Effects of short-term malnutrition in rats

D. C. Estrela[1]; C. G. C. Lemes[2]; A. T. B. Guimarães[2]; G. Malafaia[2*]

[1]*Prgrama de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Goiás, CEP: 74001-970, Goiânia, GO, Brasil.*

[2]*Laboratório de Pesquisas Biológicas do Instituto Federal Goiano – Câmpus Urutaí, CEP: 75790-000, Urutaí, GO, Brasil.*

**guilhermeifgoiano@gmail.com*

(Recebido em 12 de junho de 2014; aceito em 28 de julho de 2014)

The aim of the present study is to evaluate the effects of short-term malnutrition on physical, biochemical, hematological and histological parameters in Wistar rats. Ninety day-old female rats were submitted to food restriction by adopting a diet containing 6% protein. The animals of the control group were fed with a standard commercial diet containing 19% protein. After malnutrition (indicated by statistics differences in body mass) was established, the rats were euthanized and the absolute and relative masses of liver, spleen, heart, pancreas, brain, kidneys, lungs and adrenal glands, and biochemical and hematological parameters were analyzed, as well as the histology of the spleen, liver and kidneys. The malnutrition group presented lower concentrations of leukocytes, triglycerides and very-low-density lipoproteins (VLDL), and higher relative liver mass and fasting glucose, with incipient histopathological changes, when compared to those obtained for the control group. These data suggest that short-term malnutrition is able to induce a malnutrition condition and signs of liver alteration, but it does not cause generalized biochemical and hematological changes.

Keywords: nutritional deficiency, protein deficit, experimental models, food restriction.

Efeitos da desnutrição em curto prazo em ratos

O presente estudo objetivou avaliar os efeitos da desnutrição, em curto prazo, sobre parâmetros físicos, bioquímicos, hematológicos e histológicos em ratos Wistar. Para isso, fêmeas adultas com 90 dias de idade foram submetidas ao processo de restrição alimentar utilizando uma dieta contendo 6% de proteína. Já os animais do grupo controle foram alimentados com dieta comercial padrão contendo 19% de proteína. Após o estabelecimento da desnutrição, indicada pela diferença estatística entre as massas corpóreas dos animais estudados, estes foram submetidos à eutanásia, tendo sido avaliada a massa absoluta e relativa do fígado, baço, coração, pâncreas, cérebro, rins, pulmões e glândulas suprarrenais, parâmetros bioquímicos e hematológicos, assim como a histologia do baço, fígado e rins desses animais. Os animais do grupo desnutrição apresentaram diminuição da concentração de leucócitos, triglicérides e lipoproteínas de muito baixa densidade (VLDL) e maior massa relativa do fígado e glicemia em jejum, com presença de alterações histopatológicas incipientes quando comparados com os respectivos controles. Estes dados sugerem que a desnutrição, em curto prazo, é capaz de induzir o quadro de desnutrição e ocasionar sinais de alterações no fígado, mas não ocasiona alterações bioquímicas e hematológicas generalizadas

Palavras-chave: deficiência nutricional, déficit proteico, modelos experimentais;, restrição alimentar..

1. INTRODUCTION

Eating is considered a primordial factor in humans' daily routine, not only for being a basic necessity, but mainly because incorrect eating habits are a public health problem, either because of excesses or deficiencies [1].

The lack of food or incorrect eating can induce a nutritional deficit or malnutrition condition, which, according to Lacerda et al. [2], is characterized as a pathological condition resulting from lack of energy, proteins and/or nutrients in varied proportions. Some years ago malnutrition was associated with poverty, low educational standards, large numbers of people living in the same house, poor housing and sanitation, and maternity at less than 20 years old [3,4].

The World Health Organization (WHO) estimates that malnutrition contributes to more than a third of all infant casualties in the world, although it is rarely listed as a direct cause of mortality [5]. Even moderate levels of nutritional deficiency, detected by biochemical and/or clinical tests, can cause serious losses to human health [1].

In Brazil, according to the Ministry of Health (MS – Ministério da Saúde), there were approximately 923 thousand people at nutritional risk in 2001 [6]. This number has decreased in the last years and some studies have shown that malnutrition has diminished in Brazil [7,8]. However, chronic malnutrition has remained a major public health problem of in the majority of the developing countries, such as Brazil, Korea, South Africa, among others [5]. Short stature associated with morbidity still prevails strongly in these countries [9].

Aiming at a better understanding of the effects and consequences of malnutrition, studies using experimental models have emerged as important sources of information. Gerude et al. [10], using Wistar rats submitted to protein deficit, showed that the main changes observed in the stomach are: atrophy of the gastric mucosa with obvious papillomatosis, and decreased secretion of hydrochloric acid and pepsin, causing weakening of the gastric barrier to bacteria. Physical changes in this experimental model have also been reported, as weight loss [11,12, 13] and changes in body and organ growth [14]. Besides, the development of certain illnesses such as hepatic steatosis [15,16,17] and steatohepatitis in undernourished Wistar rats, with effects similar to those of alcoholic steatohepatitis [15], among others, has been related to malnutrition.

Biochemical parameters are also negatively affected by malnutrition, as shown by Fagundes et al. [18], who detected hypoinsulinemia and discrete hypoglycemia in adult Wistar rats, due to protein restriction during lactation. Xavier et al. [19], Borelli et al. [20] and Malafaia et al. [21] observed significant decreases in total protein concentration in mice. Pinheiro et al. [13] observed that changes in blood sugar, insulinemia and leptinemia in Wistar rats, caused by protein restriction during gestation and/or lactation, can be passed transgenerationally to the second generation of pups. Besides, hematological parameters are affected, as demonstrated by Ferrari et al. [22] and Santos et al. [23] in situations of food restriction and protein restriction respectively. In these studies, significant drop of total leukocyte concentration was detected in Wistar rats, characterizing leukopenia.

However, these studies have examined these parameters in assays conducted over long periods of malnutrition and not aimed to understand the effects of malnutrition in the early stages of this condition. Only the study by Malafaia et al. [21] aimed to fill this gap in knowledge and understanding of the effects of malnutrition in the short term in mice. For rats this gap persists, even with the potential to elucidate the parameters that can be used as indicators of initial frames of malnutrition.

In this context, the objective of this study is to evaluate the short-term malnutrition effects on physical, biochemical, hematological and histological parameters in Wistar rats. This study can contribute to elucidate the organic effects related to malnutrition, as well as to the study of malnutrition in the adopted experimental model.

2. MATERIAL AND METHODS

2.1 Animals and experimental diet

Ninety day-old female Wistar rats from the Biotério Central of the Universidade Federal de Goiás (Samambaia Campus, GO, Brazil) were used in this study. The animals were kept in individual metallic metabolic cages, under 12-hour photoperiod and mean temperature of 25 ± 2°C, in the Laboratory of Biological Research of the Instituto Federal Goiano – Urutaí Campus, GO, Brazil. Two experimental groups with seven animals each were established: a control group (C) and an undernourished group (D). Two independent experiments were performed, each lasting for 28 days.

The control group received a standard diet (Nuvilab CR1 – Nuvital), containing 19% protein, and the undernourished group was fed with a hypoproteic diet (adapted from Cabral [24]), containing 6% protein and prepared by PragSoluções Comércio e Serviços Ltda. – ME. Table 1 presents the compositions of the diets used in this study.

For the induction of the experimental malnutrition condition a protein-deficient diet was used (Tab. 1) and the volume given to the animals was limited to 50%, according to Vismara &

Furlan [14] and Mazareti & Furlan [25]. The food restriction imposed to the undernourished group was calculated daily from the control group food ingestion).

Table 1. *Composition of the experimental diets in grams for each 1000 g diet (g/kg) used in this study. Urutaí, 2014*

INGREDIENTS	**HYPOPROTEIC DIET(1) (g/Kg)**
Casein(2)	75
Corn starch	552
Dextrinized starch	130
Saccharose	100
DL-methionine	3.6
Mixture of salts(3)	35
Mixture of vitamins(4)	10
Choline bitartrate	2.5
Cellulose	50
Corn oil	40
BHT	0.8
INGREDIENTS OF THE NORMOPROTEIC DIET(5)	
Ground whole corn, soybean bran, wheat bran, calcium carbonate, dicalcium phosphate, sodium chloride, mixture of vitamins, minerals and amino acids	
SPECIFICATIONS (5)	**WARRANTY LEVELS PER kg OF THE PRODUCT (g/Kg)(5)**
Moisture	125
Casein(2)	220
Ether extract	40
Mixture of salts	90
Fibrous matter	70
Calcium	10.2
Phosphorous	8
ENRICHMENT PER kg OF THE PRODUCT(5)	
Vitamins: Vitamin A 13000 UI; Vitamin D3 2000 UI; Vitamin E 34 UI; Vitamin K3 3 mg; Vitamin B1 5 mg; Vitamin B2 6 mg; Vitamin B6 7 mg; Vitamin B12 22 µg; Niacin 60 mg; Calcium pantotene 20 mg; Folic acid 1 mg; Biotin 0.05 mg; Colin 1900 mg. **Minerals**: Zinc 60 mg; Copper 10 mg; Iodine 2 mg; Selenium 0.05 mg; Cobalt 1.5 mg; Fluorine 80 mg. **Aminoacids**: Lysine 12 g; Methionine 4000 mg. **Additives**: BHT 100 mg.	

(1) Diet prepared by *PragSoluções Comércio e Serviços Ltda. – ME*.
(2) The casein protein content was approximately 80%.
(3) Adapted from Reeves et al. [48] – AIN-93G-MX.
(4) Adapted from Reeves et al. [48] – AIN-93-GX.
(5) Nuvital.

The diets were offered as pellets and ad libitum for the control group and more restrictively to the undernourished group, as previously described. The diet for the undernourished group was offered at different hours each day, in order to induce malnutrition and to avoid the organism to adapt to nutritional restriction. Water was offered ad libitum for both experimental groups.

Evaluation of physical parameters

After the division of the experimental groups, the animals were submitted to a 7-day period of adaptation to the diets and experimental conditions. During this period, the undernourished group received the hypoproteic diet ad libitum. The evaluation actually started after the seven days of adaptation.

Body mass was determined daily, between the 8th and 28th experimental days. After that, all the animals were euthanized, and necropsy followed for the removal and weighing of liver, spleen, heart, pancreas, brain, kidneys, lungs and adrenal glands. For all organs and glands the absolute and relative masses were determined. The relative mass was calculated from the complete organ/body mass ratio, according to the method used by Oliveira [26] and Ritter et al. [27].

2.2 Evaluation of biochemical parameters

Serum measurements of glucose, total protein and its fractions, as well as the lipid profile, were carried out. The analyses were performed in a commercial clinical laboratory in Pires do Rio, Goiás State, by the automatized method A15 – Biosystems, according to Rodrigues et al. [28] and Rahamtalla et al. [29].

It is worth mentioning that for the biochemical evaluations, the animals fasted for at least 12 hours. By disrupting the brachial artery, 3 mL blood were collected in 5mL vials without the addition of anticoagulants.

2.3 Evaluation of hematological parameters

To the evaluation of hematological parameters, haemograms including erythrograms, plateletgrams and total leukocytes were obtained. The analyses were also performed in a commercial clinical laboratory in Pires do Rio, Goiás State, by the automatized method ABX – Micros 60, according to Tomczak et al. [30] and Urtiaga et al. (2013). For these analyses, the animals fasted for at least 12 hours. By disrupting the brachial artery, 1 mL blood was collected in 5 mL vials with the addition of EDTA.

2.4 Histological evaluation

For the histopathological evaluations, liver, spleen and kidneys from animals from both experimental groups were collected during euthanasia and fixed in 10% buffered formalin for at least 48 hours for following processing and inclusion in paraffin. Fragments of approximately 1 cm2 were processed in increasing concentrations of alcohols, diaphanized in two baths of xylene, embedded in two baths of liquid paraffin at 60ºC, for an hour in each battery, and included in paraffin. The paraffin blocks were cut to a thickness of 4 µm. The histological cuts were stained with hematoxylin-eosin (HE).

The histological sections were made in a commercial clinical analysis laboratory in Goiânia, Goiás State. The analyses of the histological cuts were qualitatively made under the microscope, in order to compare the tissue structures of the organs removed from animals of both experimental groups.

2.5 Statistical analysis and ethical issues

The Anderson-Darling normality test, followed by the Student t test and Repeated measured ANOVA test followed by the Post-hoc Bonferron test, were applied to all numerical data. The differences were considered statistically significant when p values were less than 0.05 ($p<0.05$). The statistical software Minitab® was used in the Anderson-Darling normality test and Student t

test. The software Statistica® 7.1 was used in the Repeated measured ANOVA and Post-hoc Bonferron test.

The methodology of this study was consistent with the ethical principles for animal experimentation and approved by the Ethics Committee for Animal Use of the Instituto Federal Goiano (protocol n. 003/20123).

3. RESULTS AND DISCUSSION

At the end of the experimental period, significant decrease ($F_{20, 240}$=29,500, p<0.001) in body mass of the undernourished group animals were observed (Fig. 1), with clear physical changes, when compared to the control group (Fig. 2).

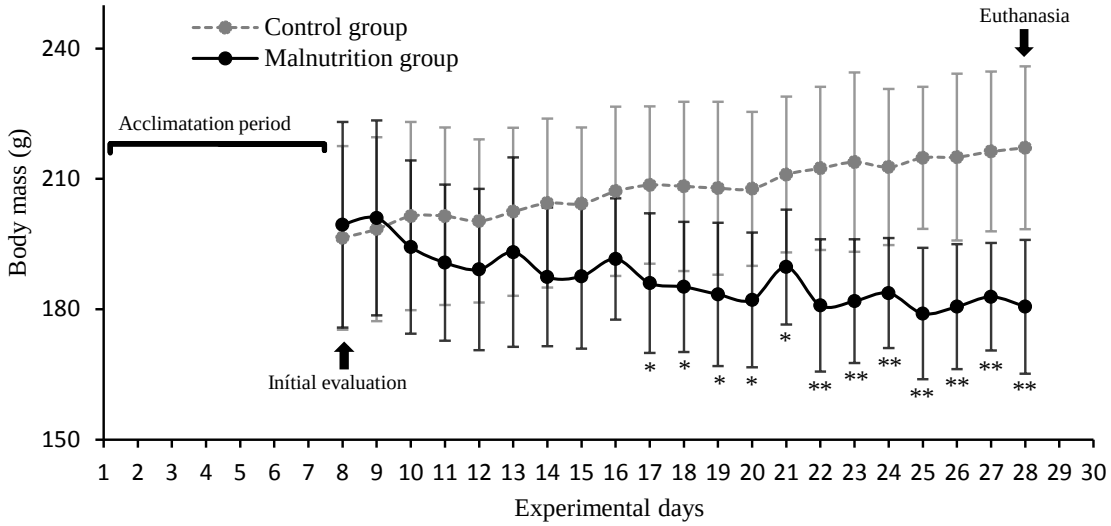


***Figure 1**: Body mass variation. The data are expressed as mean ± standard deviation. Differences between the groups are considered significant when *(p<0.05) or **(p<0.01). Significant differences from the 17th day on. The statistical analysis was performed using Repeated Measured ANOVA. The data are from two experiments carried out independently [control group (n=7) and malnutrition group (n=7)].*

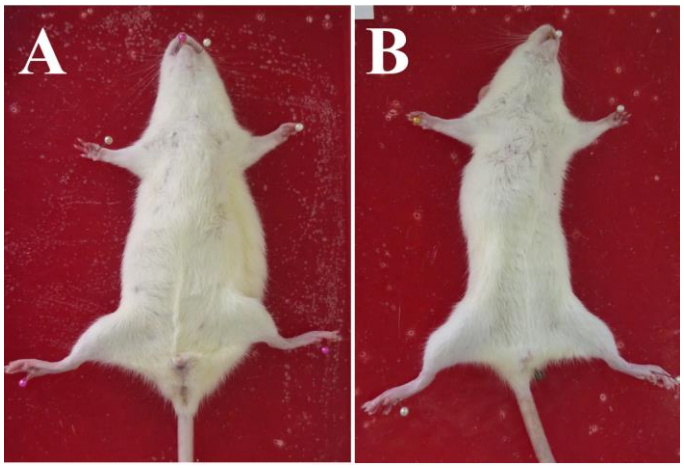


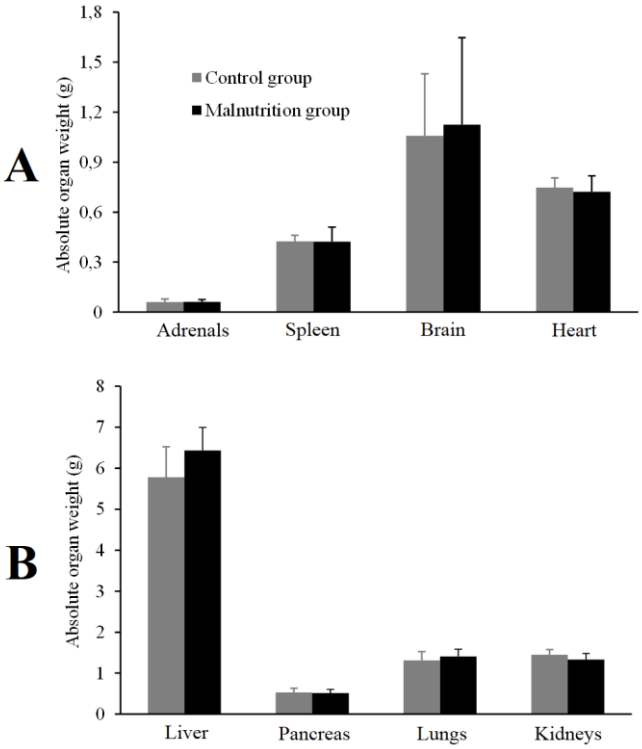
***Figure 2**: Visual aspect of the animals from the control group (A) and malnutrition group (B), photographed at the end of the experimental period.*

These results corroborate with previous studies that also evidenced significant body mass loss in animals submitted to malnutrition, but adopted experimental long-term malnutrition protocols [13,21,24]. It is worth stressing out that in the present study body mass reduction was observed in a short period of malnutrition, implying evident organic losses. Thus, we can attest that the

experimental protocol used in our study was effective in promoting a malnutrition condition, as body mass reduction can be used as a basic indicator for this condition [32].

Body mass loss in animals of the undernourished group, even in a short time period, can be related to the reduced food consumption imposed to them. As discussed by Alves et al. [33], the skeletal muscle tissue is sensitive to protein deficiency because it is a protein reservoir to the organism. When there is a protein deficiency in the diet, this tissue becomes a target for depletion [34,35,36], leading to significant muscle loss and consequently body mass loss. Besides, the lower food consumption leads to a caloric and micronutrient deficiency condition, even if the hypoproteic diet used in the present study is isocaloric in relation to the control diet.

No differences were found in the absolute masses of the organs from animals of both groups (Fig. 3A and B). Significant differences were observed only for liver and lung relative masses (Fig. 3C and D). Therefore, a three week-long malnutrition condition was insufficient to cause significant loss of absolute mass of the organs, corroborating to the hypothesis of initial depletion of skeletal muscles



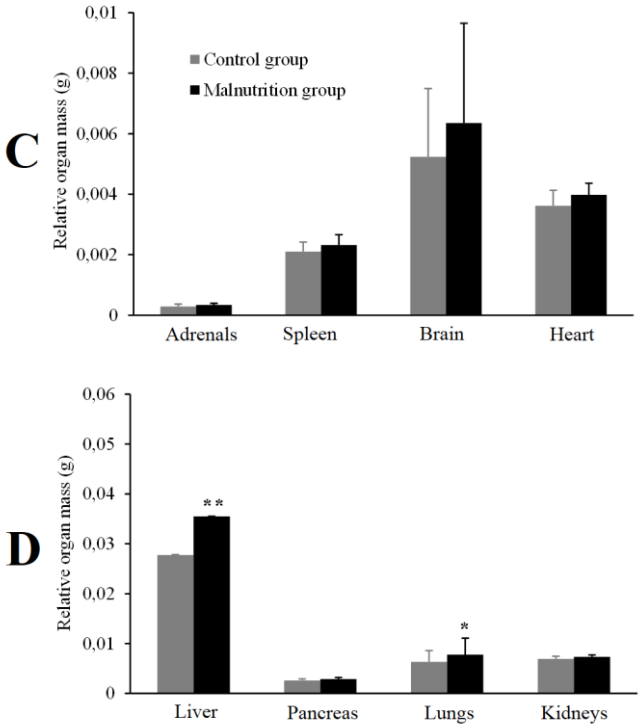


***Figure 3**: Absolute (A and B) and relative (C and D) masses of organs and glands. The data are expressed in mean ± standard deviation. . The statistical analysis was performed using Student's t test. The data are from two experiments carried out independently [control group (n=7) and malnutrition group (n=7)].*

These results corroborate to the results obtained by Malafaia et al. [13], who have not observed significant differences in the masses of some organs removed from Swiss mice submitted to aproteic diet for three weeks. These authors observed significant differences in liver and spleen masses, which contrast with the results of the present study. However, such differences could have been caused by the total lack of protein in the diet used by the authors, whereas in this study, the hypoproteic diet contained 6% protein.

Malnutrition by dietary restriction and severe hunger is known to produce a series of metabolic changes, which lead to body mass reduction, immunocompetence depression and change in the function of the digestive system, in particular liver and small intestine [37]. However, the weight of the organs of the undernourished animals was not adversely affected, indicating that the metabolic active mass of such organs was preserved, as shown by other studies [15,17,25].

Histologically, accumulation of fat was observed inside some liver cell vacuoles, with the characteristic displacement of the hepatocyte nucleus to the cell periphery (structure known as "signet ring"), suggesting a degenerative condition of initial hepatic steatosis in the undernourished animals (Fig. 4B and C – in detail). The paler and yellowish color of their livers confirms this hypothesis. Besides, the presence of a larger number of mononuclear infiltrate composed of lymphocytes, plasma cells and macrophages was observed in the liver of the undernourished animals, when compared to the animals of the control group. Additionally the hypertrophy of Kupffer cells was observed (Fig. 4D and E – in detail).

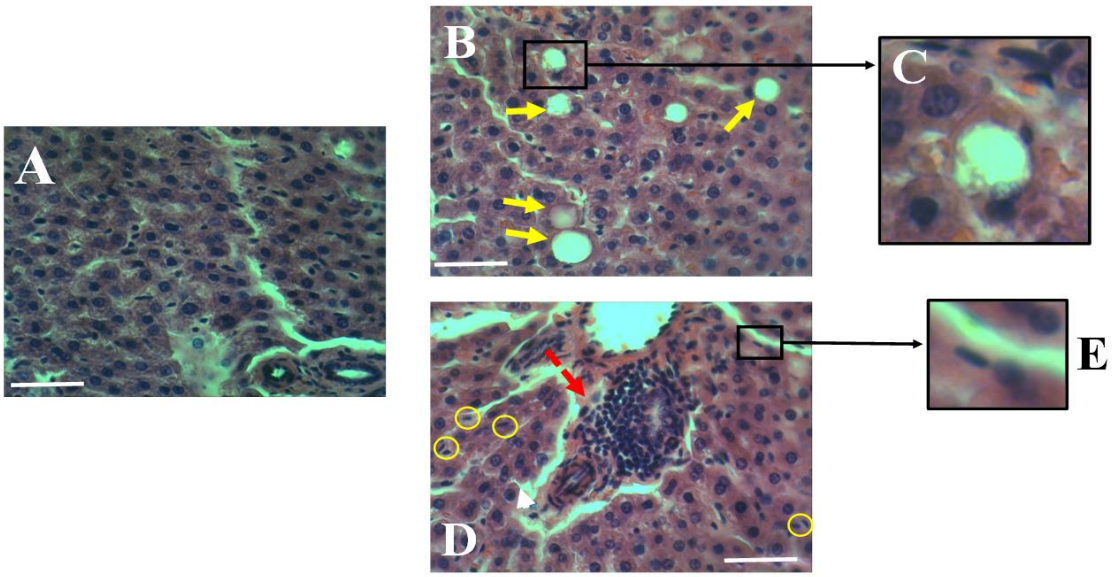


***Figure 4**: Photomicrographs of histological cuts (HE) representative of the liver from the control group (A) and the undernourished group (B, C, D and E) animals. Yellow arrows indicate fat accumulation inside some periportal cell vacuoles. Hatched red arrow indicates random inflammatory infiltrate. Circles indicate Kupffer cells. Bar = 100 µm. (A, B and D = magnitude 400 X).*

Despite these data reveal the presence of histopatological indicators characteristic of malnutrition, it is possible to infer that the malnutrition protocol imposed to the animals was not enough to promote diffuse histopathological changes. Histopathological studies of livers of undernourished murine models, as that developed by Coelho [38], have evidenced, among other changes, the presence of hydropic degeneration, inflammation of the portal tracts, hyperemia of the portal field, sinusoidal congestion, and granulomatous reaction.

Regarding the histological analyses, histopathological changes were not observed in kidneys and spleen (figure not shown). These results may be related to the short time period of malnutrition.

Regarding the biochemical parameters, it can be said that in general a few parameters changed during the malnutrition period imposed to the animals. Whereas lower glucose concentrations were detected in the control group, triglycerides and VLDL concentrations were statistically higher in undernourished animals (Tab. 2).

***Table 2**. Biochemical parameters[1] of the studied animals.*

Biochemical parameters	Control Group	Undernourished Group
Total proteins (g/dL)	7.53 ± (0.41)	7.47 ± (0.37)
Albumin (g/dL)	3.07 ± (0.30)	3.20 ± (0.26)
Globulins (g/dL)	4.46 ± (0.29)	4.27 ± (0.36)
A/G ratio (g/dL)	0.69 ± (0.08)	0.76 ± (0.10)
Glucose mg/dL	59.86 ± (18.72)	124.00 ± (45.15)**
Serum aspect	Limpid	Limpid
Total cholesterol (mg/dL)	97.57 ± (26.98)	86.86 ± (23.93)
Triglycerides (TG) (mg/dL)	109.14 ± (36.58)	62.43 ± (12.41)*
HDL (mg/dL)	50.71 ±(9.11)	48.57 ± (8.89)
LDL (mg/dL)	25.00 ± (15.21)	25.71 ± (16.21)
VLDL (mg/dL)	21.83 ± (7.32)	12.49 ± (2.48)*
Castelli index (I)	1.90 ± (0.22)	1.77 ± (0.31)
Castelli index (II)	0.46 ± (0.25)	0.51 ± (0.29)

[1] Data expressed as mean ± standard deviation. The statistical analysis was performed using Student's t test. The data are from two experiments carried out independently [control group (n=7) and malnutrition group (n=7)].
* Significant difference ($p<0.05$).
** Significant difference ($p<0.01$).

Regarding glycemia, it is suggested that malnutrition, even in a short time period, caused mitochondrial changes in liver and skeletal muscles of animals of the undernourished group. These are the organs mainly affected by insulin, according to Park et al. [39]. Such changes can explain the increase in glucose concentration seen in the undernourished animals (Tab. 2). In rats, the activities and gene expression of insulin-sensitive hepatic enzymes are altered in situations of protein malnutrition, such as the reduced activity of glucokinase and the increased activity of phosphoenolpyruvate carboxykinase [39]. Besides, metabolic changes in the pancreas occur, hindering the development of β-cells, and consequently decreasing insulin secretion in response to glucose [40].

Studies that developed long-term experimental malnutrition protocols using murine models show a decline in insulin production (hypoinsulinemia) [18,41], corroborating the hypothesis mentioned before. Galdino et al. [41] also observed that the insulin liberation is reduced in rats fed with hypoproteic diets. However, this effect is compensated by the increase in sensitivity of the insulin target tissues, which enables the maintenance of normal glycemic levels, as the animals are submitted to long periods of malnutrition.

In relation to the decrease in very-low-density lipoprotein concentrations (VLDL) and triglycerides (TGs), as suggested by Soares et al. [42], this is consistent with the reduced food consumption by the undernourished animals and lower hepatic VLDL synthesis, which is an important TG carrier [43], and also higher lipid oxidation for energy purposes. Similar data were obtained in previous studies. Madani et al. [44] observed that diets containing low casein concentrations (10%) yielded an evident reduction in TG, mainly in VLDL, plasma concentration. Nassir et al. [45] also found lower TG and VLDL concentrations in rats fed with diets containing 8% casein, when compared to diets containing 16% and 32% casein. Shaw & Huang [46] showed that rats fed with diets containing 8% lactalbumin, the rate of α-tocopherol secretion in VLDL and the activity of lipases (lipoprotein lipase, and total lipase released by heparin) in plasma and of hepatic lipase was only 50-60% of the observed in rats fed with normoproteic diets.

In relation to total cholesterol concentrations observed in both experimental groups (Tab. 2), it is suggested that the deficiency in protein synthesis caused by malnutrition did not interfere in the removal of circulating cholesterol, nor contributed to the increase in total cholesterol in the undernourished group, as observed by Oliveira [26]. It is also believed that the non-differentiation of HDL levels in the experimental groups reflects the trend observed in the total cholesterol levels (Tab. 2).

Regarding the hematological parameters, lower total leukocyte concentrations were observed in undernourished animals (Tab. 3). These data corroborate to previous studies [22,23,47], which indicates that the undernourished animals were immunocompromized, being more susceptible to infection and diseases. Besides, the decrease in total leukocytes is expected for the diet and malnutrition protocol used, once lack or deficiency in proteins affects negatively the immune system.

***Table 3**. Hematological Parameters[1] of the studied animals.*

Hematological parameters	Control group	Undernourished group
Erytrocytes (tera/L)	6.39 ± (0.47)	6.54 ± (0.28)
Hematocrit (%)	36.61 ± (2.45)	35.63 ± (2.10)
Hemoglobins (g/dL)	15.26 ± (1.23)	14.30 ± (0.49)
Total leukocytes (mm^3)	10071.43 ± (3263.80)	6828.57 ± (1731.78)*
Platelets (mm^3)	628000.00 ± (354016.01)	543285.71 ± (278408.40)

[1]Data expressed in mean ± standard deviation. The statistical analysis was performed using Student's t test. The data are from two experiments carried out independently [control group (n=7) and malnutrition group (n=7)].
* Significant difference ($p<0.05$).

4. CONCLUSION

From the exposed above, it is concluded that:

- The malnutrition protocol adopted in this study was able to induce a malnutrition condition, even in a short time period;
- The short-term malnutrition did not cause generalized biochemical or hematological changes in the animals of the study. However, it induced a leukopenia condition;
- The short-term malnutrition imposed to the animals was insufficient to cause diffuse histopathological changes in the analyzed organs.

This study is not exhaustive and therefore new research is suggested in order to investigate variables that were not considered here, as well as existing gaps that prevent a systematization of the knowledge on the subject

5. ACKNOWLEDGMENTS

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de Goiás (Fapeg) (Projetct n. 201210267001124) by financial support and Conselho Nacional de Desenvolvimento Científico and Tecnológico (CNPq) (scholarship – A.T.B. Guimarães).

1. World Health Organization (WHO). Malnutrition. Quantifying the health impact at national and local levels. Geneva: World Health Organization; 2005. 150 p.
2. Lacerda EMA, Accioly E, Faria IG, Costa VM. Práticas de Nutrição Pediátrica. São Paulo: Editora Atheneu; 2006. 250 p.

3. Olinto MTA, Victoria CG, Barros FC, Tomasi E. Determinantes da desnutrição infantil em uma população de baixa renda: um modelo de análise hierarquizada. Cad Saúde Pública. 1993 Jan/Mar;9(Supl. 1):14-27.
4. Monteiro CA, Benicio MH, Konno SC, Silva ACF, Lima ALL, Conde WL. Causas do declínio da desnutrição infantil no Brasil, 1996-2007. Rev Saúde Pública. 2009 Jan/Fev;43(1): 35-43.
5. World Health Organization (WHO) [Internet]. [Genebra]: Nutrition [cited 2014 Jun 14]. Available from: http://www.who.int/maternal_child_adolescent/topics/child/malnutrition/en/index.html.
6. Brasil. Programa de Combate às Carências Nutricionais – PCCN/ Ministério da Saúde. Brasília: Editora MS, 2001. 100 p.
7. Monteiro CA. A dimensão da pobreza, da desnutrição e da fome no Brasil. Estud Av. 2003 Maio/Ago;17(48):7-20.
8. Chagas DC, Silva AAM, Batista RFLB, Simões VMF, Lamy ZC. Coimbra LC, Alves MTSSB. Prevalência e fatores associados à desnutrição e ao excesso de peso em menores de cinco anos nos seis maiores municípios do Maranhão. Rev Bras Epidemiol. 2013 Jan;16(1):146-156.
9. Pedraza DF, Rocha ACD, Sales MC. Deficiência de micronutrientes e crescimento linear: revisão sistemática de estudos observacionais. Ciênc Saúde Coletiva. 2013 Nov;18(11):3333-3347.
10. Gerude MF, Augusto ALP, Alves DC, Mannarino IC. Terapia Nutricional. São Paulo: Editora Atheneu, 2002. 150 p.
11. Araújo EJA, Sant'ana DMG, Molinari SL, Miranda-Neto MH. Effect of protein and vitamin B deficiency on the morpho-quantitative aspects of the myenteric plexus of the descending colon of adult rats. Arq. Neuro-Psiquiatr. 2003 Fev;6(2):226-233.
12. Araujo EJA, Sant'Ana DMG, Molinari SL, Miranda-Neto MH. Biometric and food consumption parameters of rats subjected to hypoproteic and hypocaloric diet. Arq Ciênc Vet Zool UNIPAR. 2005 Jul/Dez;8(2):133-140.
13. Pinheiro AR, Salvucci ID, Aguila MB, Mandarim-de-Lacerda CA. Protein restriction during gestation and/or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. Clin Sci. 2008 Mar; 114(5):381-392.
14. Vismara MR, Furlan MM. Parâmetros biométricos como indicadores do grau de desnutrição em ratos sob restrição alimentar desde o nascimento. Arq Ciênc Vet Zool UNIPAR. 2007 Jan/Abr;11(1):3-8.
15. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology. 1996 Jun;111(6):1645-1653.
16. Okan A, Astarcioglu H, Tankurt E, Sagol O, Altekin E, Astarcioglu I, Gonen O. Effect of ursodeoxycholic acid on hepatic steatosis in rats. Digest Dis Sci. 2002 Nov;47(11):2389-2397.
17. Zamin-Junior I, Mattos AA, Mattos AZ, Migon E, Soares E, Perryy MLS. Modelo experimental de esteatohepatite não-alcoólica com dieta deficiente em metionina e colina. Arq Gastroenterol. 2009 Jan/Mar;46(1):69-74.
18. Fagundes AT, Moura EG, Passos MC, Oliveira E, Toste FP, Bonomo IT, Trevenzoli IH, Garcia RM, Lisboa PC. Maternal low-protein diet during lactation programmes body composition and glucose homeostasis in the adult rat offspring. Brit J Nutr. 2007 Nov; 98(5): 922-928.
19. Xavier JG, Favero ME, Vinolo MAR, Rogero MM, Dagli MLZ, Aranha-Chavez VE, Borojevic R, Borelli P. Protein-energy malnutrition alters histological and ultrastructural characteristics of the bone marrow and decreases haematopoiesis in adult mice. Histol Histopathol. 2007 Jun;22(6):651-660.
20. Borelli P, Blatt S, Pereira J, Maurino BB, Tsujita M, Souza AC, Xavier JG, Fock RA. Reduction of erythroid progenitors in protein-energy malnutrition. Brit J Nutrit. 2007 Fev;97(2):307-314.
21. Malafaia G, Martins RF, Silva ME. Avaliação dos efeitos, em curto prazo, da deficiência proteica nos parâmetros físicos e bioquímicos de camundongos Swiss. SaBios: Rev Saúde e Biol. 2009 Jul/Dez;4(2):21-33.
22. Ferrari F, Gabrielli PRM, Mello MAR. Restrição alimentar durante a gestação e suas implicações sobre o binômio mãe/feto. Um modelo experimental utilizando ratas jovens e adultas. Aliment Nutr. 1992 Jan/Dez;4(1):45-56.
23. Santos HB, Madruga MS, Bion FM, Antunes NLM, Mendes K, Águida R. Estudos bioquímicos e hematológicos em ratos sobre biodisponibilidade de minerais numa dieta enriquecida com multimistura. Ciênc Tec Aliment. 2004 Out/Dez;24(4):613-618.
24. Cabral CAC. Efeitos do exercício físico em cardiomiócitos de ratos desnutridos Ouro Preto (Minas Gerais) [Tese]. [Ouro Preto (MG)]: Universidade Federal de Ouro Preto; 2013. 62 p.
25. Mazereti CM, Furlan MMDP. Crescimento e parâmetros reprodutivos de ratas Wistar, em restrição alimentar desde o nascimento. Acta Scient Biol Sci. 2008 Abr/Jun;30(2):197-204.

26. Oliveira EC Avaliação bioquímica nutricional de animais treinados submetidos à desnutrição e recuperação nutricional. Ouro Preto (Minas Gerais) [Dissertação]. [Ouro Preto (MG)]: Universidade Federal de Ouro Preto; 2007. 139 p.
27. Ritter LLN, Santos WLM, Rodrigues JG, Almeida TR, Barbosa-Neto O. Treinamento físico por natação melhora perfil hepático em ratos Wistar tratados com dieta hipercalórica. Col Pesq Educ Física. 2012 Abr/Jun;11(2):183-190.
28. Rodrigues AL, Moura EG, Passos MCF, Trevensoli IHT, Conceição EPS, Bonono IT, Nogueira-Neto JF, Lisboa PC. Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. J Nutrit Biochemistry. 2011 Fev;22(2):109-117.
29. Rahamtalla FA, Elagib AA, Mahdi A, Ahmed SM. Prevalence of microalbuminuria among sudanese type 2 diabetic patients at elmusbah center at ombadda – Omdurman. IOSR J Pharmacy. 2012 Set/Out;2(5):51-55.
30. Tomczak ACT, Grilo KTM, Castro JM, Machado AMB, Leonart MSS, Nascimento AJ. Estudo de métodos laboratoriais para o controle de qualidade de unidades transfusionais eritrocitárias no Centro de Hematologia e Hemoterapia do Paraná (Hemepar), Brasil. Rev Bras Hematol Hemot. 2010 Maio/Jun;32(3):209-214.
31. Urtiaga G, Campos VF, Collares TF, Leon PMM, Deschamps JC, Seixas FK, Collares T. Associação entre proteínas do plasma seminal, motilidade e viabilidade espermática em coelhos submetidos a doping genético. Arq Ciênc Vet Zool. 2013 Jan/Fev;65(1):75-81.
32. Sawaya AL. Desnutrição: consequências em longo prazo e efeitos da recuperação nutricional. Est Av. 2006 Set/Dez;20(58):147-158.
33. Alves AP, Damaso AR, Dal-Pai V. Efeito da desnutrição proteica pré e pós-natal sobre a morfologia, a diferenciação e o metabolismo do tecido muscular estriado esquelético em ratos. J Pediatr. 2008 Mar;84(3):264-271.
34. Dubowitz V. Enzyme histochemistry of skeletal muscle. J Neurol Neurosurg Psychiatry. 1965 Dez;28(6):516-24.
35. Ihemelandu EC. Fibre number and sizes of mouse soleus muscle in early postnatal protein malnutrition. Acta Anat. 1985 Fev;121(2):89-93.
36. Oliveira FL, Oliveira AS, Schimidt B, Amâncio OM. Desnutrição energética intra-uterina em ratos: alterações músculo-esqueléticas na 1ª e 2ª gerações. J Pediatr. 1999 Jun/Jul;75(5):350-356.
37. Boza JJ, Moënnoz D, Vuichoud J, Jarret AR, Gaudard-de-Weck D, Fritsché R, Donnet A, Schiffrin EJ, Perruisseau G, Ballevre O. Food deprivation and refeeding influence growth, nutrient retention and functional recovery of rats. J Nutr. 1999 Out/Nov;129(7):1340-1346.
38. Coelho LF. Análise morfológica do fígado e baço de camundongos Balb/c submetidos à desnutrição protéico-calórica e infectados com *Leishmania infantum* [Dissertação]. [Ouro Preto (MG)]: Universidade Federal de Ouro Preto; 2011. 92 p.
39. Park KS, Kim SK, Kim MS, Cho EY, Lee HJ, Lee K, Pak YK, Lee HK. Fetal and early postnatal protein malnutrition cause long-term changes in rat liver and muscle mitochondria. J Nutr. 2003 Out;(10):3085-3090.
40. Park HK, Jin CJ, Cho YM, Park DJ, Shin CS, Park KS, Kim SY, Cho BY, Lee HK. Changes of mitocondrial DNA Content in the male offspring of protein-malnourished rats. Ann N Y Acad Sci. 2004 Jan;1011(1):205-216.
41. Galdino R, Almeida CCS, Luciano E, Mello MAR. Protein malnutrition does not impair glucose metabolism adaptations to exercise-traning. Nutr Res. 2000 Abr;20(4):527-535.
42. Soares JKB, Bion FM, Nascimento E, Medeiros MC, Pessoa DCN, Queiroz PMA. Prática de natação, consumo de aguardente e restrição alimentar em ratos adolescentes: consequências nutricionais e metabólicas. Rev Bras Ciênc Mov. 2011 Mar;19(3):58-68.
43. Gill JMR, Hardman AE. Exercise and postprandial lipid metabolism: an update on potential mechanisms and interactions with high-carbohydrate diets. J Nutr Biochemistry. 2003 Mar;14(3):122-132.
44. Madani S, Prost J, Belleville J. Dietary protein level and origin (casein and highly purified soybean protein) affect hepatic storage, plasma lipid transport and antioxidative defense status in the rat. Nutrition. 2000 Maio/Jun;16(5):368-375.
45. Nassir F, Moundras C, Bayle D, Serougne C, Gueux E, Rock E, Rayssiguier Y, Mazur A. Effect of selenium deficiency on hepatic lipid and lipoprotein metabolism in the rat. Brit J Nutr. 1997 Mar;78(3):493-500.
46. Shaw HM, Huang CJ. Secretion of alpha-tocopherol in VLDL is decreased by dietary protein insufficiency in young growing rats. J Nutr. 2000 Dez;130(12):3050-3054.

47. Melo JF, Macedo EMC, Silva RPP, Viana MT, Silva WTF, Castro CMMB. Efeito da desnutrição neonatal sobre o recrutamento celular e a atividade oxidante-antioxidante de macrófagos em ratos adultos endotoxêmicos. Rev Nutr. 2008 Jun;21(6):683-694.
48. Reeves PG, Nielsen FH, Fahey-Junior GC. 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-76A rodent diet. J Nutr. 1993 Nov;123(11):1939-1951.