EG for each parameter, we used the t-test, at the significance level of either 5% or 1%. The possible degree of dependence between variables was investigated with Pearson's correlation coefficient, to which t-test was applied for α = 5% or 1%. The results are presented as mean ±standard deviation.

Results

During the experimental period, the CG animals developed themselves as expected for this species and they showed healthy behaviors and organs. On the other hand, EG animals, in the first four weeks, were stable and healthy, although without significant body mass development. From the fifth week onwards, five from these animals lost weight continually, three lost most of their fur, and three died before the end of the experiment. However, their fecal pellets were normal, despite the proportion of fibers in the chow offered to EG being less than the one to CG.

The CG ingested less chow gradually, according to the experiment, while the rats from EG had consumed few chow during almost all experiment ($p<0.01$), except from the 10ª week. As for water consumption, the values for EG are predominantly lower than for the CG ($p<0.01$). The chow and water consumption by CG and EG are presented in figures 1 and 2, respectively.

As for body weight, there was a steady increase for the CG animals (≈ 13.9 g/week), while the weight of most of the EG animals did not vary significantly during the experimental weeks (≈ -0.375g/week). The body weight evolution of both groups is presented in figure 3.

The values referring to the nose-tail tip length, final body weight, retroperitoneal and periepididymal fat, and creatinine level are presented in table 2.

Discussion

The experimental protocols for the investigation of malnutrition make it possible to understand the morphologic and biochemical alterations that this illness causes. Through studies with laboratory animals, it is possible to establish limits, albeit feeble, between the pre-clinical situation and the moderate and severe clinical pictures of malnutrition. From the animal models employed for experimental research in nutrition, the rat has been the most used, due to its reduced size, short gestation period, adaptability to several diets and easiness of handling (NATIONAL RESEARCH COUNCIL, 1995). Protein deficiency in young rats can result in growth retardation, anemia, hypoproteinemia, depletion of body protein, muscle loss, edema, and when sufficiently severe, death (NATIONAL RESEARCH COUNCIL, 1995). Through our results, we discuss the severity of the protein malnutrition caused in rats fed with a chow containing only 4% protein in terms of biometric and food consumption parameters.

Our results demonstrate that the animals from EG ingested a significantly smaller amount of chow relative to CG ($p<0.01$). We aimed at understanding why the rats from EG ingested a smaller amount of chow, although this was offered *ad libitum*. The regulatory mechanism of food ingestion in mammals is made primarily by the hypothalamus. It is a complex mechanism involving several hypothalamic nucleus, but the lateral nucleus, directly linked to hunger, and the ventromedial nucleus, considered as the satiety center, are highlighted (GUYTON & HALL, 2002). The hypothalamus is inhibited when the nutrients are properly leveled in the blood, when insulin level rises, and by gastrointestinal hormones and leptin; on the other hand, hunger arises when the hypothalamic receptors notice that the plasma levels of glucose and/or amino acids (especially the essential ones) and/or fatty acids are low (GUYTON & HALL, 2002). As for the case of the EG animals of this study, the chow intake

TABLE 2 – Evaluation of body growth (nose-tail tip length) and comparison of body weight, retroperitoneal and periepididymal fats, and creatinine level between the groups

	Control Group (CG)		*Experimental Group (EG)*	
	Beginning	End	Beginning	End
Nose-tail tip length(cm)	40.03±1.86^{a}	44.15±1.45^{ac}	40.67±1.39^{b}	42.33±1.37^{bc}
Body weight (g)	294.2±43.28^{a}	368.45±26.37^{ac}	288.83±33.78^{bc}	267.63±29.10^{c}
Retroperitoneal fat (g)	----	4.14±1.53^{a}	----	2.65±1.35^{a}
Retrop. fat (mg) / body weight (g)	----	11.01±3.96^{a}	----	12.74±5.29^{b}
Periepididymal fat (g)	----	3.95±1.00^{a}	----	2.16±0.91^{a}
Periepid. fat (mg) / body weight (g)	----	10.71±2.60^{a}	----	10.64±3.11^{b}
Creatinine* (g/dl)	----	0.51±0.02^{a}	----	0.43±0.03^{a}

Means followed by the same letter in the same line are significantly different ($p<0.01$). Values are given as mean ±standard deviation; n = 15 for CG and n = 12 for EG; *n = 5 for both groups.

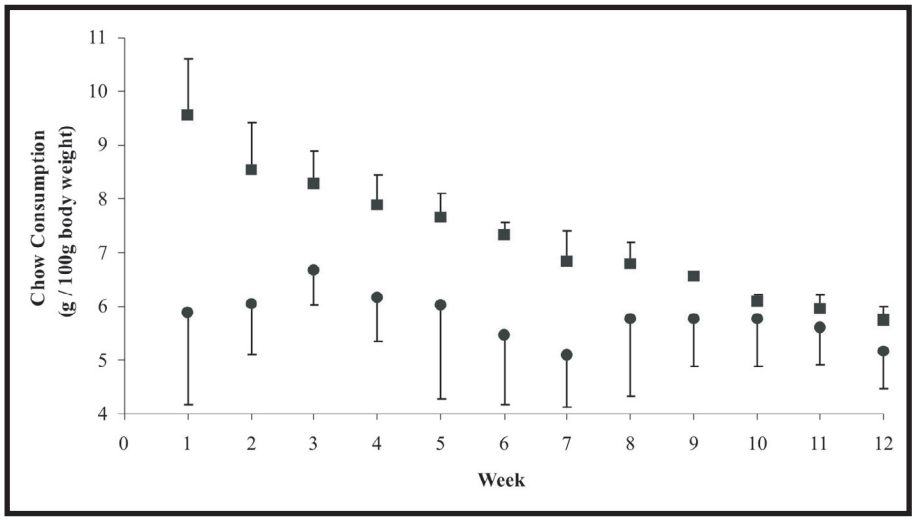

Figure 1 – Chow consumption by the animals receiving balanced diet (■) and by the animals receiving 4%-hypoproteic diet (●). Data are arithmetic means of the daily chow consumption by the animals of each group (n = 15) at each week of the experiment

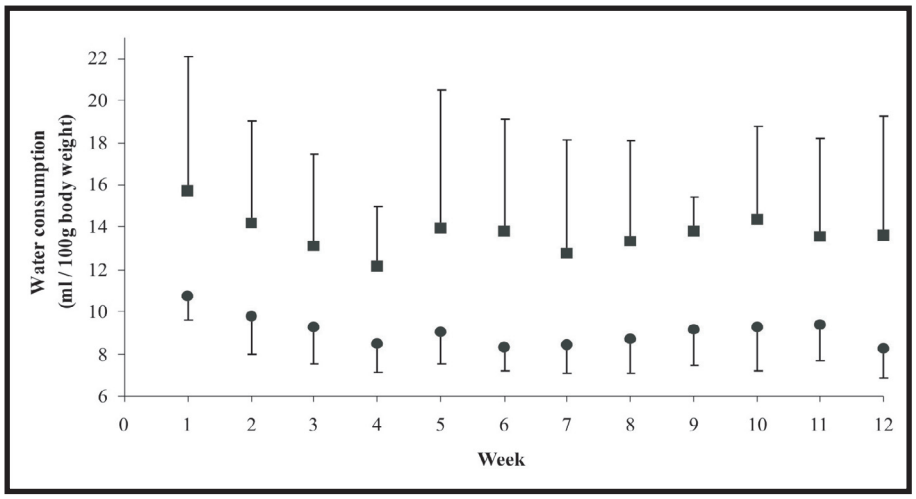

Figure 2 – Water consumption by the animals receiving balanced diet (■) and those receiving 4%-hypoproteic diet (●). Data are arithmetic means of the daily water consumption by the animals of each group (n = 15) at each week of the experiment

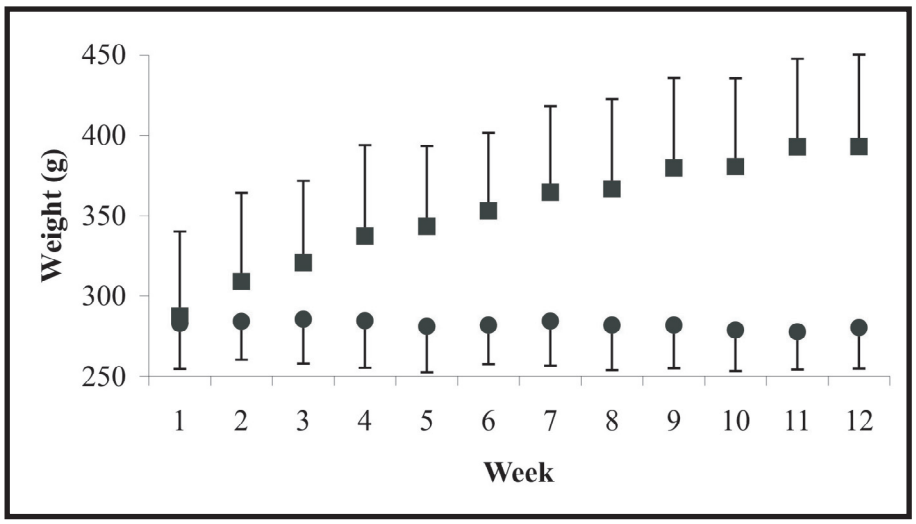

Figure 3 – Evolution of the weight of the animals from the Control Group (■) and those which are receiving 4%-hypoproteic diet (●). Data are arithmetic means of the weight of all the animals of each group (n = 15) at the end of each week of the experiment

was smaller than the ones from the CG, probably lowering the plasma levels of nutrients; this would have stimulated the lateral hypothalamic nucleus to trigger hunger, but the ingestion was still small. A possible explanation for this fact is the action of leptin, whose plasma concentrations are increased in animals subjected to PEM (STAPLETON *et al.*, 2003). Leptin is a hormone produced by adiposities and it is one of the major modulators of the hypothalamus concerning to food ingestion (GUYTON & HALL, 2002). Recent studies show that the increase in the plasma concentration of leptin causes the release of the anorexigenous neuropeptide α-MSH from the arcuate nucleus, inhibiting the lateral hypothalamic nucleus (NISWENDER & SCHWARTZ, 2003). Other studies demonstrate that the PEM in rats during lactation leads to an increase in the volume of the ventromedial nucleus, as well as it makes the neurons of this nucleus richer in Nissl's corpuscles, an indicative of metabolic increase in these cells (PLAGEMANN *et al.*, 2000). The possible inhibition of the hunger center and stimulation of the satiety center may characterize a process of adaptation due to an unbalanced diet, although it is available at larger amounts. A smaller ingestion of food was also observed in monkeys (ENWONWU *et al.*, 1973) and in rats (ANTHONY & FALOONA, 1974; EDOZIEN & SWITZER, 1978a,b; PLAGEMANN *et al.*, 2000; STAPLETON *et al.*, 2003) fed with hypoproteic diets. Other investigations are needed to elucidate the biochemical mechanisms concerned with the diminished hunger and/or prolongation of the satiety sensation in instances of PEM.

EG ingested less water (≈ 47.5%; $p<0.01$) than CG. The maintenance of a relatively constant volume and a steady composition in the body fluids are essential for homeostasis in every animal. The amount of water to be ingested depends primarily on the amount of body water lost daily, urine formation by the kidneys being the major route (GUYTON & HALL, 2002). Urine formation has the major purpose of freeing the organism of undesirable materials ingested or produced by metabolism. Among these, urea (from amino acid metabolism), creatinine (from muscle creatine), uric acid (from nuclei acids) and final products of hemoglobin degradation (such as bilirubin) are the most significant (GUYTON & HALL, 2002). As these compounds are markedly reduced in the blood of EG rats (PEREIRA *et al.*, 2003; TRISTÃO *et al.*, 2003; ANDRADE *et al.*, 2004), it is expected that the amount of water lost as urine is smaller, which could explain the smaller water ingestion observed. No record was found in the literature concerning water ingestion of rats subjected to hypoproteic diet.

It is commonsense that malnutrition, either as marasmus or *kwashiorkor*, alters the body composition, diminishing both fat and lean masses (GONÇALVES & WAITZBERG, 1989; WAITZBERG *et al.*, 1989; GONÇALVES *et al.*, 1990).

As for the fat mass, it was assessed in this study using the retroperitoneal and the epididymal fats; these were in smaller amounts in EG than in CG ($p<0.01$). However, when we considered the retroperitoneal fat weight/body weight ratio, as well as the epididymal fat weight/body weight ratio, we verified that there are no significant differences between the animals from both groups ($p>0.05$). The development of fat mass depends essentially on two factors: (a) that the energy balance is positive and (b) that the adipocytes are plastic enough to store more lipid, which is directly proportional to the availability of aminoacids for protein synthesis. As for the energy balance, the EG animals ingested less calories than CG. Additionally, the smaller formation of fatty mass in EG animals could also be attributed to the smaller plasticity of the adipocytes due to the smaller availability of aminoacids. These results are in disagreement with those observed in rats subjected to protein malnutrition at the 8% level (SANT´ANA *et al.*, 2001), which demonstrated a significant gain of retroperitoneal fat. This prompts us to think that the severity of a 4%-hypoproteic diet is sufficient to reduce the formation of fat mass. A decrease of the fat mass was also verified in rats subjected to protein diet for six weeks (WAITZBERG *et al.*, 1989; GONÇALVES *et al.*, 1990). Through correlation analysis, we tried to infer which elements from the fat mass contributed the most to the final weight of the EG rats and verified, in the first place, the retroperitoneal fat ($r = 0.88$ $p<0.01$) and second the epididymal fat ($r = 0.78$ $p<0.01$). The retroperitoneal and the epididymal fats also contributed to the final weight of the CG animals ($r = 0.48$ $p<0.05$ and $r = 0.29$), but less intensely. This difference is probably due to the fact that the CG animals had more muscle mass and then greater amounts of lean mass.

Another element of body composition affected by malnutrition is the muscle mass, one of the major constituents of the growing rats lean mass. In this study, we followed the muscular dynamics through an indirect method, that is, creatinine concentration in blood. Creatinine is a non-protein nitrogenous organic compound formed from creatine dehydration. Creatine is synthesized in the kidneys, liver and pancreas and transported to other organs such as muscle and brain, where it is phosphorilated to phosphocreatine (GUYTON & HALL, 2002). The phosphocreatine-creatine controversy is a particular feature of the metabolic process of muscle contraction. Some free creatine in muscle do not take part in this reaction and they are spontaneously converted to creatinine (VOET *et al.*, 2000). Thus, the amount of endogenous creatinine produced is proportional to the muscle mass. Based on the creatinine values obtained in this investigation, we suggest that the EG animals had a smaller muscle mass relative to the CG rats at the end of the experiment ($p<0.01$). AKINGBEMI & AIRE (1994) subjected rats to a hypoproteic diet, but having 8% protein, and observed that creatinine concentration increased. Elevated values of creatinine may be indicative of chronic failures of the renal excretory function, especially in conditions where there is "pre-renal" alteration of urea metabolism, as it occurs with the ingestion of hypoproteic diets (OBATOLU *et al.*, 2003). AKINGBEMI *et al.* (1995) subjected rats to a 6%-hypoproteic diet for a month, and OBATOLU *et al.* (2003) subjected rats to a protein diet also for a month; neither of them observed a significant difference in serum creatinine concentration, probably because the time period of these studies was not enough to change the muscle mass and/or the renal function. On the other hand, when DAL PAI *et al.* (2003) subjected lactating rats to protein malnutrition (8%) for 28 days, they noticed a decrease in the muscle fiber diameter, indicating that the protein catabolism in these cells increases, probably with the aim of providing aminoacids

to supplement the plasma content of exogenous origin. The smaller muscle mass in the EG animals of our study could be accounted for the less intense protein synthesis because of amino acid and/or insulin lack, as well as by a presumptive increase of muscle proteolysis. As already presented earlier, animals fed with hypoproteic diets have lower circulating insulin levels, favoring protein catabolism and non favoring synthesis. Nevertheless, more specific studies are necessary to attest for this possibility.

The smaller protein-energy intake in rats during the growth period may cause complex metabolic changes that usually decrease the growth rate and the body weight evolution. Even knowing that genetic and environmental factors can influence the growth rate and the body weight, anthropometric data have been widely used by health services and scientific studies (LESSA *et al.*, 2003) as a means of diagnosing children at nutritional risk or disnatured. However, weight and/or height below the reference values are not specific to diagnose the types of malnutrition. In this study we verified that the protein level supplied to the rats from EG (4%) was not enough to allow weight gain by these animals, that on average remained at about the initial value after 12 weeks of treatment ($p>0.05$). We may suggest that this is partially due to the smaller fat mass of EG, their reduced lean mass as assessed through muscle mass, and the smaller water ingestion. A reduction of both, the fat and the lean masses was observed as well as in rats subjected to protein diet during six weeks (WAITZBERG *et al.*, 1989; GONÇALVES *et al.*, 1990). Experimental studies using rats observed a lag in weight gain by the animals when they were fed with hypoproteic diets containing at least 5% protein (ENWONWU *et al.*, 1973; EDOZIEN & SWITZER, 1978b; NATALI *et al.*, 2000; SANT´ANA *et al.*, 2001; ARAÚJO *et al.*, 2003; MELLO, 2004). Other studies demonstrated absence of weight gain and even weight loss when the animals were fed with diets having protein levels of 0.5% (ENWONWU & SREEBNY, 1970; ANTHONY & FALOONA, 1974; PHILBRICK & HALL, 1974; ANTHONY & EDOZIEN, 1975), 2% (ENWONWU et al. 1973; EDOZIEN & SWITZER, 1978b) and 4% (ANTHONY & EDOZIEN, 1975).

We additionally verified in this study that the animals from EG grew less than those from CG. This result is based on the distance from the nose to the tail tip of each animal. Knowing that proteins play an essential role in the hypertrophy and hyperplasia of any cell, this finding was expected. In addition to the smaller availability of substrates (aminoacids) for the construction of new cells, we know that the vertebrate growth depends primarily on the mitotic index of the cartilaginous tissues at strategic sites (GUYTON & HALL, 2002). During growth, this type of cartilage undergoes hyperplasia and some of them are subjected to ossification (JUNQUEIRA & CARNEIRO, 2004). Bone elongation induces muscle fiber elongation and growth of the other tissues. The control of this process is made primarily by growth hormone and insulin (GUYTON & HALL, 2002). The release of growth hormone by the pituitary leads to the synthesis and release of somatomedins, which stimulate chondrogenic cells, chondroblasts and chondrocytes to proliferate (GUYTON & HALL, 2002). As the environmental variables of this study were the same for both groups (except for the diet), the smaller growth of the EG animals was attributed to the smaller availability of aminoacids for cellular division and/or to hormonal variations. SMITH *et al.* (1981) noticed that children with marasmus or *kwashiorkor* below their age-matched mean height had a greater cortisol production and a lower somatomedin activity. These authors suggest that the high levels of cortisol inhibit the stimulatory action of somatomedin over the epiphyseal plate, leading to a derangement of the morphology and metabolism of the chondrocytes; additionally, the authors put forward that cortisol inhibits somatomedin synthesis, and thus could be one of the reasons of the diminished growth. Finally, several investigations demonstrate that insulin concentration in the blood of protein-disnatured animals is significantly reduced (ANTHONY & FALOONA, 1974; ANTHONY & EDOZIEN, 1975; HEARD *et al.*, 1977; SMITH *et al.*, 1981); as protein catabolism increases and protein synthesis ceases when insulin is not available (GUYTON & HALL, 2002), maybe this is another reason why the retarded growth was observed in the EG animals of this research.

In this way, we can conclude that malnutrition triggered in young (90-180 days old) Wistar rats by the ingestion of a diet having 4% protein is severe enough to account for: smaller ingestion of food and water, lack of weight gain, growth retardation, smaller production of fat mass (retroperitoneal and epididymal) and reduction of the muscle mass.

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