Consequences on anthropometry and metabolism of offspring maintenance with low-protein diet since pregnancy to adult age

Consequências sobre a antropometria e o metabolismo em ratos submetidos a dieta com baixo teor de proteínas da gravidez até a idade adulta

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ABSTRACT

The pre and early post natal is a period high susceptible to environmental insults for instance nutrition. There is a high metabolic demand necessary for the multiplication and differentiation of cells for the formation of tissues and organs and unbalanced diets affect metabolism at long term. The present study aimed to analyze physiological and metabolic parameters in male offspring submitted to the low protein diet in the perinatal life followed by a normoprotein diet or kept on the same maternal low protein diet after weaning. 12 female Wistar rats were matched with male of same strainand according maternal diet forming normoproteic and low-protein groups during gestation and lactation. At weaning three groups were randomly formed: CC

(control-control), LP (low-protein-low protein) and LPC (low-protein-control). Somatic growth, feed intake, organ weight, biochemical parameters, liver fat, blood cell count and glucose tolerance test were analysed. The post-weaning "nutritional recovery" diet improved body mass and longitudinal length. But, the maintenance with low protein diet post weaning caused weight and length deficiency (P<0.001). Other parameters such as food intake, murinometric measurements, fasting gliscemia, visceral fat, organ weight, OGTT and biochemical parameters observed in the LPC were similar to CC. The LP groups caused lower area under the glycemic curve, lower visceral fat, but similar blood count, tibial growth and liver fat compared to control. The parameters evaluated in offspring submitted to nutritional recovery corroborate previous study, but the maintenance of offspring with low protein diet minimizes catch-up growth, but alters metabolic response to glucose.

Keywords: low-protein diet, catch-up growth, metabolismo, rats.

RESUMO
O pré e pós-natal precoce é um período altamente suscetível a insultos ambientais como, por exemplo, a nutrição. Há uma alta demanda metabólica necessária para a multiplicação e diferenciação das células para a formação de tecidos e órgãos e dietas desequilibradas afetam o metabolismo a longo prazo. O presente estudo teve como objetivo analisar parâmetros fisiológicos e metabólicos em descendentes masculinos submetidos à dieta de baixa proteína na vida perinatal seguida por uma dieta normoproteica ou mantidos na mesma dieta maternal de baixa proteína após o desmame. 12 ratos Wistar fêmeas foram acasalados com machos da mesma linhagem e de acordo com a dieta materna, formando grupos normoproteicos e de baixa proteína durante a gestação e lactação. No desmame, três grupos foram formados aleatoriamente: CC (controle-controle), LP (proteína baixa em proteína) e LPC (proteína baixa em proteína). Foram analisados o crescimento somático, ingestão de ração, peso dos órgãos, parâmetros bioquímicos, gordura hepática, contagem de células sanguíneas e teste de tolerância à glicose. A dieta de "recuperação nutricional" pós-desmame melhorou a massa corporal e o comprimento longitudinal. Mas, a manutenção com dieta de baixa proteína pós-desmame causou deficiência de peso e comprimento (P<0,001). Outros parâmetros tais como ingestão de alimentos, medidas murinométricas, gliscemia de jejum, gordura visceral, peso de órgãos, OGTT e parâmetros bioquímicos observados no LPC foram semelhantes ao CC. Os grupos LP causaram menor área sob a curva glicêmica, menor gordura visceral, mas contagem de sangue semelhante, crescimento tibial e gordura hepática comparados ao controle. Os parâmetros avaliados nos descendentes submetidos à recuperação nutricional corroboram o estudo anterior, mas a manutenção da descendência com dieta pobre em proteínas minimiza o crescimento, mas altera a resposta metabólica à glicose.

Palavras-chave: dieta pobre em proteínas, crescimento de recuperação, metabolismo, ratos.

1 INTRODUCTION

Population studies since the 1970s have shown that severe food restriction early in life is capable of promoting consequences in short and long term on development and health adult. Increase in the body in adult life, especially cardiovascular disease, type II diabetes, dyslipidemias (Symonds et al., 2009; Song et al., 2017) has been associated with the sedentary lifestyle in adulthood, as well as food habits and food pattern inherited early in life (Hales et

al., 1991) in descendants who maternal diet had nutritional and/or energetic deficit. The vulnerability of pre and postnatal environmental insults with emphasis on nutrition comes from the fact that this period is caractherized by a high nutritional and energetic demand necessary for the multiplication and differentiation of cells and formation of tissues and organs (Barker 2001; Von Ehr and Von Versen-Hoynck, 2016).

The premise for these associations refers to changes in the environment in which the fetus develops, which to ensure its survival, adapts to this adverse environment by optimizing the use of nutrients. Several studies advocate that an increase in nutrient availability after this period cause metabolic changes associated with obesity and type II diabetes for example (Barker, 2007). The mechanism involved in this context still need further clarification, but, may be associated with a "programming event", and / or "phenotypic plasticity". Plasticity has been used to explain that, during ontogenesis, the development of each organ or system passes through a critical window of sensitivity or plasticity, in which environmental factors, such as diet, can generate adjustments in the phenotype that remain along the life (Gluckman and Hanson, 2007; Jimenez-Chillaron et al., 2016). At the molecular level, phenotypic plasticity can be defined as several stimuli can modify the ability of expression of several phenotypes associated with a single genotype (West-Eberhard, 1998).

Another model related to the consequences of pre and postnatal aggression is Predictive Adaptive Response (PAR), suggesting that the developing organism has the ability to predict the environment in which it will grow, from maternal hormonal signals that cross the placenta and / or through milk maternal (lactation) (Gluckman and Hanson, 2004). These signals, therefore, would make the individual adjust his physiology according to this inference. Thus, the nature of the PAR is determined by the predicted and the prenatal and postnatal enviroment. So, if the actual postnatal enviromente matches the prenatal prediction then PAR is appropriate and disease risk is low. But, if it is not match, the disease risk is high. Thus, in the model PAR the relative difference in nutrition between the pre and postnatal environment, rather than an absolute level of nutrition that determines disease risk (Gluckman and Hanson 2004).

To date, the effects of low protein continuous on anthropometric parameters and metabolism at offspring have not been yet reported. We hypothesized that low-protein continuous in offspring is less deleterious to metabolism that change of abundant nutrients diet after weaning. Therefore, the present study aimed to evaluate growth parameters and metabolism of offspring derived from rats submitted to low protein diet during pregnancy and lactation, followed the continuity of this diet until adulthood.

2 METHODS

2.1 ANIMALS

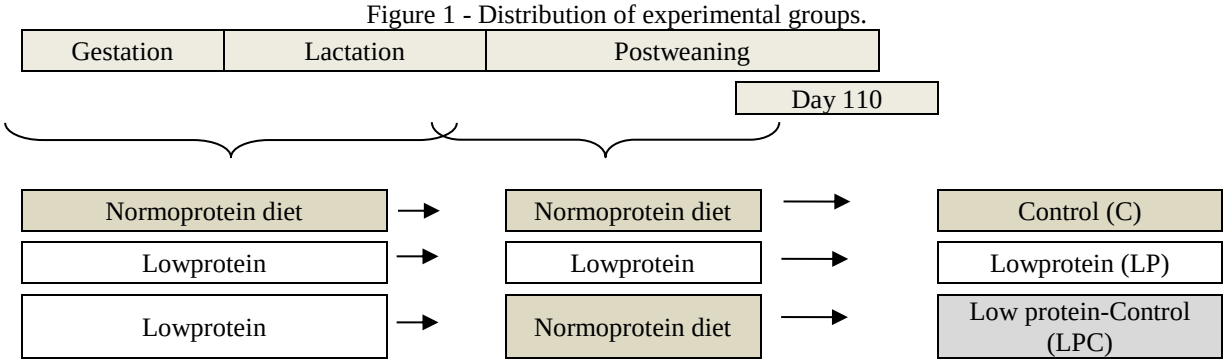
The project was approved by the Ethics Committee on Animal Use (CEUA) of UFPE with process nº: 23076.044215 / 2012-04 and followed the norms suggested by the Brazilian College for Animal Experimentation and with the international norms established by the National Instituteof Health Guide for Careand Use of Laboratory Animals (Bayne, 1996). The animals were maintained in an environment with a temperature of 22 ± 1°C in a light-dark cycle, light on (8:00 to 20:00) and light off (8:00 to 20: 00h).

Eleven female *Wistar* rats (*RattusNorvegicus*) weighing 220-250g and with 90-120d old were mated with male of same strain obtained from the Department of Nutrition of Federal University of Pernambuco (UFPE). The day on which spermatozoa were present in a vaginal smear was designed as the day of conception (day 0 pregnancy) followed by weekly body mass gain.

2.2 EXPERIMENTAL GROUPS

After confirmation of pregancy, the rats were isolated in individual cages and arbitrarily divided into 2 groups forming the control group (C, n = 6) and low protein group (LP, n = 5) that received normal protein diet (protein 17g%) or a low protein (8g% protein) respectively throughout gestation and lactation. The 24h after delivery the offspring were arbitrarily distributed between mothers to form litters of eight animals, composed of four males and four females (in both, control and low protein groups). At weaning, only male offspring remained in the study and female offspring were used for other experiments.Thus, the groups formed were: Control (CC, n = 13) - animals that remained consumed normoproteic growth diet (AIN-93G, 17g% protein) until 60 days of life and after 60 days of life receiving normoproteic maintenance diet (AIN93-M, 12% protein) (Reeves et al, 1993). The low protein group was subdivided into two groups: Low protein (LP, n = 13) continued to receive the same low protein diet of dams (8g% protein) until the end of the experiment; and Low protein - Control (LPC, n = 11) that received low protein diet during pregnancy and lactation, and after weaning received a control diet. The control diet (AIN-93G, 17g% protein) until 60 days of age followed of maintenance diet with 12g% of protein (AIN93-M) form 61 to 110 days (Figure 1) of life.

Figure 1 - Distribution of experimental groups.



2.3 COMPOSITION OF DIETS

Normoprotein diets were formulated according to AIN-93G and AIN-93M recommendations, with 17 and 12% protein, respectively. The low-protein diet was formulated with protein reduction (8%) but remained isocaloric similar to AIN-93 (REEVES, 1997) (Table 1).

Table 1. Nutritional composition of the normoprotein and low protein diets offered during the gestation, lactation and post-weaning periods

Nutrients	Growth		Maintenance	
	Normoprotein diet (17g%)	**Lowprotein diet (8g%)**	**Normoprotein diet (17g%)**	**Lowprotein diet (8g%)**
g%	100	100	100	100
Protein	17.4	8.3	12.0	8.3
Carbohydrate	61.9	73.1	71.6	76.1
Lipid	7.0	7.0	4.0	4.0
Fiber	5.0	5.0	5.0	5.0
Vitamin	1.0	1.0	1.0	1.0
Mineral	3.5	3.5	3.5	3.5
Methionine	0.3	0.2	0.3	0.2
% Kcal	**380.2**	**388.6**	**370.4**	**373.6**

Source: Adapted values of the recommendations of AIN-93G and AIN-93M according to Reeves, 1997.

2.4 STUDIES IN VIVO

2.4.1 Somatic growth

Somatic growth was assessed in terms of body mass, longitudinal length and tibia length. Offspring body mass was performed weekly between 7:30 am and 8:00 am. The body mass gain of the animals in the period was evaluated by means of the body mass gain percentage, by the following formula: % WG = [Weight of the day (g) x 100 / Weight of the 1st day (g)] - 100 (Bayol et al., 2004).

The length longitudinal (LL, distance for nose to anus) was measured weekly from birth to the end of the experiment, containing the animal gently (Santana Muniz et al., 2013) was

measured with digital caliper (Starret ®, series 799, São Paulo, Brazil) with a 0.01mm precision.

After sacrifice the left tibia was removed. The bone was submerged in a solution of water (2/3) and sodium hypochlorite (1/3) for 48 hours, so that excess muscle detaches from the bone portion (Liu et al., 2003). After the established period, the solution was discarded and the tibia dried at ambient temperature. After this procedure, the tibia was weighed and with the aid of a digital caliper (Mark Starrett®, with accuracy of 0,01mm) was carried out the measurement of the upper diameter (millimeters). The choice of tibia measurement is based on the fact that it provides support for the evaluation of the animal's growth.

2.4.2 Measurement of food intake

Offspring were housed in pairs (constituting a sample unit) and animal´s daily food consumption was determined by the difference between the amount of provide (50 to 60g) at the onset of the light phase and the amount of food remaining 24h later. Body and food weight was measured with a digital electronic scale was used, Marte XL 500, class II, maximum capacity 500g (smaller division 0.001g) (Santana Muniz et al., 2013)

2.4.3 Fasting glycemia and oral glucose tolerance teste (OGTT)

At 22, 45 and 90 days old, and after fasting (12 h), blood glucose was determined. The OGTT was prerformed at 90 days of age with the same fasting time interval, with the first blood collection considered zero time. A small aliquot of blood was collected by small puncture at the tail end of the animal, and then a 50% glucose solution was given by gavage (Equiplex Indústria Farmacêutica Ltda., Aparecida de Goiânia, GO, Brazil 62), at a dose of 2 mg / kg body weight. Subsequently samples were collected at times 30, 60, 90 and 120 minutes. The sample was deposited on the test strip (glucose, Roche®) and analyzed on the Accutrend Glucose (Roche®) apparatus. The area under the glucose curves (ΔG), obtained by the blood glucose values at 0, 30, 60, 90 and 120 minutes, and was calculated by the trapezoidal method. (Le Floch, Escuyer et al. 1990; Mathews, Xue et al. 2015).

2.4.4 Biochemical assesssment

The offspring were euthanaized, at 110 days of life and blood was collected in serology tubes and centrifuged at 1048 g for 20 minutes. The serum was transferred to a cryogenic tube and stored in a freezer at -80º C for subsequent biochemical analyses of glucose, cholesterol, triglycerides, total proteins and albumin. Thevery-low-density-protein cholesterol (VLDL-c)

was obtained by the Friedewald formula (Martins et al., 2015). These analyzes were determined with commercially available kits (Labtest Diagnóstica S.A®, Lagoa Santa-MG, Brazil).

2.4.5 Wet weight of organs and determination of visceral fat in the offspring

After euthanasia the abdominal and thoracic cavity were opened through a large medial incision. The animals had the heart, liver, and stomachs removed, cleaned and quickly weighed. Subsequently, fat removal from the retroperitoneal and gonadal regions served as a parameter for the evaluation of visceral fat depots. The values obtained were used for calculations of the relative weights (g/100g). All were weighed in digital analytical balance (Shimadzu, BL320H with sensitivity of 0.001g).

2.4.6 Determination of blood counts and hepatic fat content of offspring

After animals`seuthanasia, the blood was collected in heparinized tube and submitted to slight homogenization. This material was used to complete haematological analysis using automatic cell counter (ABX Micros 60 Horiba).

The liver was weighted and removed and the hepatic fat was determined by the SOXHLET method (Instituto Adolfo Lutz, 2008). For the calculation of lipid percentage, the following formula was used: L = (W / Wa) x 100, where L = lipids, total fat or ethereal extract, W = B1 - B0 (Weight of the extract), B1 = Balloon weight + extract, B0 = Balloon tare and, Wa = Sample weight (g). The results were expressed in g%.

2.4.7 Statistical analyses

Normality of the measurements was assessed using the Shapiro-WilK´stest. The parametric data for two samples were analyzed by the unpaired and paired Student t test as required. For multiple comparisons between groups, analysis of variance (ANOVA) was used. For the measures of interaction between two factors, the two-way ANOVA test was used. When the difference between groups was detected, the Bonferroni post-test was used.Statistical significance was set at $p \leq 0.05$, whereas $0.05 < P < 0.10$ was considered a trend toward significance. Data analysis was performed using the statistical program Graphpad Prism5® (GraphPad-Software Inc., La Jolla, CA, USA).

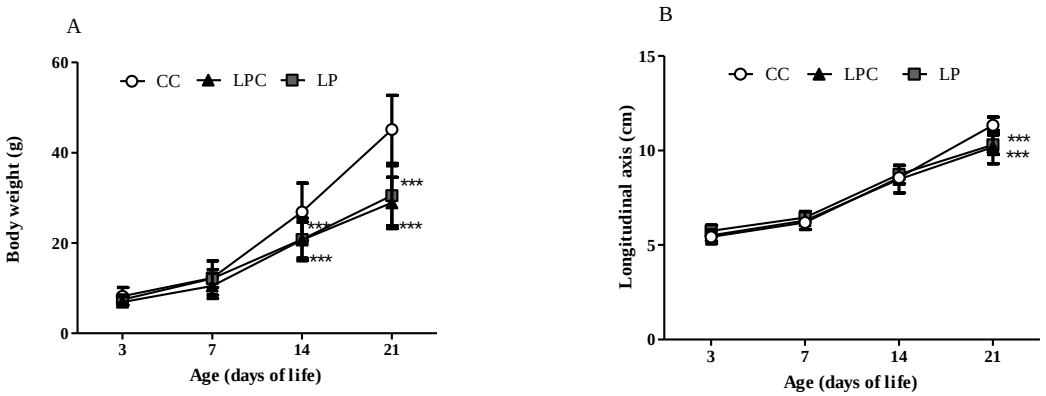
3 RESULTS

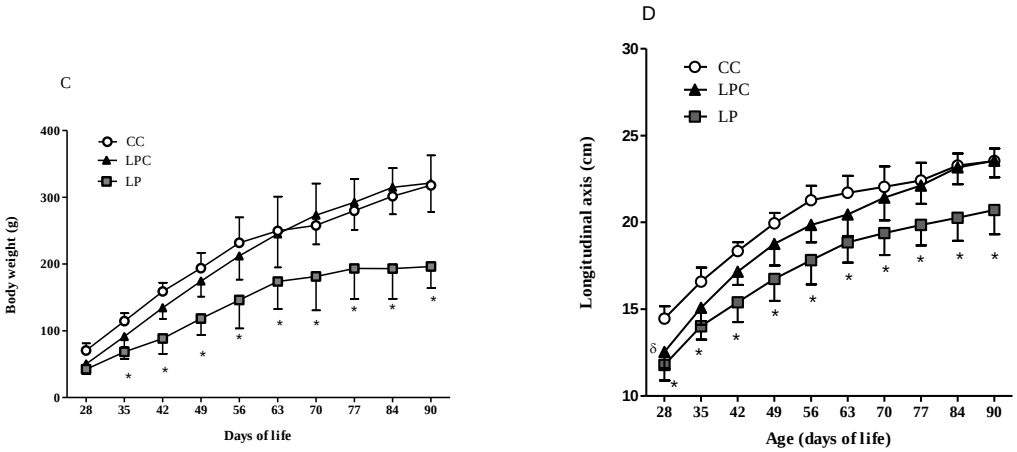
3.1 SOMATIC GROWTH

From the 2nd week of life, the offspring from of dams submitted to low-protein diet showed deficits of body mass (LP = 20.49 ± 4.72, n = 13; LPC = 20.67 ± 4.01, n = 11; CC = 26.58 ± 6.36, n = 13; p <0.01) and they will remain with low body mass until 21 days old (Figure 1A). At end of the weaning, a reduction in longitudinal length was also observed compared to control (LP = 10.31 ± 0.52, n = 11, LPC = 10.18 ± 0.89, n = 11; CC = 11.33 ± 0.44, n = 9, p <0.001) (Figure 1B). At 90 days old, the body mass of the LP group (maintenead with the same diet of mothers during gestation and lactation) was about 38% lower than the control, but the low-protein recovered group was similar to control (CC = 317.96 ± 44.7, n = 12, LP = 196.26 ± 32.22, n = 12; LPC = 321.23 ± 43.03, n = 9; P<0.001). (Figure 1C). This increase of percentage body mass reached un value of 403% grater when compared to control group since weaning.

Regarding longitudinal growth, a similar percentage of gain was observed between CC and LP. While the LPC group showed a growth 27% higher than the CC (Figure 1D). On the other hand, when the tibia growth was relativized, the LP group showed higher values compared to the CC and LPC groups. These data suggest that bone growth was not impairment by maintained of low protein diet intake after weaning.

Figure 1 - Evolution of body weight (A, C) and longitudinal length (B, D) of males born to mothers fed control or low protein diet during gestation and lactation and after weaning (C, D), respectively. Values presented as mean and standard deviation of the mean. Groups CC, n = 12; LP = 12; LP = 9. Values represented in mean and standard deviation. Two-way ANOVA test was used to analyze the data, followed by the Bonferroni post-test, p <0.05.





3.2 FOOD CONSUMPTION

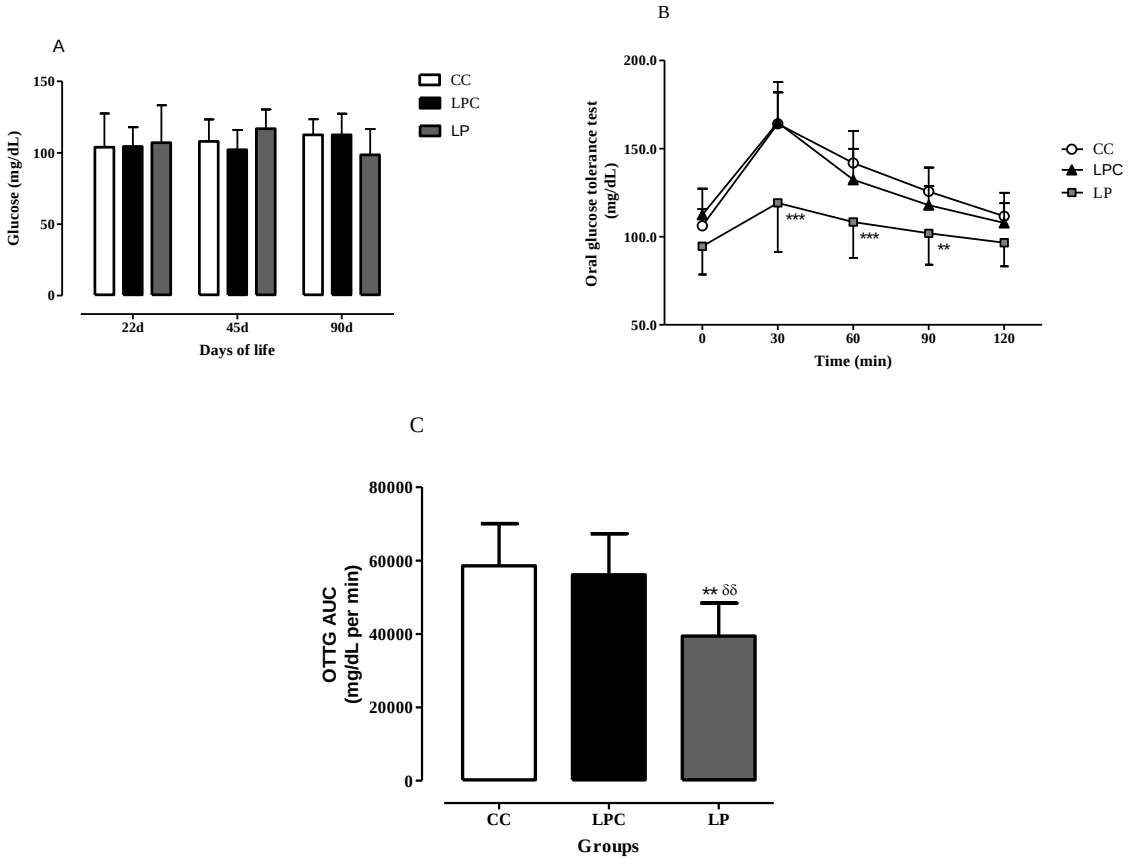
Food intake accumulated during the period observed (10 weeks) not showed difference among the groups (CC = 150.0 ± 21.9g, LP = 125.8 ± 19.8g, LPC = 151.5 ± 25.5 g; P = 0.23). These results suggest that even without detecting differences in the total amount of ingested food or energy, since all had similar energetic value, the LPC group showed a greater weight gain than the control group feature the catch-up growth, but, the LP had a weight gain lower than both, CC and LPC.

3.3 FASTING GLUCOSE AND ORAL GLUCOSE TOLERANCE TEST

Fasting glycemia was determined at three different ages: after weaning (22 days old), at 45 days old (the end of the highest growth period) and at the beginning of adult life (90 days old). No differences were observed amongthe ages in accordance to dietary treatments of the groups (Figure 2A).

The oral glucose tolerance test (OGTT) did not show differences between LPC and CC groups, but revealed a greater tolerance to the glucose load in the LP group when compared to the CC (Figure 2B) demonstrating a smaller area under on curve (Figure 2C). The area under on curve expresses the average concentration of glucose per minute compared to the other groups.

Figure 2 - Fasting glycemia on days 22, 45 and 90 (A), oral glucose tolerance test (B) and area on the glycemic curve (C) of males from mothers fed control or low protein diet during gestation and lactation, which were or were not recovered with normoprotein diet after weaning. Groups CC (control, n = 7), LP (low protein, n = 8), LPC (recovered low protein, n = 9). Data expressed as mean and standard deviation. Results obtained by two-way ANOVA (A and B) and one-way ANOVA (C). ** δδ<0.01 vs CC; ***P <0.001 vs CC; δP <0.05 vs LPC; δδδP <0.001 vs LPC.



3.4 BIOCHEMICAL PARAMETERS AND WET WEIGHT OF ORGANS

The continuity of the low protein diet after weaning caused reduction of albumin and total protein in the blood (Table 2), which is suggestive of existing of malnutrition. In the organs, no differences were found between LPC and CC groups. But due to the lower body mass in the LP group compared to CC and LPC groups was founded that the LP animals presented higher weights of the liver, stomach and testicles when relativized by body mass (Table 2).

Table 2 - Effects of maternal low protein diet and post-weaning diet, control-control or control-low protein biochemistry and on the wet weight of the organs of the offspring at the age of 90 days.

GROUPS	CC Mean ± standard deviation	LPC Mean ± standard deviation	LP Mean ± standard deviation	Pvalue
Glucose(mmol/L)	6.77±1.04	6.77 ± 0.80	7.01 ± 1.98	0.92
Albumin (mg/dL)	3.85± 0.11	3.49±0.47	**3.19±0.54*δ**	<0.00
Total Protein (mg/dL)	6.93± 1.39	7.54±1.53	**5.30±0.19*δ**	0.01

Triglycerides(mg/dL)	86.55 ± 27.75	68.68±26.90	66.38 ±32.66	0.25
Cholesterol (mg/dL)	78.51 ±6.33	80.53 ±9.29	77.45 ± 7.87	0.79
VLDL-c (mg/dL)	17.31 ± 5.55	13.74 ± 5.38	13.27±7.53	0.25
ABSOLUTE WEIGHT OF ORGANS(g)				
Heart	1.34 ±0.15	1.23 ± 0.11	**0.94±0.18*$^{\delta}$**	<0.00
Liver	9.87 ±1.58	9.88 ±0.74	8.28±1.84	0.11
Stomach	1.60 ±0.27	1.66 ± 0.03	1.72± 0.26	0.52
Visceral fat	11.69 ±4.45	9.29 ± 4.80	**2.65 ± 1.27*δ**	<0.00
Righttesticle	1.56 ±0.07	1.59± 0.08	**1.28±0.10*δ**	<0.00
Lefttesticle	1.56 ±0.08	1.62± 0.04	**1.30±0.12*δ**	<0.00
Brain	1.90 ±0.07	1.91± 0.06	**1.68 ±0.09*δ**	<0.00
RELATIVE WEIGHT(g/100g)				
Heart	0.42 ±0.05	0.42 ± 0.05	0.46 ± 0.08	0.38
Liver	3.08 ± 0.49	3.38 ± 0.49	**4.04 ± 0.86*δ**	0.01
Stomach	0.51±0.14	0.59 ± 0.09	**0.88 ± 0.28*$^{\delta}$**	<0.00
Visceral fat	3.56 ±0.96	2.99 ±1.19	**1.44 ± 0.46*$^{\delta}$**	<0.00
Righttesticle	0.49 ±0.08	0.55 ± 0.12	**0.64 ± 0.15*$^{\delta}$**	0.02
Lefttesticle	0.49 ±0.08	0.56 ± 0.13	**0.64 ± 0.16*$^{\delta}$**	0.02
Brain	0.63 ±0.09	0.66 ± 0.11	**0.96 ± 0.11*$^{\delta}$**	<0.00

CC = Control, LP = Low Protein, LPC= "Low-protein-Control Diet" Data expressed as average ±DP. Differences detected through one-way ANOVA test. * *vs* CC;; $^{\delta}$*vs*LPc;

3.5 HEMOGRAM AND LIVER FAT

Continuous low-protein diet or only during pregnancy and lactation (LP or LPC) did not cause significant changes on hematological parameters. But was observed a trend to accumulation of fatty liver in both LP groups (LPC and LP) compared to control group (C= 3.6±0.9; LPC=.4.6±2.0; LP= 5.6±1.2g/100g; P=0.08) – Table 3.

Table 3 - Percentage of liver fat, blood count and growth parameters of the offspring of the tibia according to dietary manipulation pre and post-weaning.

	C, n=7	**LP, n=8**	**LPC, n=9**	**p**
Liver fat	3.99 ± 1.33	5.56 ± 1.19	3.99 ± 2.23	0.20
Blood count				
Red blood cells (106/mm3)	7.45 ± 1.20	6.26 ± 0.89	6.76 ± 0.21	0.08
Hemoglobin (g/dL)	13.64 ± 1.32	12.02 ± 1.54	13.00 ± 0.51	0.09
Hematocrit (%)	40.42 ± 5.84	34.37 ± 5.11	38.34 ± 1.06	0.09
Platelet count (103/mm3)	348.30 ± 139.30	363.00 ± 174.20	540.60 ± 138.80	0.09
Total leukocytes (103/mm3)	5.63 ± 3.70	2.50 ± 2.18	3.18 ± 1.62	0.12
Absolute tibia parameters				
Weight (g)	0.59 ± 0.10	0.63 ± 0.10	0.78 ± 0.29	0.11
Length (cm)	3.70 ± 0.15	3.76 ± 0.29	3.86 ± 0.38	0.54
Upper diameter (mm)	6.60 ± 0.14	6.99 ± 0.61	7.30 ± 1.02	0.11
Mean diameter (mm)	3.43 ± 0.36	3.05 ± 0.14	3.34 ± 0.40	0.05
Minor diameter (mm)	6.19 ± 0.39	6.03 ± 0.15	6.40 ± 0.29	0.13
Relative parameters of the tibia				
Weight (g)	0.19 ± 0.02	0.33 ± 0.12 **	0.26 ± 0.06	<0.00
Length (cm)	1.22 ± 0.18	1.89 ± 0.51 **	1.32 ± 0.12 δ	<0.00
Upper diameter (mm)	2.17 ± 0.32	3.49 ± 0.89 ***	2.49 ± 0.23 δ	<0.00
Mean diameter (mm)	1.12 ± 0..12	1.55 ± 0.45 *	1.15 ± 0.13	0.01
Minor diameter (mm)	2.03 ± 0.32	3.06 ± 0.87 **	2.21 ± 0.32	<0.00

Data expressed in average ±DP.*One-way* ANOVA test was used. *P<0,05 vs C; **P<0,01 *vs* C; ***P<0,001 *vs* C; $^{\delta}$ P<0,05 *vs* LPC.CC = Control, LP = LowProtein, LPC = LowProtein-Control.

4 DISCUSSION

Studies on offspring outcome derived from mothers feed with low-protein diet during pregnancy and lactation and recoverd after weaning generally cause catch-up growth, metabolic alterations and higher risk of chronic disesase in adult life (Alexandre-Gouabau et al., 2012; Berends et al., 2013) is well documented. But, the physiological and metabolic outcomes in the offspring following low-protein diet for a long time is less known. Thus, we found that maintenance of low proteic throughout life promotes permanent lower body mass and length, altered glucose tolerance, reduced visceral fat, and suggestive chronic protein malnutrition, but without changes in tibial growth. It is believed that the change in dietary pattern in adulthood is one of the relevant factors for the appearance of future metabolic changes. This work is one of the few studies that evaluated the effect of maintaining the low protein diet of pregnant until adult life on metabolic factors and parameters of body growth.

Nutritionally recovered animals presented body mass similar to control, different of the other studies, who the body mass of the recovered group was lower (Dudele et al., 2012). We attribute this difference to the type of diet used among the studies. Standard chow diet has different nutritional composition compared to casein-based diet, used in present study. In previous studies that utilized standard chow diet the recovered group had low body mass compared to control group. The group maintained with the low protein diet not reaches the same body mass that control group or recovered group, but the body weight gain after weaning was similar to control group. The association of weight gain and longitudinal growth in animal previously malnourished reveals the ability of diet change after weaning to stimulate catch-up growth in animals. This rapid growth has been seen in several species between humans and animals (Coupe et al., 2009; Ziegler 2015) that received defficient diet in pregnancy and/or early life and subsequently receive diet high in energy and nutrients.

Malnutrition during pregnancy and lactation is related to changes in the brain control of feeding behavior, which can promote overeating (Orozco-Solis et al., 2009). Studies have demonstrated that hypothalamic pathways are altered in expression of orexigenic and anorexigenic peptides, causing an imbalance with increased expression in orexigenes peptides and reduction of anorexigenes peptides, and consequently, increase food intake (Coupe et al., 2009; Bharne et al., 2015). However, in the present study, animals nutritionally recovered do not present hyperphagia. This fact can be possibly due to the type of diet during the period of nutritional recovery respecting energy demand and distribution of other nutrients. Dietary changes may modify feed preferences. It is common for animals submitted to perinatal undernutrition and consuming normoprotein diet after weaning to present in adulthood

metabolic changes (Ravelli et al., 1976; Orozco-Solis et al., 2009) and feed preference (Bellinger et al., 2004).

Our data revealed that animals recovered nutritionally showed no change in relevant metabolic parameters such as blood glucose, triglycerides and cholesterol or glucose intolerance. However the animal group maintenance with the same low protein diet since pregnancy showed high responsivity to glucose. The model of predictive adaptive response (PAR) explains that the organism is able to predict what the situation will develop, this can prevent the emergence of wrong answers and deleterious outcomes (Gluckman et al., 2005). Another important factor is that the organism presents the capacity of plasticity according to the energy demand (Norman et al., 2012). These two factors can be considered as key points for the nutritional recovery of animals without presenting metabolic alterations at risk of non-transmissible metabolic diseases.

The liver weight can express several metabolic outcomes. The hepatic steatosis is an index used to demonstrate the metabolic answer of the organism to the diet consumption including resistance to insulin and excessive accumulation of lipids (Bugianesi et al., 2010). The fat in liver brings damage to the hepatocytes that get occupied with droplets of fat rather than storing glucose in the form of glycogen (Orman et al., 2012). As a consequence, both glucose and lipid metabolism can be impaired. We didn´t observe significance in accumulation of fatty liver but had a tendency ($P<0.10$) in accumulation in both LP groups (LPC and LP). A study demonstrated with use of low-protein diet in utero changed genes expression relationed to lipidic metabolism regulation lead to the development of fatty liver (Carr et al., 2014).

In this study the monitoring of fasting glycemia in different periods of the life span it was not influenced by the use low protein diet throughout the experimental period or by recoverd nutritional. Previous study with monitoring of glycemia at ages of 28, 56, 70 and 84 days also found no glycemic changes (Dahri et al., 1991) in recovered nutritionallly rat. However, we found intriguing changes in glucose tolerance test where no change was observed in the recovered group (LPC) and greater tolerance was observed in the group maintained on a low protein diet (LP). This result is completely opposite of that found in several previous studies that show the resistance to return to glucose at basal levels in animals from dams fed with low-protein diet (Dahri et al., 1991; Zheng et al., 2017) and this resistance may to be associated to epigenetic changes such as hepatic microRNAs expression relationed glucose regulation (Zheng et al., 2017).

In the other hand, the greater sensibility observed in oral glucose tolerance in group maintained on a low protein diet may be due to the initial increase in insulin sensitivity in

response to the increase in receptors of insulin in the skeletal muscle (Ozanne et al., 1996; Ozanne et al., 2003). This hypersensitivity to insulin would be associated with an increase in the initial steps of the signaling intracellular expression of the hormone (Latorraca et al., 1999). However, this hypersensitivity may be modified over time because it is suggested that a schedule occurs in the offspring of low protein mothers in the perinatal life favoring a hyperinsulinemia with tissue resistance, manifestations of glucose intolerance and Type 2 diabetes (Ozanne et al. 2005; Guzman-Quevedo et al., 2013). Another relevant point is the low amount of visceral fat found in the low protein group. The accumulation of visceral and hepatic fat is reported in malnourished rats after recoverd nutritional period is associated increase of the risk of metabolic disorders and chronic diseases (Lesage et al., 2006) and this result probably is associated genic expression in fatty regulation in liver (Carr et al., 2014).

In this study, our animals in continuous low protein diet do not show impaired bone growth of the tibia, showing preservation of their bone growth capacity. The deleterious effects of maternal nutrition on inadequate bone growth can occur in the epiphyseal plate morphology compromising the longitudinal bone growth (Even-Zohar et al., 2008) or affecting the formation and calcification of bone matrix affecting the growth of long bones (Nakamoto and Miller, 1979). In mammals, longitudinal bone growth occurs on the epiphyseal plate, a thin band of cartilage located near the ends of long bones (Abad et al., 1999). The epiphyseal plaque is highly regulated through the integration of signals produced by systemic and local hormones (Ballock and O'Keefe 2003), but it can also be influenced by environmental factors. In rats, the acceleration phase of bone growth is the period 21 to 35 days of life, which occur in high rates of longitudinal bone growth (Hunziker and Schenk, 1989). Although we saw a lower growth in the group kept under low protein diet, we found in this study changes on the growth of the tibia at 90 days of life.This result contrasts with a previous study that associated to low energy die showed reduction in the weight and diameter of the tibia of pups at 60 days of life (Nascimento et al., 2014).

Moreove, the data show some deleterious outcomes with maintenance of low protein diet in young rat such as low plasmatic protein**.** So, this exploratory experimental study requires further studies to investigate the short- and long-term repercussions as well as a molecular level assessment of cellular changes. In addition, follow-up until young adulthood does not allow us to infer what might happen in later life as increased metabolic disorders and chronic disease facilities such as obesity, dyslipidemias and diabetes.

5 CONCLUSION

The continuous low-protein diet of offspring by life span caused less body mass and body length, altered sensitivity to glucose but without difference in basal levels and reduced **visceral** fat. In addition, did not reduce the relative vital organs weight or length and diameter of tibia. However, maintaining a low protein diet not supports the hypothesis of pedictive adaptive responses that advocate less adverse metabolic outcome in offspring.

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