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Maternal and post-weaning exposure to a high fat diet promotes visceral obesity and hepatic steatosis in adult rats

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Abstract

Aim: **considering the frequent consumption of fat-rich diets by women of reproductive age, the aim of the present study was to investigate the effects of maternal consumption of a high-fat diet during the perinatal and/or post-weaning period on the liver parameters and lipid metabolism of young rats.**

Methods: Wistar **female rats were fed a high-fat (H) or control (C) diet during pregnancy and lactation. The offspring were allocated to four groups: Control Control (CC, n = 11), offspring fed a control diet after weaning; Control High-fat (CH, n = 10), offspring fed a high-fat diet after weaning; High-fat High-fat (HH, n = 10), offspring of mothers H fed a high-fat diet after weaning; and High-fat Control (HC, n = 9), offspring of mothers H fed with control diet after weaning.**

Results and discussion: **the food intake did not differ among the groups, however, the relative weight of the adipose tissue was higher in animals from the HC, HH and CH groups ($p \leq 0.005$). Liver steatosis was found in the CH and HH animals, which also exhibited hypercholesterolemia ($p \leq 0.05$). The levels of the liver enzymes alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) were higher in the HH group, and the LDL level was higher in the CH group compared to the CC. The consumption of an obesogenic diet during critical periods of development may contribute to the occurrence of visceral obesity, liver steatosis and hypercholesterolemia in adult rats, even in the absence of changes in dietary intake.**

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Key words: *Critical period of development. High fat diet. Liver. Rats.*

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LA EXPOSICIÓN EN EL PERIODO PERINATAL Y EL POSTDESTETE A UNA DIETA ALTA EN GRASAS PROMUEVE LA OBESIDAD VISCERAL Y LA ESTEATOSIS HEPÁTICA EN RATAS ADULTAS

Resumen

Objetivo: **teniendo en cuenta el consumo frecuente de dietas ricas en grasas por las mujeres en edad reproductiva, el objetivo del presente estudio fue investigar los efectos del consumo materno de una dieta alta en grasas durante el período perinatal y/o post-destete en el hígado y el metabolismo de los lípidos en ratas jóvenes.**

Métodos: **ratas hembra Wistar fueron alimentadas durante el embarazo y la lactancia con un alto contenido de grasa (H) o de control (C). La descendencia se asignó a cuatro grupos: Control (CC, n = 11), descendencia alimentada con una dieta de control después del destete; Control de dieta alta en grasa (CH, n = 10), crías alimentadas con una dieta alta en grasas después del destete; Alta en grasas de alta en grasa (HH, n = 10), hijos de madres H alimentados con una dieta alta en grasas después del destete; y Control de alta en grasa (HC, n = 9), hijos de madres H alimentados con dieta de control tras el destete.**

Resultados y discusión: **la ingesta de alimentos no difirió entre los grupos; sin embargo, el peso relativo del tejido adiposo fue mayor en los animales de los grupos HC, HH y CH ($p \leq 0{,}005$). La esteatosis hepática se encontró en los CH y HH, que también presentaban hipercolesterolemia ($p \leq 0{,}05$). Los niveles de las enzimas hepáticas alanina aminotransferasa (ALT) y gamma-glutamil transpeptidasa (GGT) fueron mayores en el grupo de HH, y el nivel de LDL fue mayor en el grupo CH en comparación con el CC. El consumo de la dieta propició la obesidad durante los períodos críticos del desarrollo y puede contribuir a la aparición de obesidad visceral, esteatosis hepática e hipercolesterolemia en ratas adultas, incluso en ausencia de cambios en la ingesta dietética.**

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Palabras clave: *Período crítico del desarrollo. Dieta rica en grasa. Hígado. Ratas.*

Abbreviations

AGL: Ácidos Graxos Livres.
ALT: Alanina Aminotransferase.
AST: Aspartato Transaminase.
C: Control.
CC: Control.
CH: Control High-Fat.
NAFLD: Nonalcoholic Fatty Liver Disease.
GGT: Gama Glutamil Transferase.
H: High-Fat.
HC: High-Fat Control.
HDL-C: High Density Lipoprotein Cholesterol.
HH: High-Fat.
LDL–C: Low Density Lipoprotein Cholesterol.
VLDL–C: Very Low Density Lipoprotein Cholesterol.

Introduction

Epidemiological, clinical and experimental studies[1-3] have shown that maternal dietary inadequacy during pregnancy and lactation is a significant risk factor for metabolic disorders in the offspring in the short and long terms.

Because of the current increase in the intake of so-called fast food, which is rich in energy and fat[4], by women of reproductive age[5], there is an increasing interest in the effects of the maternal diet on the offspring in both childhood and adulthood.

A high intake of fat-rich foods promotes an increase in the triglyceride content of the liver and may cause nonalcoholic fatty liver disease (NAFLD)[6]. Although NAFLD is diagnosed worldwide, the prevalence of this disorder varies, reaching 20-30% in the Western world[7].

In the present study, we used a maternal diet that mimetizasse the current obesogenic food, nutritionally unbalanced, during critical developmental periods, namely, pregnancy, lactation and post-weaning, on the liver histology and enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT)], visceral adipose tissue and lipid profile of young adult rats.

Methods

Animals

All the procedures involving animals were approved by the Animal Research Ethics Committee of the Federal University of Bahia (UFBA), protocol no. 02/13. The animals were kept under stable temperature conditions with a 12-hour light-dark cycle. Unrelated, primiparous *Wistar* rats, 90-100 days old and weighing 220-280 g, were mated with non-consanguineous males (2:1 ratio). Pregnancy was confirmed by means of the vaginal smear test, in which the presence of spermatozoa in the vaginal secretion was considered indicative of the onset of pregnancy.

Experimental groups

After pregnancy was confirmed, the rats were allocated to two experimental groups based on the diet to be given during pregnancy and lactation: Control group (C, n = 4), fed a standard commercial diet for rats; High-fat group (H, n = 4), fed a high-fat diet. The day after birth, the litters were standardized to eight pups, which were kept with the lactating mothers until day 21, when they were weaned. The offspring of the rats in groups C and H were allocated to subgroups according to the diet fed after weaning as described in table I.

Diets

The control diet was a standard commercial diet for rats (Nuvilab® CR1) containing approximately 22.0% protein, 57.0% carbohydrates, 4% fat and 9% vitamins and minerals, corresponding to approximately 3.5 kcal/g. The high-fat diet consisted of a mixture of hypercaloric foods, including the commercial diet (Nuvilab®), roasted peanuts, milk chocolate and Marie biscuits, containing 17.0% protein, 46.0% carbohydrates, 23% fat and 4% vitamins and minerals, corresponding to 4.5 kcal/g[3].

Dietary intake

From 45 to 60 days of age, the animals were kept for 15 days in individual cages with a feeder and drinker. A standard amount of food (70 g) was offered every day, and the leftovers in the feeders were weighed 24 hours later[8] to estimate the 24-hour dietary intake.

Visceral adipose tissue and liver

At 60 days of age, the animals were sacrificed by means of the cardiac puncture technique. A longitudi-

Table I
Experimental design

Group Name (n)	*Pregnancy*	*Lactation*	*Post-weaning*
CC (11)	Control	Control	Control
CH (10)	Control	Control	High-Fat
HC (9)	High-Fat	High-Fat	Control
HH(10)	High-Fat	High-Fat	High-Fat

Experimental design. Rats were fed rodent standard commercial diet (C) or with the high-fat diet (H) in pregnancy and lactation or post-weaning up to 8 weeks of age. The numbers in parentheses indicate the number of offspring in each nutritional group.

nal incision was performed on the abdomen to dissect the visceral adipose tissue and liver. The adipose tissue was weighed on a digital electronic scale (Marte® model S-400) with a 4-kg capacity and a 0.001-g sensitivity. The liver was kept in a 10% formaldehyde solution for the subsequent preparation of histological slides. The liver lobes were transversely sectioned and immersed in the formaldehyde-buffered solution. The sections were stained with hematoxylin-eosin. A microscopic analysis was performed by an independent pathologist blinded to the experimental protocol, who assessed the liver architecture and reported the presence of fat and inflammation. The histological results were classified based on the presence of microvesicular or macrovesicular steatosis.

Biochemical parameters

To analyze the biochemical parameters, the animals were anesthetized at 60 days of age (0.5 mL xylazine and 2.0 mL ketamine in normal saline, final volume 10 mL; 0.1 mL anesthetic solution/10 g body weight) before the cardiac puncture was performed. Once it was established that the animals were fully sedated, blood was collected and centrifuged to separate the plasma fraction. The levels of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, ALT, AST and GGT were measured at a specialized veterinary laboratory by means of enzymatic methods using a commercial kit (Biosystems, Spain) and an A15 Biosystems device.

Statistical analysis

The various groups were compared regarding the parametric data by means of an analysis of variance (ANOVA) using the GraphPadPrism version 6.04 software for Windows. When the ANOVA indicated a significant difference, Tukey's test was used to identify the differences between groups. The significance level was set to 5% in all the analyses.

Results

The mean dietary intake during the investigated period (days 45 to 59) was similar among all the animals, with no significant differences (Fig. 1).

The relative weight of the visceral (mesenteric) adipose tissue was significantly greater (Fig. 2) among the offspring of the mothers fed the high-fat diet, as well as among the offspring that were maintained on the high-fat diet until day 60, compared to the control group.

The animals fed the high-fat diet from weaning to 60 days of age (Fig. 3) exhibited macrovesicular steatosis (90%, characterized by the presence of a large

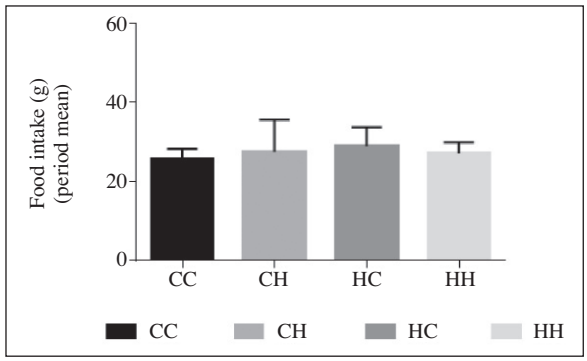

Fig. 1.—Mean dietary intake of the offspring of rats subjected to a control or high-fat diet during pregnancy and lactation that were kept or not on the diet following weaning. The results are expressed as the means ± SEM as calculated by a one-way ANOVA followed by Tukey's multiple comparison test.

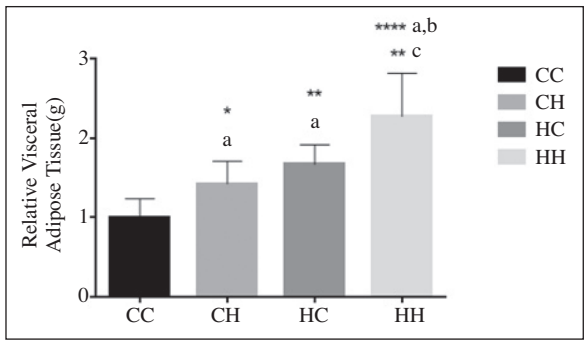

*Fig. 2.—Relative weight of the visceral adipose tissue of the offspring of rats subjected to a control or high-fat diet during pregnancy and lactation that were kept or not on the diet following weaning. The results are expressed as the means ± SEM as calculated by a one-way ANOVA followed by Tukey's multiple comparison test. Significance level: *p < 0.05, **p < 0.005, **** p < 0.0001; "a": compared to CC, "b": compared to CH and "c": compared to HC.*

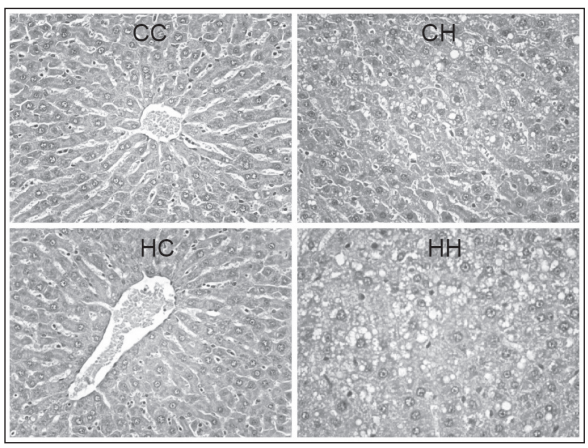

Fig. 3.—Histological analysis of liver specimens from the offspring of rats fed a control or high-fat diet during pregnancy and lactation that were maintained on the diet or not after weaning (200x magnification).

fat vacuole that pushes the nucleus to the periphery of the cell) and microvesicular steatosis (50%, many small cytoplasmic vacuoles that cause indentations in the nucleus, which is in a central position). In the case of the HC pups, which were born from mothers fed the high-fat diet, the liver architecture was preserved, with an absence of changes in the liver parenchyma in 100% of the animals. However, it was observed that the liver adaptations to the consumption of a high-fat diet during postnatal life can be aggravated by the use of such a diet in the perinatal period, with the consequent appearance of macrovesicular and microvesicular steatosis in 100% of the offspring.

Regarding the biochemical tests (Table II), the Total Cholesterol and VLDL levels were elevated in the CH group compared to the CC group. The Total Cholesterol and GGT levels were elevated in the offspring of the mothers fed the high-fat diet that were also fed that diet after weaning (HH) compared to the control group; in addition, the ALT levels were higher in group HH compared to group CH.

Discussion

The diet used in the present study was meant to represent the dietary habits of the population worldwide because there is presently a wide availability of high-fat, high-calorie foods. It is indisputable that the habit of consuming foods rich in fat, especially saturated fat, is being established increasingly earlier, thereby perpetuating changes that take place in critical developmental stages such as pregnancy and lactation[9,10].

Although high-fat diets are associated with hyperphagia[11,12], the average food intake at 45 days of age did not differ among the animals during the 15 days they were analyzed. These findings corroborate the results reported by Khan et al.[13], who did not find differences in food intake from weaning until age 180 days. Similar results were also reported in other studies[14,15]. Nevertheless, others studies have pointed to an increase in food intake by the offspring of rats fed a high-fat diet that were then maintained on this diet or not until adulthood[3,12].

Although no changes were found in the dietary intake of the animals, the amount of visceral adipose tissue was greater in all the groups fed the high-fat diet, either in the perinatal period only (HC), post-weaning period only (CH) or both (HH). These findings confirm the strong influence that a high-fat diet has on the development of visceral obesity.

Other studies have found similar results; i.e., the amount of adipose tissue was greater in animals fed a high-fat diet[16,17]. In addition, long-term observation has shown that a high-fat diet not only increases the body adipose tissue but also induces a similar increase in subsequent generations[18].

Adipose tissue is the main organ for energy storage in the human body. It is recognized as a multifunctional organ with significant endocrine functions, which maintains extensive communication with the other organs and systems of the body[19].

The high amount of fat in the diet used in the present study also promoted changes in the hepatic parameters. The accumulation of fat in the liver mostly depends on the elevation of the level of FFAs in the bloodstream. When excess FFAs are not oxidized or transported to the circulation as low-density lipoproteins, these FFAs are converted to triacylglycerols and deposited in the liver, thus initiating NAFLD[20]. The wide availability of fat in the diet used in the present study, particularly saturated fat, may have increased the levels of circulating FFAs in the animals.

According to Diehl (1999)[21], NAFLD is mainly characterized by the presence of macrovesicles of fat within hepatocytes. When this phenomenon takes place in the absence of inflammation, it is known as liver steatosis. Studies using different methods have indicated that rats fed a high-fat diet in the neonatal period exhibit an in-

Table II
Biochemical parameters of the offspring of mothers fed a control or high-fat diet during pregnancy and lactation that were maintained or not on the diet after weaning

	CC	*CH*	*HH*	*HC*
Total Cholesterol (mg/dL)	64,18 ± 2,28	**83,60**^{***a} ± 5,30	**78,00**^{*a} ± 2,24	72,78 ± 1,67
HDL-C (mg/dL)	38,14 ± 3,02	39,88 ± 2,24	42,12 ± 1,59	38,21 ± 1,20
LDL-C (mg/dL)	18,40 ± 1,54	27,52 ± 4,71	25,29 ± 3,06	26,44 ± 1,47
VLDL-C (mg/dL)	4,64 ± 0,56	**10,80**^{**a} ± 1,60	7,23 ± 0,82	8,40 ± 1,42
TGL (mg/dL)	66,66 ± 2,73	73,31 ± 1,79	67,08 ± 1,80	63,97 ± 3,13
ALT (U/L)	50,35 ± 2,37	48,84 ± 3,95	**62,19**^{*a} ± 4,66	53,20 ± 2,44
AST (U/L)	227,53 ± 13,79	225,20 ± 13,88	238,47 ± 7,78	213,72 ± 10,53
GGT (U/L)	1,0 ± 0,05	1,02 ± 0,05	**1,39**^{**a} ± 0,09	1,18 ± 0,09

The values are expressed as the means ± SEM (standard error); **p < 0.005; ***p < 0.0005 (one-way ANOVA followed by Tukey's multiple comparison test). H&E stain of representative liver section.

crease in the size of the liver and the accumulation of fat vesicles within the liver in adulthood[22,23]. These findings conflict with the results reported by Ahmed[24], who fed animals a fat-rich diet over 3 weeks and did not detect the development of liver steatosis.

Liver steatosis is manifested in two forms: microvesicular and macrovesicular. Microvesicular steatosis, which was found in 100% of the animals in group HH and in 50% of the animals in group CH, is usually associated with severe liver dysfunction related to abnormal changes in the FFA beta-oxidation pathway. Macrovesicular steatosis, in contrast, results from chronic pathophysiological changes, which involve increased synthesis, deficient oxidation and reduced secretion of fat by the liver, and occurs primarily in obesity[25].

The greatest amount of visceral adipose tissue was detected in group HH; in addition, the liver steatosis observed in the rats fed the high-fat diet after weaning was aggravated by the adoption of a high-fat diet by the mothers in the perinatal period. Having been subjected to an inadequate diet during critical developmental periods, the rats' livers underwent physiological and structural changes resulting in a more severe degree of steatosis, which was confirmed by the greater paleness of the livers. Other studies conducted with similar methods found similar results[12,26].

This phenomenon may be related to the large supply of saturated fatty acids obtained via the placenta or the breast milk, resulting in increased lipogenesis and oxidative stress in the fetal liver[27]. The supply of FFAs was kept high for the animals fed the high-fat diet after weaning, promoting a more severe liver steatosis in the animals in group HH compared to those in group CH. These results provide further evidence regarding the paramount importance of a balanced diet in the critical periods of fetal development.

The increase in visceral fat may have also contributed to the abnormalities in liver metabolism and the consequent liver steatosis in groups CH and HH because excess fat increases the rate of lipolysis and FFA uptake by the liver. Although liver steatosis is considered a benign condition, an increasing number of studies demonstrate that NAFLD may cause complications in the long term, such as liver cirrhosis and carcinoma, as well as an increased mortality rate in liver diseases[28]. In addition, by itself or in combination with other liver disorders, NAFLD is a risk factor for diabetes and cardiovascular diseases[29,30].

The results of the biochemical tests indicated that the animals in group CH exhibited greater total cholesterol and VLDL levels, while group HH exhibited an elevation of total cholesterol and ALT. High total cholesterol and VLDL levels are common findings among obese individuals, mainly due to hyperlipogenesis in the liver[31]. Hypercholesterolemia is described as one of the main independent risk factors for atherosclerotic disease, and saturated fat, which was abundant in the diet used in the present study, is the main predictor of an increase in lipid and plasma lipoprotein levels[32].

The results of the present study corroborate the importance of a balanced diet not only for the mother during pregnancy and lactation but also for the offspring after weaning, as the maintenance of an obesogenic environment after weaning exacerbated the noxious effects of the high-fat diet.

Liver steatosis may be associated with the abnormal results of the liver function tests, including the elevated ALT and AST levels, which may result from cell damage and the extravasation of these enzymes into the bloodstream. High-cholesterol diets, such as the one used in the present study, can promote increased oxidative stress in the liver[33], with a consequent elevation of the activity of these enzymes. In the present study, an increase in ALT was found only in group HH. It is a well-established fact that liver disease, including advanced degrees of NAFLD, may be accompanied by normal liver enzyme levels[34].

The GGT levels were also increased in group HH. Although this enzyme is produced by extrahepatic tissues, such as the kidneys, epididymis and lungs, among others, most of the serum GGT is from the liver; therefore, GGT is used in combination with other and more specific enzymes as a biomarker of liver or biliary disease[35].

In the present study, we confirmed that the consumption of a high-fat diet during critical developmental periods can contribute to the occurrence of visceral obesity, liver steatosis and hypercholesterolemia in young adult rats, even in the absence of changes in the dietary intake. These conditions were exacerbated in the offspring of rats fed the high-fat diet during pregnancy and lactation that were maintained on that diet until adulthood.

The present study emphasizes the need to encourage the development of healthy dietary habits during pregnancy and lactation to prevent the occurrence of visceral adiposity and metabolic disorders in the offspring as a strategy to promote long-term health in future generations.

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Conflict of interest

None.

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