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COMMUNICATION

A Perinatal Palatable High-Fat Diet Increases Food Intake and Promotes Hypercholesterolemia in Adult Rats

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Abstract The main goal of the present study was to evaluate the long-term effects of a perinatal palatable high-fat diet on the food intake and cholesterol profile of adult rats. Male Wistar rats (aged 22 days) were divided into two groups according to their mother's diet during gestation and lactation (C_p, $n = 10$; pups from control mothers; and HL_p $n = 10$; pups from mothers fed a palatable high-fat diet). At the 76th day, pups were housed individually for 14 days, and daily food consumption was determined during a period of 6 days. Blood from 100-day-old rats was sampled by cardiac puncture. Fasting (12 h) serum glucose, total cholesterol, LDL-C, HDL-C, triglycerides (TG), and VLDL-C levels were determined. The measurement of food intake was higher in the animals submitted to a hyperlipidic diet during the perinatal period. Serum total cholesterol, LDL-C, HDL-C, TG, VLDL-C and glycemia were increased in the HL_p group compared to the control group. Our findings show that an early life environment with a high-fat diet can contribute to metabolic disease in later life.

Keywords Hyperlipidic diet · Cholesterolemia · Rats · Developmental plasticity · Critical period of development

Abbreviations

C_p	Control pups
HL_p	Hyperlipid diet pups
HDL-C	High-density-lipoprotein cholesterol
LDL-C	Low-density-lipoprotein cholesterol
MUFA	Monounsaturated fatty acids
ND	Not detected
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
TFA	Trans fatty acids
VLDL-C	Very-low-density-lipoprotein cholesterol

Introduction

The etiology of metabolic syndrome has been associated with the perinatal environment, according to previous evidence from experimental studies and epidemiological research [1–3]. Developmental plasticity is the term used to explain the events operating at a critical or susceptible period of development and the lasting consequences for the structure or function of the organism [4]. Our previous studies using rats demonstrated that postnatal undernourishment

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(multi-deficient diet with 7% protein) may influence brain growth spurt, feeding behavior, ontogeny of reflexes, skeletal muscle mechanical properties and locomotor activity in adult rats [5–7].

Developmental plasticity is now observed when an organism is exposed to very high caloric nutrition before birth [4]. In fact, human studies have shown that high-fat diet availability during gestation and lactation, as well as gestational diabetes, may predispose offspring to increased fat mass and incidence of metabolic syndrome as children and adults [8]. In animals, maternal high fat or cholesterol over-feeding during the perinatal period is associated with long-lasting effects on the offspring such as: dyslipidemia, hyperleptinemia, increased adiposity and blood pressure, elevated blood glucose and triglycerides [9, 10]. Litter size reduction (3 pups/litter) resulted in postnatal overfeeding during the suckling period and elevated blood pressure in adulthood [11]. Previous studies have related early adiposity to faster growth and hyperphagia (suppressed orexigenic signals), as seen in 20-postnatal-day offspring from mothers submitted to a high-fat diet during gestation [12]. Out of this context, the main goal of the present study is to evaluate the long-term effects of a palatable high-fat diet during gestation and lactation on the food intake and lipids profiles of offspring.

Materials and Methods

Animals and Diet

The experimental procedures were approved by the Ethical Committee of the Center of Health Science, Federal University of Bahia (protocol no. 018/2008-13), and followed the Guidelines for the Care and Use of Laboratory Animals [13]. Ten pregnant Wistar rats (aged 90–100 days and weighing 180 ± 11 g) were fed either a control diet (*C*, $n = 5$) or a palatable hyperlipidic diet (HL, $n = 5$) during gestation and lactation. The compositions of the diets are shown in Table 1. The fatty acids were analyzed on a gas chromatograph (Shimadzu GC-9A, Japan) with an FID detector. The column was WAX (25 m × 0.25 mm × 0.2 μm), with a flow of 1.3 mL min^{-1} of helium [14]. During the suckling period, the offspring were kept in groups of 8 pups/litter. At weaning (on the 22th day of age), two male offspring from each mother were randomly chosen and assigned to either control (C_p $n = 10$, pups from control mothers) or palatable hyperlipidic diet (HL_p $n = 10$; pups from HL mothers). The offspring were housed 3–4 per cage and received the animals' standard laboratory chow (Nuvilab® CR1, Brasil) ad libitum.

Table 1 Analysis of fatty acid percentage composition of the diets

Ingredients (g/100 g)	Control diet	Hyperlipidic diet
Carbohydrate	57	46
Protein	22	17
Lipids	4	23
Ashes	9	4
Humidity	8	10
Energy (kcal/g)	3.5	4.5
Individual fatty acids (g/100 g of total fatty acid)		
C12:0	ND	13.81
C14:0	ND	5.81
C16:0	15.86	12.65
C18:0	3.31	6.08
C18:1n-9 *cis*	26.24	34.52
C18:1n-9 *trans*	1.18	0.41
C18:2n-6 *cis*	49.68	21.68
C18:3n-3	3.72	0.27
C20:0	ND	0.77
C20:1n-9	ND	0.80
C22:0	ND	1.58
C24:0	ND	1.01
Total fatty acids		
Total SFA	19.17	41.71
Total MUFA *cis*	26.24	35.32
Total PUFA *cis*	53.4	21.95
Total TFA	1.18	0.41
PUFA:SFA	2.78	0.53
n-6:n-3	13.35	80.3

Control diet included commercial chow (Nuvilab® CR1, Brasil). The palatable food included commercial chow (Nuvilab® CR1, Brasil), peanut, milk chocolate bars, and biscuits in the proportion of 3:2:2:1. The main source of fat in the control diet was soybean oil. The main sources of fat in the HL were lard, animal fat, butter and safflower oil

SFA Saturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* Polyunsaturated fatty acids, *TFA* Trans fatty acids, *ND* Not detected

Measurement of Food Intake and Fasting Serum Glycemia and Cholesterol Profile

On day 76, pups were housed individually for 14 days in a metabolic cage. The first 4 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 (50 g) at the onset of the light cycle and the amount of food remaining 24 h later. Body and food weights were recorded by a Marte Scale (AS-1000), in increments of 0.01 g [15]. At 100 days 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®). Low-density-lipoprotein cholesterol (LDL-C) and very low-density-lipoprotein cholesterol (VLDL-C) were obtained using Friedwald calculations [16].

Statistical Analyses

Results are presented as means ± standard errors of the mean. Data for all analyses were performed using the statistical program Graphpad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA). For statistical analysis of body weight and food intake, a two-way repeated measures ANOVA was used, with the mother's diet (HL) and time (weeks) as factors. Bonferroni's post hoc test was used, and Student's *t* test was used to compare groups in terms of blood biochemical parameters. Significance was set at $P < 0.05$.

Results and Discussion

Offspring's body weight in the HL_p group was higher than that of the control group throughout the weeks of development (Fig. 1a). The effects of a palatable high-fat diet during the perinatal period and the consequences of offspring body weight have also been seen in previous studies [8, 17]. The present study shows that life-time effects on body weight are seen in rats in response to perinatal (gestation and lactation) and that adult animals fed by mothers that were fed a perinatal high-fat diet were permanently heavier than their controls.

The measurement of food intake during a period of 6 days was higher in the HL_p group (Fig. 1b). High food intake can be associated with a high adiposity, high leptinemia and leptin resistance [18]. There is a correlation between high leptin resistance and the relative food intake per gram of body weight [18]. Although the concentrations of leptin were not evaluated in the present study, previous studies have found that a perinatal high-fat diet or gestational diabetes can lead to hyperinsulinism and a malprogramming in central regulators of body weight and metabolism [17].

Serum total cholesterol, LDL-C, HDL-C, TG, VLDL-C and glycemia were higher in the HL_p group than controls group (Table 2). In addition, these animals were markedly heavier and had significantly increased serum concentrations of glucose, cholesterol, and triglycerides, suggesting impairments in carbohydrate and lipid metabolism. Khan et al. [19] reported an increase in plasma triglycerides and a decrease in HDL-cholesterol concentrations in the female offspring born to mothers fed a rich in lard (25.7% fat) diet 10 days before and throughout pregnancy and lactation, although these changes were only apparent in one-year-old offspring. Although previous studies have used different protocols to induce developmental plasticity by a maternal high-fat diet, our results are in accordance with previously reported results in terms of blood lipid profiles [8, 10, 17,

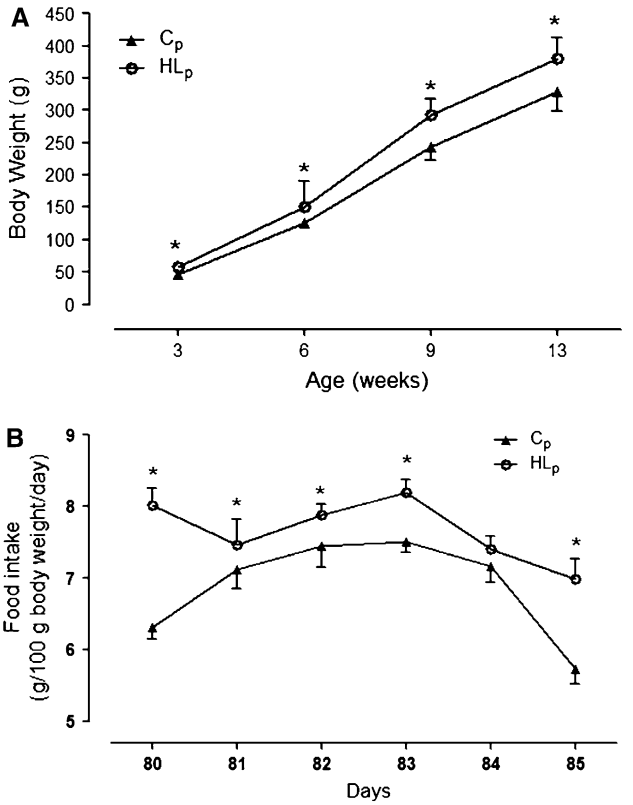

Fig. 1 Body weight of pups from mothers submitted to either a control or a hyperlipidic diet during gestation and lactation (**a**). Body weight was evaluated weekly until the 90th day of life. Food intake by pups at 80 days old from mothers submitted to either a control or a hyperlipidic diet during gestation and lactation (**b**). Food intake was evaluated daily until the 85th day of life. Control (C_p, $n = 10$) and hyperlipidic pups (HL_p, $n = 10$). The values are presented as means + SEM. *$P < 0.05$ using two-way ANOVA and Bonferroni's post hoc test

Table 2 Fasting serum glycemia and cholesterol profile of pups at 90 days old from mothers submitted to either a control or a hyperlipidic diet during gestation and lactation

	C_p	HL_p
Total cholesterol (mg/dL)	35.1 ± 1.0	56.4 ± 3.3*
LDL-C (mg/dL)	9.6 ± 1.1	19.14 ± 2.2*
HDL-C (mg/dL)	16.6 ± 0.7	25.5 ± 1.3*
Triglycerides (mg/dL)	44.2 ± 4.1	58.8 ± 5.5*
VLDL-C (mg/dL)	8.84 ± 0.8	11.76 ± 1.1*
Glycemia (mg/dL)	171.6 ± 11.1	217 ± 10.6*

Control (C_p, $n = 10$) and hyperlipidic pups (HL_p, $n = 10$) were evaluated after 24 h of fasting

LDL-C Low-density-lipoprotein cholesterol, *HDL-C* High-density-lipoprotein cholesterol, *VLDL-C* Very low-density-lipoprotein cholesterol

* $P < 0.05$ by using Student's *t* test

20–22]. The alterations could be associated with the concentration of saturated fatty acids (41.71%) in the mother's diet, even though the post-weaning diet was balanced for both groups in terms of carbohydrates, lipids and protein. The novelty of the present study is that a perinatal high-fat diet induces altered plasma cholesterol and triglyceride levels in the offspring and can predispose the offspring to developing obesity in a manner that is independent of postnatal diet.

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]. Most models have used malnutrition as a stimulus for developmental plasticity, and relatively few studies have seen the effects of a high-fat diet during gestation and lactation. In this regard, we have presented data that confirm our hypothesis by demonstrating that a palatable high-fat diet during perinatal period increases food intake and induces hypercholesterolemia. In conclusion, our observations extend the evidence that both gestation and lactation are critical periods for the development of metabolic disease in later life.

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References

1. Barker DJ (2007) The origins of the developmental origins theory. J Intern Med 261:412–417
2. Gluckman PD, Hanson MA, Spencer HG, Bateson P (2005) Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. Proc Biol Sci 272:671–677
3. Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in male mice. Nature 427:411–412
4. Gluckman PD, Hanson MA (2007) Developmental plasticity and human disease: research directions. J Intern Med 261:461–471
5. Barreto-Medeiros JM, Feitoza EG, Magalhaes K, Cabral-Filho JE, Manhaes-De-Castro FM, De-Castro CM, Manhaes-De-Castro R (2004) Malnutrition during brain growth spurt alters the effect of fluoxetine on aggressive behavior in adult rats. Nutr Neurosci 7:49–52
6. Orozco-Solis R, Lopes de Souza S, Barbosa Matos RJ, Grit I, Le Bloch J, Nguyen P, Manhaes de Castro R, Bolanos-Jimenez F (2009) Perinatal undernutrition-induced obesity is independent of the developmental programming of feeding. Physiol Behav 96:481–492
7. Toscano AE, Manhaes-de-Castro R, Canon F (2008) Effect of a low-protein diet during pregnancy on skeletal muscle mechanical properties of offspring rats. Nutrition 24:270–278
8. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJ, Badger TM (2008) Maternal obesity at conception programs obesity in the offspring. Am J Physiol Regul Integr Comp Physiol 294:R528–R538
9. Buckley AJ, Keseru B, Briody J, Thompson M, Ozanne SE, Thompson CH (2005) Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism 54:500–507
10. Chechi K, Cheema SK (2006) Maternal diet rich in saturated fats has deleterious effects on plasma lipids of mice. Exp Clin Cardiol 11:129–135
11. Boubred F, Buffat C, Feuerstein JM, Daniel L, Tsimaratos M, Oliver C, Lelievre-Pegorier M, Simeoni U (2007) Effects of early postnatal hypernutrition on nephron number and long-term renal function and structure in rats. Am J Physiol Renal Physiol 293:F1944–F1949
12. Chen H, Morris MJ (2009) Differential responses of orexigenic neuropeptides to fasting in offspring of obese mothers. Obesity (Silver Spring) 17:1356–1362
13. Bayne K (1996) Revised Guide for the Care and Use of Laboratory Animals available. Am Physiol Soc. Physiologist 39:199, 208–111
14. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
15. Lopes de Souza S, Orozco-Solis R, Grit I, Manhaes de Castro R, Bolanos-Jimenez F (2008) Perinatal protein restriction reduces the inhibitory action of serotonin on food intake. Eur J Neurosci 27:1400–1408
16. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
17. Srinivasan M, Katewa SD, Palaniyappan A, Pandya JD, Patel MS (2006) Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood. Am J Physiol Endocrinol Metab 291:E792–E799
18. Zambrano E, Bautista CJ, Deas M, Martinez-Samayoa PM, Gonzalez-Zamorano M, Ledesma H, Morales J, Larrea F, Nathanielsz PW (2006) A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. J Physiol 571:221–230
19. Khan IY, Taylor PD, Dekou V, Seed PT, Lakasing L, Graham D, Dominiczak AF, Hanson MA, Poston L (2003) Gender-linked hypertension in offspring of lard-fed pregnant rats. Hypertension 41:168–175
20. Tamashiro KL, Moran TH (2010) Perinatal environment and its influences on metabolic programming of offspring. Physiol Behav 100:560–566
21. White CL, Purpera MN, Morrison CD (2009) Maternal obesity is necessary for programming effect of high-fat diet on offspring. Am J Physiol Regul Integr Comp Physiol 296:R1464–R1472
22. Rodrigues AL, de Moura EG, Passos MC, Trevenzoli IH, da Conceicao EP, Bonono IT, Neto JF, Lisboa PC (2011) Postnatal early overfeeding induces hypothalamic higher SOCS3 expression and lower STAT3 activity in adult rats. J Nutr Biochem 22:109–117