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ORIGINAL ARTICLE

Hypocaloric Diet in Perinatal Life Followed by Obesity Exacerbates Metabolic Disorders in the Offspring of Wistar Rats

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Highlights

1. Low-calorie diet during pregnancy promotes changes in glucose/lipid profiles.
2. Obesogenic diet after low-calorie diet during pregnancy promotes metabolic changes.
3. Poor nutrition encourages the development of obesity and comorbidities

ABSTRACT

The effects of an obesogenic post-weaning diet on the growth and metabolic parameters of adult offspring submitted to a hypocaloric diet in perinatal life were evaluated. Male Wistar rats were divided into two groups according to maternal diet during pregnancy and lactation: Control (C, received normocaloric diet) and hypocaloric diet during pregnancy and lactation (H, received hypocaloric diet). At weaning, half the number of animals in each group was divided into two more groups according to the post-weaning diet: control (CC, n=12), control and subject to the obesogenic diet (CO n=11), hypocaloric diet and control (HC, n=14) and hypocaloric and obesogenic diet (HO, n=9). Maternal body weight, food intake, and energy intake were recorded daily. In the offspring, birth weight, growth rate, and physical characteristics were evaluated. At 120 days, relative food consumption, glucose tolerance test (GTT), biochemical profile, and organ weight were analyzed. Mothers on a low-calorie diet showed no difference in body weight during pregnancy or lactation even with lower energy intake. In offspring, litters from mothers fed a low-calorie diet showed a deficit in physical characteristics (growth restriction and low weight). The effect of an obesogenic diet on visceral fat weight, GTT, and hypercholesterolemia was most pronounced in animals subjected to a perinatal hypocaloric diet followed by a lifelong obesogenic diet. Conclusion: Our observations expand the evidence that social environments with food scarcity and/or obesogenic environments determine greater susceptibility to obesity.

Keywords: Adaptive plasticity, high-fat diet, obesity, rats

DIETA HIPOCALÓRICA NA VIDA PERINATAL SEGUIDA DE OBESOGÊNICA EXACERBA DISTÚRBIOS METABÓLICOS NA PROLE DE RATOS *WISTAR*

RESUMO

Avaliou-se os efeitos de uma dieta obesogênica pós-desmame sobre o crescimento e parâmetros metabólicos da prole adulta submetida a uma dieta hipocalórica na vida perinatal. Ratos *Wistar* machos foram divididos em dois grupos de acordo com a dieta materna durante a gestação e lactação: Controle (C recebeu dieta normocalórica) e dieta hipocalórica durante a gestação e lactação (H recebeu dieta hipocalórica). Ao desmame, metade do número de animais de cada grupo foi dividido em mais dois grupos de acordo com a dieta pós-desmame: controle (CC, n=12), controle e submetido à dieta obesogênica (CO n=11), dieta hipocalórica e controle (HC, n=14) e dieta hipocalórica e obesogênica (HO, n=9). O peso corporal materno, a ingestão de alimentos e a ingestão de energia foram registrados diariamente. Na prole foi avaliado o peso ao nascer, taxa de crescimento e características físicas. Aos 120 dias foram analisados o consumo alimentar relativo, teste de tolerância à glicose (GTT), perfil bioquímico e peso dos órgãos. As mães submetidas à dieta hipocalórica não apresentaram diferença no peso corporal durante a gestação ou lactação mesmo com menor consumo de energia. Na prole as ninhadas de mães alimentadas com uma dieta hipocalórica mostraram um déficit nas características físicas (restrição do crescimento e baixo peso). O efeito de uma dieta obesogênica sobre o peso da gordura visceral, GTT e hipercolesterolemia, foi mais pronunciado em animais submetidos a uma dieta hipocalórica perinatal seguida por uma dieta obesogênica ao longo da vida. *Conclusão*: Nossas observações ampliam a evidência de que ambientes sociais com escassez alimentar e/ou ambientes obesogênicos determinam uma maior suscetibilidade à obesidade.

Palavras-chave: plasticidade adaptativa; dieta hiperlipídica; obesidade; ratos

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INTRODUCTION

The maternal low-protein diet model during gestation and/or lactation is one of the most extensively studied animal models of developmental plasticity[1]. Feeding a low-protein diet (8% casein) during gestation and lactation is associated with growth restriction, age-dependent loss of glucose tolerance, insulin resistance, hypertension, and dyslipidemia, even when the offspring are weaned into a control diet[1]. Diets of total food restriction or maternal calorie restriction have been also studied as a model of experimental perinatal undernutrition. Nutritional deprivation (50% of *ad libitum* intake) in pregnant rats resulted in an increase in adipogenic potential and pro-inflammatory markers in the offspring, in addition to causing a differential expression of sexual hormones and adipocyte receptors[2]. Furthermore, adult offspring exposed to a maternal dietary restriction of 50% in the intrauterine period present intrauterine growth restriction, high levels of glucocorticoids, dysfunction of the hypothalamic-pituitary-adrenal axis with consequences in poor hippocampal feedback and hyperexcitability of the hypothalamus[3]. Indeed, variations in the quality and quantity of the intra-uterine and post-natal environment are linked with a later risk of cardiovascular and metabolic disease[4] This phenomenon, termed “developmental plasticity” or metabolic programming, refers to the property of a given genotype to produce different phenotypes in response to distinct environmental conditions[5].

The low-energy diet was developed to minimize the impact of maternal food restriction which is related to high levels of stress of starvation caused by meal-feeding behavior once rats consume all their food within a short period time[6]. Maternal non-restricted hypocaloric diet during gestation and lactation did not affect offspring in the short-term, but there is a more deleterious effect on later somatic growth and structural parameters[7]. When a low protein diet was offered to the mothers followed by a high lipid diet for the pups, there was greater growth and food intake, dyslipidemia, and abdominal fat accumulation[8] which demonstrates responses different according to the worsening of the energy and/or nutritional deficit at the beginning of life. A study using 50% calorie restriction in the maternal diet followed by a standard weaning diet found in offspring reduced hepatic autophagy, increased oxidative stress, and reduced antioxidant enzymes[9]. Maternal energy restriction during late gestation and through lactation following of control diet did not significantly affect litter size or litter birth weight, but caused lower body weight, lower body length, and delayed physical maturation at weaning in offspring. In adult life, the pups from dams submitted to maternal energy restriction showed delayed growth, but, better lipidic and glycemic profiles at 60th old age[10].

In the literature, studies are scarce with a proposal to evaluate the relationship between a maternal diet with caloric reduction, not protein, followed by a westernized diet in physiological and metabolic outcomes in offspring, which highlights the originality of the present study. The body of evidence points out that a post-weaning palatable hyperlipidic diet probably amplifies the risk of metabolic disorders in the adult life of offspring submitted to maternal undernutrition[11–12]. When perinatal undernourished offspring (30% of *ad libitum* intake) are exposed to a high-fat diet during growth, increased visceral fatness, resulting in obesity development, and diabetes type 2 are seen[13]. In fact, increased susceptibility to diet-induced obesity develops if a mismatch between the anticipated and the actual conditions are encountered[14].

Due to the adverse consequences generated among the offspring of mothers with energy restriction during pregnancy and lactation and weaned to a standard diet, such as the presence of accumulation of adipose tissue, increased body weight, alteration in the expression of lipogenic and lipolytic genes in the tissue adipose or even cardiovascular risk[(15)]. We hypothesize is that a post-weaning obesogenic diet amplifies the effects of an *ad libitum* low-energy diet with more

pronounced consequences in terms of metabolic parameters when compared a standard diet. Thus, to test the interaction between a perinatal hypocaloric diet and an obesogenic diet, the main goal of the present study was to evaluate the effects of a post-weaning obesogenic diet on growth and metabolic parameters of adult offspring submitted to a hypocaloric *ad libitum* diet during gestation and lactation.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethics Committee of the Biological Sciences Center (protocol number 23076.048926/210-88) Federal University of Pernambuco, Recife, PE, Brazil, and we followed the Guidelines for the Care and Use of Laboratory Animals.

Animals

Virgin female albino *Wistar* rats (*Rattus norvegicus*) were obtained from the Department of Nutrition, Federal University of Pernambuco. The female rats were 90-120-days old, 220-260g weight, when they mated. The day on which spermatozoa were present in a vaginal smear, followed by body weight gain was designated as the day of conception, day 0 of pregnancy. Pregnant rats were then transferred to individual cages, and they were maintained at a room temperature of 22 ± 1ºC with a controlled light–dark cycle (light 06.00-18.00 hours). Pregnant rats were randomly divided in two groups (n=6/each): normocaloric diet – control (Presence®) (C, received normocaloric diet during gestation and lactation [3.6 Kcal/g]) and hypocaloric diet (H, received low-energy diet during gestation and lactation [2.3 Kcal/g]). At the time of delivery, the litter size and pups' birth weights were recorded. On the first day after birth (24 hours after delivery), litters were standardized to eight pups, and during the suckling period, their mothers continued to be provided with either a normocaloric or hypocaloric diet. The litters of eight pups represent the sample that was evaluated during lactation. At weaning (on the 22nd day of age), only three or four randomly chosen male pups from each mother were used.

Thus, animals of each group were divided in two more groups according to their diet after weaning: control continued to normocaloric diet (CC, n=12), normocaloric and submitted to obesogenic diet (CO, n=11), hypocaloric diet submitted to normocaloric diet (HC, n=14), and hypocaloric diet submitted to obesogenic diet (HO, n=9). Water and standard chow diet were given *ad libitum*. Female pups were used in another experiment. Percentage of caloric contribution of macronutrients in the diets according to the total energetic value (TEV) and chemical composition of the experimental diets offered to animals are described in Table 1 and 2

The diet was prepared in the Department of Nutrition from the acquisition of semi-purified foods (casein as a source of protein, starch, and sucrose as a source of carbohydrates, soybean oil as a source of fat, cellulose as a source of fiber and a mix of vitamins and salts minerals) offered to the rats during pregnancy and lactation ad libitum and providing about 30% less energy value due to the composition with higher fiber and moisture content per 100g of feed. Previous studies by our group have shown that the use of a hypocaloric diet followed by a standard diet has repercussions on changes in the growth and maturation of the nervous system[11].

Table 1: Percentage of caloric contribution of macronutrients in the diets according to the total energetic value (TEV).

Diet	Protein (% kcal TEV)	Carbohydrates (% kcal TEV)	Lipids (% kcal TEV)	TEV (kcal/g)
Hypocaloric*	18.59	63.88	18.1	2.3
Normocaloric*	19	63	18	3.51
Presence®**	26	63	11	3.58
Hyperlipic**	20.8	47.0	32.2	4.2

*The calculations of chemical composition of macronutrients were based in nutritional information provided by products' suppliers and in the Brazilian Table of Food Composition (TACO).

**Nutritional information obtained from Adolfo Lutz Institute, 1985.

Table 2. Chemical composition of the experimental diets offered to animals.

INGREDIENTS	AIN-93G (g/100g) *	Hypocaloric (g/100g) **	Obesogenic (g/100g)
Corn starch (88% carbohydrates)	51.70	30.00	13.10
Soy flour	-	-	8.50
Wheat flour	-	-	13.50
Cookie cornstark	-	-	8.0
Casein (80% protein)	21.25	13.00	13.00
Guar gum	-	3.50	0.10
Sucrose	10.00	10.00	21.0
Concentrated soluble sucrose to 30%	-	33.00	-
Soybean oil	7.00	4.50	4,00
Animal lard	-	-	5.6
Cellulose	5.00	12.00	0.3
Margarine (65% lipids)	-	-	3.5
Milk Cream (20% lipids)	-	-	6.0
Mineral mixture (AIN-93G)	3.50	2.80	2,50
Vitamin mixture (AIN-93G)	1.00	0.80	0,70
L-Metionina	0.30	0.20	0,20
Choline bitartrate (41.1% choline)	0.25	0.25	0,25
Tert-Butylhydroquinone (TBHQ). *mg*	14.00	14.00	0.014
NaCl (39.34% Na)	-	-	0.36
Calories (g/100g)	3.51	2.30	4.20

* Source: Reeves et al [18]. The calculations of chemical composition of macronutrients were based in nutritional information provided by products' manufacturer and in the Brazilian Table of Food Composition (TACO).

**Nutritional information obtained from Adolfo Lutz Institute, 1985.

Mother´s Body Weight, Food Intake and Energy Intake Assessment

The mother's body weight was recorded daily during gestation and lactation for calculation of the range of body weight percentage. Daily food intake was determined by the difference between the amount (weight, in grams) of food provided at 08 a.m. and the amount (weight, in grams) of food remaining 24 h later[18]. Body weight was recorded with a Marte Scale (XL-500, II class) with a 0.001g accuracy. The energy intake was calculated by multiplying the amount of food intake during gestation and lactation by the energetic value of both low-energy and isocaloric diets.

Pup´s Assessment

Somatic growth

Somatic growth was assessed in terms of body weight, tail length, and laterolateral and anteroposterior head axis measurements performed from the first to 21st postnatal day between 13.00 and 15.00 hours as follows: body weight of the pups was recorded at 3rd, 12th, 21st, 30th, 60th, 90th, and 120th day throughout the experiment with a Marte scale with 100mg precision. Body weight gain was calculated as follows: Percentage weight gain = [body weight (g) x 100/birth weight (g)] – 100. Tail length (distance from tail typo to tail base), length of the laterolateral skull axis (distance between the ear holes) and length of the anteroposterior axis of the head (distance between snout and head-neck articulation) were measured with a digital caliper (Starrett®, Series 799, São Paulo, Brazil) with a 0.01mm precision.

Food intake

On the 110 to 120-day in the life, pups were housed individually for 10-days in a metabolic cage. The first five days were designed for adaptation to the cage. Next, the animal's daily food consumption was determined by the difference between the amount of food provided (50g) at the onset of the light cycle and the amount of food remaining 24h later. Body and food weights were recorded by a Marte Scale (AS-1000), in increments of 0.01g[12].

Glucose tolerance test (GTT) of offspring

The GTT was performed at 60, 90 and 120-day in the animals fasted overnight. The blood sample collections were performed by cutting the tip of the tail to remove approximately 10 µL of blood. The first blood sample was collected (time zero) before the injection of glucose. In the GTT, a glucose solution 50% (Equiplex Pharmaceutical Ltd., GO, Brazil) at a dose of 1 mg/g of body weight was administered intraperitoneally. Blood samples were then collected at 30, 60, 90 and 120 minutes after administration. The area under the glucose curve was obtained using the trapezoidal method[13].

Biochemical assessment

At 120-day old, and after fasting (12 h), serum glucose, total cholesterol, high-density-lipoprotein cholesterol (HDL-c) and triglyceride (TG) levels were determined with commercially available kits (BioSystems, Spain – A 25 Clinical Chemistry Analyse®). Very low-density-lipoprotein cholesterol (VLDL-c) was obtained using Friedwald calculations[14].

Weight of organs

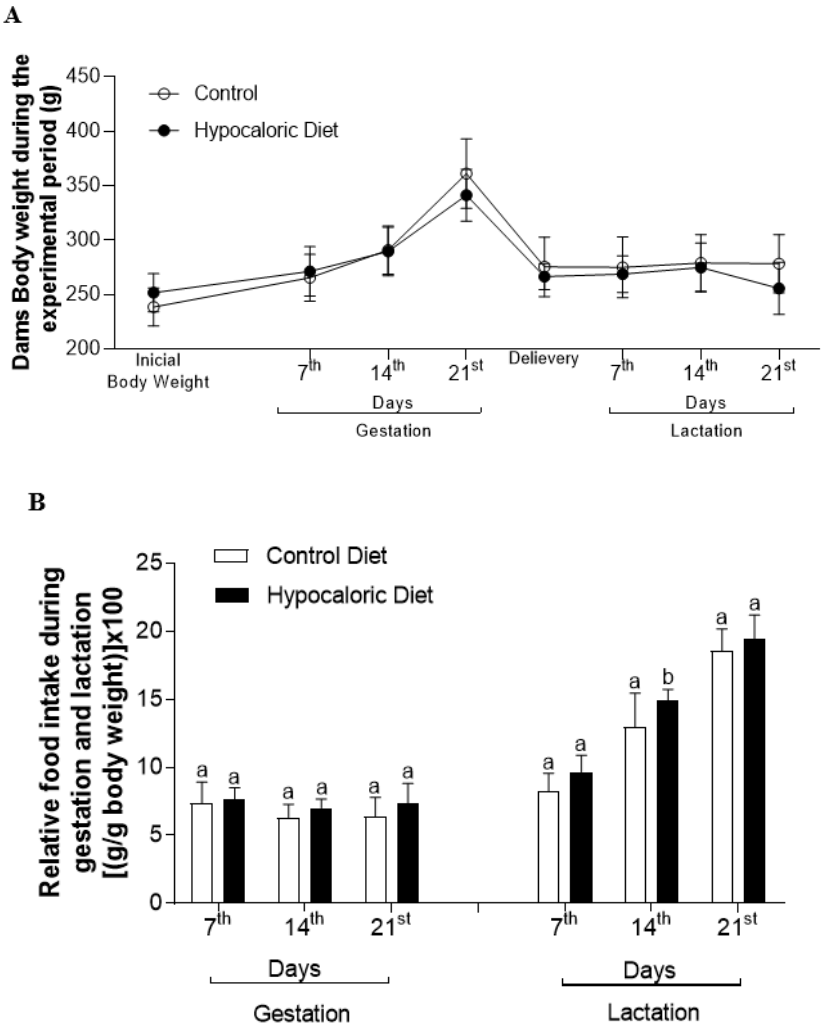
After the euthanasia, liver, heart, right and left kidney, right and left adrenal, stomach, fat (epididymal and retroperitoneal) and spleen were removed and weighted to calculate absolute and relative organs weight.

STATISTICAL ANALYSES

Values are presented as means ± standard deviation (S.D.). Intra-litter analyses were performed and found not to be significant. Each litter of eight pups was considered one sample during lactation, and statistical analyses were performed using Student's t-test to compare the mean values of each litter. Pearson's correlation coefficient was used to correlate the number of pups that were born and the mother´s body weight gain during gestation. For statistical analysis, a two-way Anova and Tukey's *post hoc* test were used. Significance was set at p <0.05. Data analysis was performed using the statistical program Graphpad Prism 7® (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

During gestation and lactation there was no difference in terms of body weight when comparing the groups of dams (Fig. 1A). However, at the end of lactation (14th and 21st days) there was an increase in food intake (g) in dams exposed to the hypocaloric diet (Fig. 1B), however this increase in intake was not enough to equal or exceed the energy value consumed (Fig. 1C). Data were adjusted for the number of pups born to each dam [C, 11.0 (9–12) and H, 10.0 (8–13) and values expressed as median (minimum and maximum)]. Pearson's correlation coefficient between number of pups and body weight gain of the mother was not significant (r^2 = 0.13, p >0.05).



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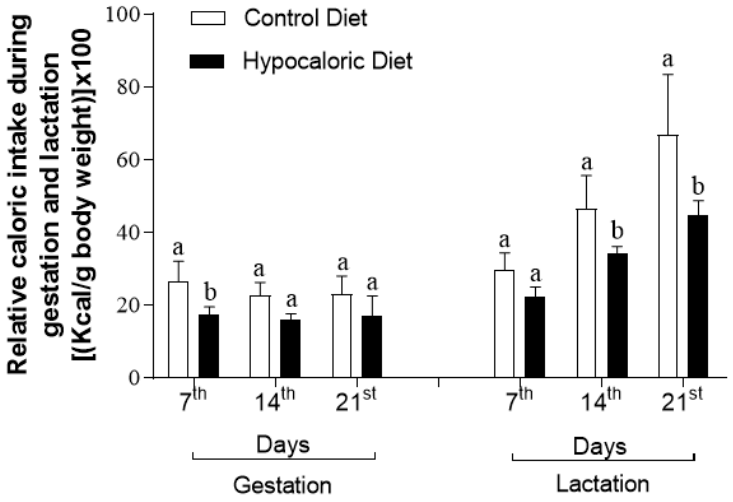


Figure 1 – Body weight, food intake and caloric intake during gestation and lactation by dams fed either a normocaloric diet (control, *n* = 6) or hypocaloric diet (H, *n* = 6). Body weight during the experimental period (g) (A); relative food intake during gestation and lactation [(g/g of body weight)] x100 (B); relative caloric intake (C) during gestation and lactation [(kcal/g body weight)]x100. The values are presented as means ± S.D. Columns followed by different letters, differ statistically (p<0.05), using two-way ANOVA and Tukey 's *post hoc* test.

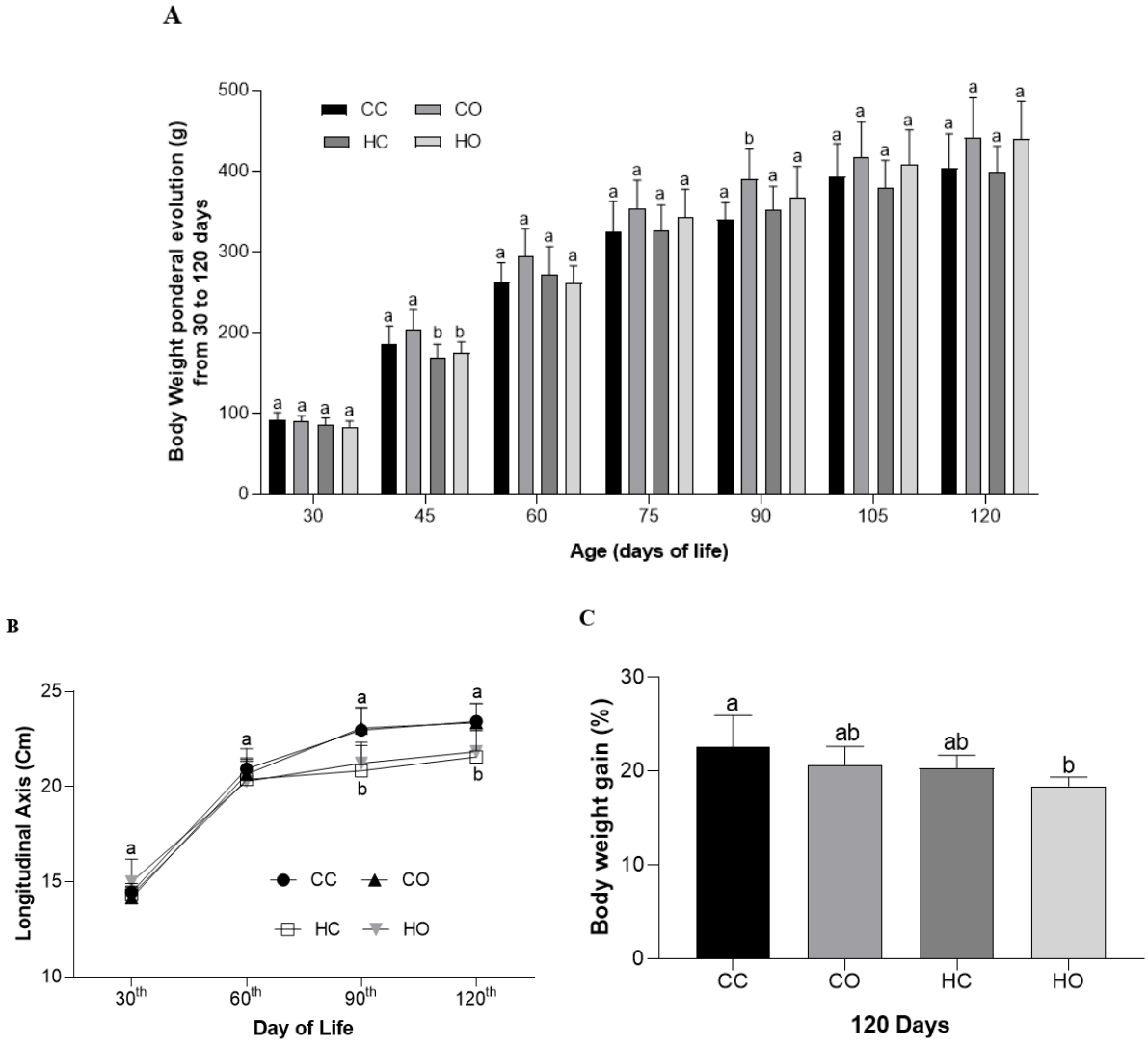
Table 3. Indicators of somatic growth during lactation of litters (8 pups per litters in each group).

EVALUATED PARAMETERS		Offspring´s days of life		
		3rd	12th	21st
	Groups	Mean ± S.D	Mean ± S.D	Mean ± S.D
Body weight (g)	C	7.69 ± 0.87[a]	26.44 ± 2.11[a]	49.99 ± 2.12[a]
	H	7.34 ± 0.58[a]	22.98 ± 274[b]	38.90 ± 3.22[b]
Tail length (mm)	C	21.03 ± 2.42[a]	44.32 ± 3.90[a]	71.54 ± 6.12[a]
	H	19.39 ± 1.41[a]	40.32 ± 3.24[a]	63.42 ± 6.32[b]
Longitudinal axis (mm)	C	57.76 ± 3.34[a]	85.91 ± 3.52[a]	117.28 ± 4.38[a]
	H	55.91 ± 2.91[a]	83.85 ± 2.19[a]	109.81 ± 2.71[b]
Laterolateral skull axis (mm)	C	11.34 ± 0.33[a]	15.62 ± 1.01[a]	17.79 ± 0.78[a]
	H	10.99 ± 0.45[a]	15.29 ± 0.12[a]	16.84 ± 0.29[b]
Anteroposterior axis of the head (mm)	C	19.84 ± 0.81[a]	30.06 ± 1.57[a]	36.81 ± 0.98[a]
	H	19.77 ± 0.64[a]	29.48 ± 0.52[a]	35.32 ± 0.63[b]

During gestation and lactation, the dams were submitted to either low-energy diet or normocaloric diet. The pups into each litter were evaluated during lactation. Groups: hypocaloric diet (H, n=6) and normocaloric diet (C, n=6). The values are presented as means ± S.D. Lines followed by different letters, differ statistically (p<0.05), using two-way ANOVA and Tukey 's post hoc test.

Assessment of somatic growth showed no difference between birth weight when groups of control dams were compared. However, at 12st d, pups from H dams showed a deficit in body weight (20% lower) and at 21st d, deficits in tail length (12% lower), body length (7% lower), laterolateral skull axis (6% lower) and anteroposterior axis of the head (4% lower) and when compared with litters from the control group (Table 3).

At weaning, half of the puppies in each group were placed on an obesogenic diet. There was no effect of the obesogenic diet on offspring body weight throughout the entire experiment (120 days). However, when evaluating the percentage weight gain of offspring's, there was a statistical difference between HO and CC. Thus highlighting the influence of the dams low-calorie diet on the weight gain of adult offspring, where the obesogenic diet was not able to recover the body weight deficit promoted by the low-calorie diet during gestation and lactation. However, the average food and energy intake in the obesogenic groups (CO and HO) was lower than that of their control diet-fed peers (CC and HC) (Figure 2D and E). Thus, they showed a reduction in food intake of 35% less than the control groups and a reduction in energy intake of around 20% less than the control groups. This scenario occurs due to the satietogenic power of the obesogenic diet. In this case, food intake was not influenced by the maternal diet. On the other hand, the longitudinal axis that reflects the growth of the offspring was lower in the groups where mothers were fed a low-calorie diet (Fig. 2B).



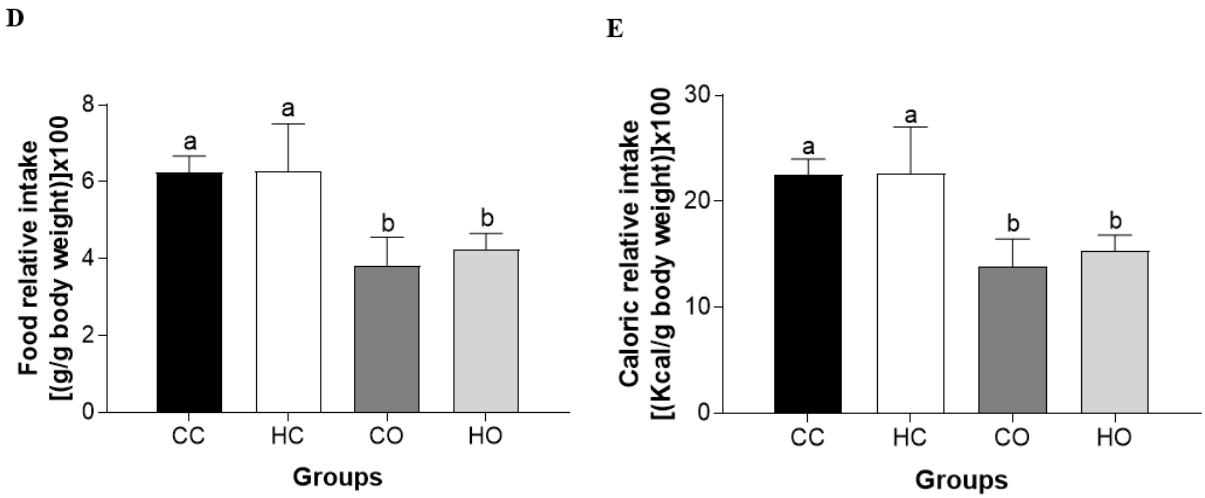


Figure 2 – (A) Evolution of body weight (g), longitudinal axis (B), body weight gain in percentage (C) from 30 to 120 d, Relative food intake (D), and relative caloric intake (E) of offspring submitted normocaloric diet (control CC, n=12), control and submitted obesogenic diet (CO, n=11), hypocaloric diet submitted control diet (HC, n=14), and hypocaloric diet and submitted obesogenic diet (HO, n=9). The values are presented as means ± S.D. Columns followed by different letters, differ statistically ($p<0.05$), using two-way ANOVA and Tukey 's ***post hoc*** test.

At 60, 90 and 120 d, pups were submitted to a glucose tolerance test (GTT). At 60d, according area under the blood glucose curve in all groups were different from the control group except HC group. From 90 days, puppies submitted to the obesogenic diet showed an increase in the area under the glycemia curve compared to puppies submitted to the control diet. And at 120 days the area under the glycemic curve differed in all groups, with CO and HO presenting the highest values. These results show the effect of the obesogenic diet in altering the glycemic profile of adult offspring, regardless of the type of diet exposed during the perinatal period (Figure 3).

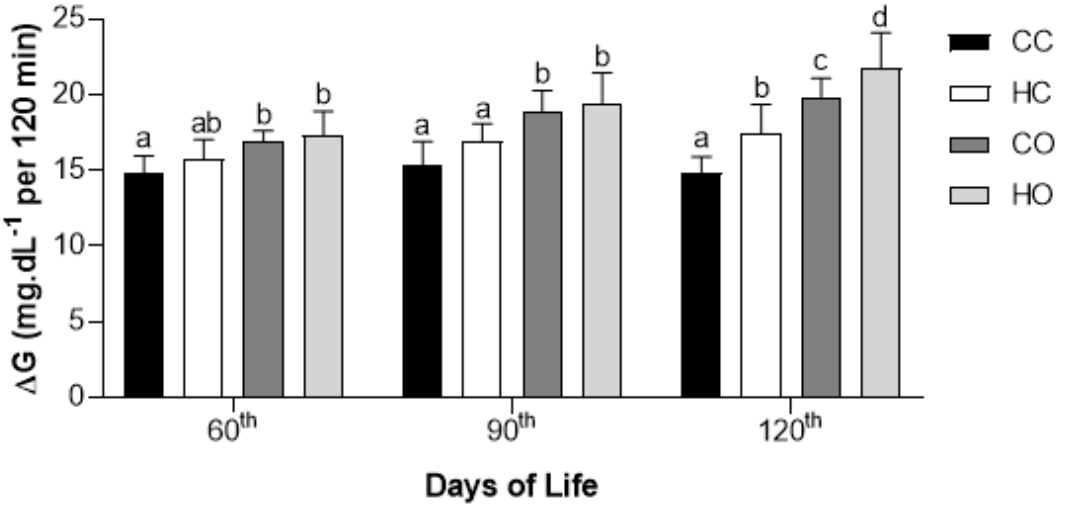


Figure 3 **–** Area under the glycemic curve of offspring submitted normocaloric diet (control CC, n=12), control and submitted obesogenic diet (CO, n=11), hypocaloric diet submitted control diet (HC, n=14), and hypocaloric diet and submitted obesogenic diet (HO, n=9).The values are presented as means ± S.D. Columns followed by different letters, differ statistically ($p<0.05$), using two-way ANOVA and Tukey 's ***post hoc*** test.

At 120 days, the obesogenic diet in puppies induced an increase in visceral fat weight (71%) and a reduction in stomach weight (Table 4) compared to the control group. The effect of obesogenic diet on visceral fat weight (absolute and relative) was observed in both groups fed obesogenic diet, but there was synergic effect in HO group that differed of HC and C group (P< 0.05) in values above 50%. In set, the effect of diet postnatal was more pronounced in visceral body fat than body weight.

Table 4. Body weight and organs weight (absolute and relative) of offspring at 120 d old whose mothers were submitted to either a normocaloric diet (C) or a hypocaloric diet (H). From weaning on, pups were fed an obesogenic diet (CO and HO) or normocaloric diet (HC and C).

	CC Mean ± S.D	CO Mean ± S. D	HC Mean ± S. D	HO Mean ± S. D
Final Body Weight	403.7 ± 42.60^a	441.52 ± 49.60^a	399.22 ± 32.09^a	440.19 ± 46.18^a
Heart (g)	1.39 ± 0.08^a	1.29 ± 0.15 ^{a}	1.33 ± 0.12 ^{a}	1.28 ± 0.43 ^{a}
Relative Weight of Heart [(heart weight/body weight)x100]	0.35 ± 0.04^a	0.30 ± 0.03^a	0.33 ± 0.02^a	0.29 ± 0.10^a
Liver (g)	12.57 ±2.14^a	11.77 ± 2.03^a	11.59 ± 1.51^a	11.25 ±3.83^a
Relative Weight of liver [(liver weight/body weight)x100]	3.15 ±0.39^a	2.72 ±0.39^a	2.85 ±0.34^a	2.58 ±0.86^a
Stomach (g)	2.05 ± 0.18^a	**1.47 ± 0.13^b**	1.83 ± 0.37^a	**1.44 ± 0.48^b**
Relative Weight of Stomach [(Stomach weight/body weight)x100]	0.52 ± 0.07^a	**0.34 ± 0.03^b**	0.45 ± 0.10^a	0.33 ± 0.11^a
Visceral Fat (g)	12.57 ± 3.73^a	**20.07 ± 5.01^b**	13.15 ± 3.32^a	**23.37 ± 9.04^b**
Relative Weight Visceral Fat [(fat weight/body weight)x100]	3.12 ±0.65^a	**4.68 ± 1.25^b**	3.22 ±0.70^a	**5.36 ± 2.08^b**

Offspring submitted normocaloric diet (control CC, n=12), control and submitted obesogenic diet (CO, n=11), hypocaloric diet submitted control diet (HC, n=14), and hypocaloric diet and submitted obesogenic diet (HO, n=9). The values are presented as means ± S.D. Columns followed by different letters, differ statistically (p<0.05), using two-way ANOVA and Tukey 's *post hoc* test.

The offspring fed obesogenic diet post weaning and pups derived from dam´s fed hypocaloric diet showed an increase in several biochemical parameters compared to control. But the HO group presented higher atherogenic risk compared to CO group and increased of Triglycerides, total cholesterol, glucose and VLDLc levels corroborating the potentiation of the interaction hypocaloric and obesogenic diets (Table 5).

Table 5. Plasma biochemical parameters of offspring at 150 d old whose mothers were submitted to either a normocaloric diet (C) or a hypocaloric diet (H). From weaning on, pups were fed an obesogenic diet (CO and HO) or normocaloric diet (HC and C).

	C Mean ± S.D	CO Mean ± S. D	HC Mean ± S. D	HO Mean ± S. D
Glucose (mg.dL^{-1})	117.62 ± 11.84^a	130.62 ± 18.01^b	101.17 ± 17.74^a	150.12 ± 16.33^b
Triglycerides (mg.dL^{-1})	87.05 ± 8.45^a	116.68 ± 12.25^b	162.24 ± 14.31^b	190.54 ± 15.15^b
Total Cholesterol (mg.dL^{-1})	77.83 ± 10.48^a	94.46 ± 10.24^b	92.25 ± 10.78^b	106.95 ± 9.76^b
LDL – Cholesterol (mg.dL^{-1})	21.07 ± 8.22^a	34.01 ± 11,07^b	19.11 ± 3,65^a	27.35 ± 3.64^b
VLDL – Cholesterol (mg.dL^{-1})	17.41 ± 1.69^a	23.34 ± 2,86 ^{b}	32.44 ± 2,86^b	38,10 ± 3.03^b
HDL – Cholesterol (mg.dL^{-1})	39.35 ± 9.24^a	37.53 ± 5.26^a	45.70 ± 9.42^a	44.98 ± 11.37^b
Rate TG/HDLc	2.39 ± 0.61^a	3.05 ± 0.62^a	3.66 ± 1.04^b	4.48 ± 1.50^b

Offspring submitted normocaloric diet (control CC, n=12), control and submitted obesogenic diet (CO, n=11), hypocaloric diet submitted control diet (HC, n=14), and hypocaloric diet and submitted obesogenic diet (HO, n=9). The values are presented as means ± S.D. Columns followed by different letters, differ statistically (p<0.05), using two-way ANOVA and Tukey 's *post hoc* test.

DISCUSSION

The environmental stimuli as diet during critical periods of pre- and immediate post-natal mammalian development can print lasting epigenetic marks inducing permanent changes in metabolism and modulating chronic disease susceptibility. The metabolic programming model exhibit many forms of developmental plasticity as instead the Predictive Adaptive Response (PAR). The PAR is the form of development plasticity in which environment cues in early life influence the development of phenotype adapted to a predictive response. Thus, when the predicted and actual environments differ, e.g., when the environment actual is different to environment early life the adverse consequences can be more deleterious to health[15]. In the other hand, a constant adverse environment can be some outcomes less harmful to health in some metabolic parameters as previously found by Nascimento et al.[16]

In the present study, we investigated whether a hypocaloric diet offered in the perinatal period followed by an obesogenic diet after weaning maximizes the adverse effects on the physiometabolic parameters in the life of the adult offspring. Thus, we investigated the interactions between perinatal maternal hypocaloric diet and postnatal and obesogenic diet on growth, food intake and biochemical parameters in rats. Hypocaloric diet did not induce changes in the body weight or food intake of dams during gestation. Only at the end of lactation did mothers fed a hypocaloric diet ingest more food, but this intake was not enough to equal or exceed the energy value consumed by mothers on an obesogenic diet. Our results demonstrated the efficiency of this model diet once the relative caloric intake was reduced in dams causing outcomes adverse in the somatic growth of pups. The main difference of this model refers to the lower stress of the animals because the diet is offered *ad libitum* and the distribution of macronutrients is following the energy value of the diet. Animal models using a low protein diet cause reduced intake of protein and energy because the mothers reduce food intake by more than 30%[17] providing a deficit protein-energetic that exacerbates the results from protein deficit in contrast to our results of energetic deficit.

Ours findings are in accordance with previous study that used the same experimental model to induce maternal undernutrition[9]. Low-energy diet is rich in dietary fiber, and it can be related to similar food intake seen in the groups due to higher satiety level limiting an energetic compensation or perhaps the amount of fiber in the diet wasn't high enough to reduce food intake or yet, a low-energy diet encouraged consumption equaling to control group. Fiber lowers energy density and sometimes diminishes the palatability of foods, increases chewing time, and delays gastric emptying with increases gastric volume which activates satiation[18]. Thus, in the present study, dams consumed the same amount of food during gestation until the beginning of lactation in a diet with a low content of energy different to food intake observed by dams fed low-protein diet[19].

Interestingly, the mother´s body weight did not decrease towards the end of lactation but reflected in lower body weight of the pups at weaning. These findings contrast with the paradigm that in an energy deficit status, the mother's organism is depleted to the detriment of the growth of the offspring. Similar results were observed in a study conducted during pregnancy and lactation with dams fed a low-fat, high-fat or control diet that found no difference in energy intake or body weight during pregnancy but observed lower consumption energy intake of dams fed a low-fat diet compared to a high-fat diet, but there was no difference in the dam´s body weight. However, at weaning, their pups had lower body weight[20]. Adaptative and compensatory mechanisms in maternal organisms during pregnancy and lactation are still unclear, despite much research over time.

Pups from mothers fed a low-energy diet showed a deficit in the body weight, tail length, laterolateral skull axis and anteroposterior axis of the head when compared with litters from the control group at the end of lactation. Our data showed that low-energy diet is associated with low stores of maternal nutrients and, subsequently, less transfer of nutrients to the offspring, which is related with

reduced postnatal growth as seen in the same modeland other models of perinatal undernutrition[9]. Both groups submitted to the maternal hypocaloric diet diet did not differ in body weight throughout the experimental period independent of post-weaning diet. In contrast, both groups submitted to an obesogenic diet reduced relative food intake and caloric intake when compared to their respective controls. However, there is an influence of the maternal diet on the percentage of body weight gain, as there was a reduction in the group exposed to the maternal hypocaloric diet followed by an obesogenic diet concerning the group that since gestation was exposed to the control diet, indicating metabolic changes in the control of long-term body weight.

In contrast, previous studies have reported that a high-fat diet post-weaning is associated with high body weight gain and body length in maternal restricted calorie diets (30% of *ad libitum* of control diet)[14-16]. The obesogenic diet or control diet were not able to recover the animal´s length of offspring from dams fed a low-energy diet but the obesogenic diet influenced increase of the visceral fat of both (control and hypocaloric) groups. It can be associated with accumulation of adipose tissue once obesogenic diet as such western-style, high-fat or cafeteria diet are well-established models to induce obesity[15] or central adiposity[22]. One of the mechanisms by which a high-fat diet may increase visceral deposits independent of the subcutaneous fat may be increased expression and activity of stearoyl-CoA desaturase-1 (SCD1), a key enzyme of FA metabolism. SCD1 converts saturated FAs, e.g. palmitate and stearate, to monounsaturated FAs, palmitoleate and oleate, which are the predominant substrates for triglyceride synthesis observed in pups from mothers fed high-fat diet during lactation[23]. Other mechanisms observed in Wistar rats fed cafeteria diet was the expression of metabolism genes as such, Slc27a3, a fatty acid transporter was 9.6-fold higher in visceral adipose tissue and 6.3-fold lower in subcutaneous adipose tissue of cafeteria group compared to standard-diet fed rats indicating a epigenetic programming[24]. As a limitation, in this study we did not investigate cellular mechanisms, but we did observe consistent phenotypic changes in pups derived from mothers fed a low-energy diet followed by an obesogenic diet and changes in phenotypic expression is strong indicator that several underlying cellular mechanisms occur.

It is well established that early offspring undergo a greater age-dependent loss of glucose tolerance[25]. Our results showed that, at 90 and 120 d, the area under the glycemia curve was elevated in puppies exposed to the obesogenic diet, therefore the obesogenic diet potentiated this effect at all moments in both normocaloric (CO) and hypocaloric diet (HO). Similarly, Thompson et al. (2007) have observed that a maternal caloric restriction (70% of *ad libitum* diet of control) followed by a high-fat diet increases fasting serum glucose with probable changes in its metabolism in skeletal muscle and liver[15]. In agreement with previous findings[26], rats fed an obesogenic diet developed glucose resistance and decreased the glucose metabolism regardless of perinatal nutritional history. The study by Alejanto et al.[27] identified that a maternal diet low in protein during pregnancy increases the predisposition to metabolic alteration and type 2 diabetes in the offspring's adult age, by generating dysfunction in pancreatic β cells, in addition to altering microRNAs (miRs) that regulate insulin secretion, insulin resistance, and obesity, miRs 342, 143; miR143 and miR219, respectively. Thus, the influence of obesogenic diet on adult offspring independent of the maternal diet in a fundamental role in the glycemic control.

In the present study, the perinatal hypocaloric diet did not affect the visceral fat weight of adult offspring. However, when submitted to an obesogenic diet, offspring showed an increased visceral fat weight. Previous studies have demonstrated that a hyperlipidic diet is able to increase the amount of adipose tissue independently of early environment conditions[28]. Our results showed that a perinatal low-calorie diet negatively influences the longitudinal growth of offspring, but adherence to obesogenic eating habits is a predictor of weight gain and greater deposition of visceral fat (central obesity) in adult life. This adipogenesis can be associated with serum concentration of insulin and

corticosterone found in the previous study of our team using the western-style diet[29]. In addition, other factors associated as microbiota altered, high levels of inflammatory adipokines, hepatic steatosis in rats fed obesogenic diets[15] can be associated. A high level of corticosterone was found in adult rats submitted to the intrauterine growth restriction model and high-fat diet after delivery[30].

As expected, a perinatal hypocaloric diet followed by an obesogenic-induced diet induced a deleterious profile of plasma dyslipidemia such as increased triglycerides, total cholesterol, and VLDL-cholesterol in adult animals. It was interesting to note that the obesogenic diet amplified the effects of a perinatal hypocaloric diet including high risk for cardiovascular disease observed in the increase of rate TG/HDL-c. Perinatal nutrition-induced hypercholesterolemia has been previously described in animal models, and the offspring's susceptibility to programmed obesity risk has been shown to be dependent on the timing and severity of diet manipulation[3]. According to previous studies using different models of perinatal undernutrition[15-17], and to the predictive adaptive response hypothesis, a developing organism adjusts its physiology to be appropriate for its predicted mature environmental range, such that a mismatch between the developmental and the adult environment may lead to an increased risk of disease development[20]. Our data support this hypothesis and propose that changes in the amount of energy in early life may be key pathways that modify susceptibility to the development of obesity and its comorbidities.

CONCLUSION

Most models have used caloric restriction as a stimulus for developmental plasticity, and relatively few studies have seen the effects of a low-energy diet during gestation and lactation. In this regard, we have presented data that confirm our hypothesis by demonstrating that a hypocaloric diet offspring shows significant discernible effects on glucose/lipid profiles that are more pronounced when submitted to an obesogenic diet throughout the life-spam. In conclusion, our observations extend the evidence that mismatched early developmental and mature environments determine a higher susceptibility to obesity development and co-morbidities.

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