Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Correspondence:

Maternal high-fat diet inversely affects insulin sensitivity in dams and young adult male rat offspring*#

Roxana KARBASCHI[1], Forouzan SADEGHIMAHALLI[2], Homeira ZARDOOZ†‡[1,3]

([1]*Department of Physiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*)

([2]*Education Development Center, Mazandaran University of Medical Sciences, Sari, Iran*)

([3]*Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*)

†E-mail: homeira_zardooz@sbmu.ac.ir

http://dx.doi.org/10.1631/jzus.B1600131

1 Introduction

Environmental factors such as maternal nutrition during intrauterine development may program the neuroendocrine system and alter its function in later life (Cerf *et al.*, 2012). It is demonstrated that metabolic diseases have their origin in early life nutritional experience such as maternal high-fat (HF) diet consumption during gestation and lactation (Srinivasan *et al.*, 2006). The changes in energy balance and cells' response to insulin are the important metabolic changes in the offspring of HF-fed dams (Sullivan *et al.*, 2015). Maternal HF diet consumption increased abdominal fat in dams at the end of the lactation period with increased (Jacobs *et al.*, 2014) or without any change (Desai *et al.*, 2014) in leptin plasma level, while fasting plasma insulin concentration was increased. In the offspring of these dams, the plasma leptin concentration was higher than that in the control rats in adulthood. Also reported are an impaired glucose tolerance and increased fasting insulin, associated with higher (Desai *et al.*, 2014) or normal (Jacobs *et al.*, 2014) fasting glucose concentrations. Moreover, some studies showed that HF diet consumption in rats (Cerf *et al.*, 2012) or mice (Tuohetimulati *et al.*, 2012) in pregnancy and lactation periods resulted in glucose intolerance and increased the homeostasis model assessment of insulin resistance (HOMA-IR) index (Cerf *et al.*, 2012; Miotto *et al.*, 2013) in offspring. The issue of the impact of maternal HF diet consumption on glucose homeostasis in both mother and offspring has attracted much attention and needs to be further clarified. We say this in the light, on one hand of varying reports on the effect of HF diet consumption during pre-pregnancy, pregnancy, and lactation periods on energy balance and response to insulin in both mother and offspring, and on the other considering the adverse metabolic consequences of maternal HF diet in critical periods of life (fetal and neonatal), as mentioned above. The present study investigated the effects of chronic maternal HF feeding (in pre-pregnancy, pregnancy, and lactation periods) on corticosterone, leptin, glucose, and insulin plasma levels as metabolic parameters, as well as glucose tolerance and the HOMA-IR index in dams and adult male rat offspring.

2 Materials and methods

2.1 Animals

Animals were kept in a room ((22±2) °C) with a 12-h light/dark cycle. Twenty female virgin Wistar

‡ Corresponding author

* Project supported by the Grant from the Research Deputy of Faculty of Medicine, Shahid Beheshti University of Medical Sciences (No. 13/542), Iran

This paper has been extracted from the thesis written by Mrs. Roxana KARBASCHI in the School of Medicine, Shahid Beheshti University of Medical Sciences (Registration No. 449), Iran

ORCID: Homeira ZARDOOZ, http://orcid.org/0000-0001-8307-1848

rats ((180±20) g) were randomly divided into control (CON dams) and HF (HF dams) groups and were simultaneously fed (during pre-pregnancy (4 weeks), pregnancy, and lactation periods) by normal diet (standard pellets, 4.75% kcal (1 kcal=4.1868 kJ) as fat, produced by Pars Company of animal food producer, Iran) and HF diet (cow butter (35%, w/w) mixed with standard pellets, 58.2% kcal as fat). The fatty acid profile of the diets has been measured according to Institute of Standards and Industrial Research of Iran (ISIRI) Nos. 4090–4091 (Table 1). All diets and tap water were provided ad libitum for the animals except during fasting conditions.

Table 1 Fatty acid composition of the normal and HF diets

Type of fatty acid	Common name	Normal diet (%)	HF diet (%)
C12:0	Lauric acid	0.30	5.00
C14:0	Myristic acid	0.27	2.88
C16:0	Palmitic acid	14.40	18.50
C16:1c *n*-7	Palmitoleic acid	0.00	0.16
C17:0	Margaric acid	0.00	0.32
C18:0	Steric acid	3.25	3.83
C18:1c *n*-9	Oleic acid	32.34	32.85
C18:2c *n*-6	Linoleic acid	44.96	33.06
C18:3c *n*-6	γ-Linolenic acid	3.90	2.66
C20:0	Arachidonic acid	0.11	0.11
C20:1c *n*-7	Paullinic acid	0.17	0.11
C22:0	Behenic acid	0.10	0.10
C24:0	Lignoceric acid	0.10	0.07
Other		0.09	0.36

At weaning, the male offspring from CON dams (CON offspring) and HF dams (HF offspring) of 8 different litters were housed (2 per cage) and fed normal diet until postnatal day (PND) 70. The body weight, food, calorie, and water intakes in all groups were measured twice a week; in offspring food and water intakes were assessed from weaning to PND 70. All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the Ethical Committee of the Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Iran (No. IR.SBMU.MSP.REC.1395.129).

2.2 Blood sampling and intraperitoneal glucose tolerance test (IPGTT)

Blood sampling was performed in fasting status (16 h) under pentobarbital anesthesia (Sigma, USA; 60 mg/kg, intraperitoneally (IP)), between 8:00 a.m. and 10:00 a.m. on 22 d after post-partum in dams and on PND 70 in offspring by cutting the tail. Blood was collected into a microtube containing 5 μl/ml heparin (5000 IU/ml) and immediately centrifuged at 3000*g* for 5 min at 4 °C and plasma was kept at −80 °C. In the anesthetized rats, glucose (20% (0.2 g/ml) solution in water) was injected IP (2 g/kg) and blood samples were taken after 15, 30, 60, 90, and 120 min in order to measure plasma glucose and insulin concentrations.

After blood sampling, the anesthetized animals were decapitated and intra-abdominal fat was removed and weighed.

2.3 Assays

Plasma glucose concentration was determined using the glucose oxidase method (Pars Azmoon, Iran). The rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden), corticosterone ELISA kit (DRG, Germany), and rat leptin ELISA kit (CUSABIO, China) were used to measure plasma insulin, corticosterone, and leptin concentrations, respectively.

2.4 HOMA-IR index

The HOMA-IR index as a method of assessing insulin resistance was calculated using the following formula: HOMA-IR=(C_i×C_g)/22.5, where C_i is fasting insulin level (μU/ml) and C_g is fasting glucose level (mmol/L) (Rostamkhani *et al.*, 2012).

2.5 Statistical analysis

All data are expressed as the mean±standard error of the mean (SEM). A mixed analysis of variance followed by least significant difference (LSD), and the unpaired *t*-test were used to compare groups. A *P*-value below 0.05 was considered to be statistically significant.

3 Results and discussion

The results show that in HF dams the food (P<0.01) and water (P<0.01) intakes were lower, but the calorie intake (P<0.001) was higher than those in CON dams, while their body weight was not different from controls (data not shown). HF diet increased

intra-abdominal fat weight and plasma level of corticosterone, whereas it decreased plasma leptin and did not change plasma glucose or insulin concentration, nor the HOMA-IR index (Table 2). In HF dams during IPGTT, the plasma glucose level did not show any significant difference compared to CON dams except for 120 min; however, they showed a higher plasma insulin level (Figs. 1a and 1b).

In HF offspring, food (P<0.01), calorie (P<0.001), and water (P<0.001) intakes were lower than those in CON offspring, also after birth to PND 70, they showed a lower body weight (P<0.001) (data not shown). Moreover, maternal HF diet decreased the intra-abdominal fat weight and plasma levels of corticosterone and leptin, without any change in fasting plasma glucose or insulin concentration nor HOMA- IR index (Table 2). After glucose injection, the plasma glucose levels at 60 and 90 min were significantly decreased in HF offspring, while their plasma insulin levels did not show any difference compared with the control rats (Figs. 1c and 1d).

The decrement of plasma leptin level in HF dams could be due to reduced insulin-dependent glucose metabolism in adipocytes, which is necessary for leptin production and secretion (Mueller *et al.*, 1998). In addition, the lower food intake (yet hypercaloric intake) despite decreased plasma leptin level was perhaps induced by elevated levels of insulin (Clegg *et al.*, 2011) and corticosterone (Desai *et al.*, 2014) in HF dams, which both inhibit food and energy intakes.

Table 2 Effect of maternal HF diet on fasting plasma parameters, intra-abdominal fat weight, and HOMA-IR index

Group	Glucose (mg/dl)	Insulin (μg/L)	Corticosterone (nmol/ml)	Leptin (ng/ml)	Intra-abdominal fat weight (g)	HOMA-IR index
CON dams	127.50±5.65	0.55±0.06	2.91±0.69	3.04±0.39	0.54±0.06	4.29±0.48
HF dams	134.63±3.62	0.60±0.05	4.95±0.28††	1.03±0.19†††	1.35±0.22††	4.80±0.46
CON offspring	108.10±3.18	0.46±0.04	1.76±0.25	2.09±0.32	1.01±0.13	3.00±0.17
HF offspring	110.56±2.78	0.46±0.06	0.85±0.12††	0.95±0.19†††	0.80±0.06†	3.06±0.41

Values are expressed as mean±SEM (n=10, group for dams; n=8, group for offspring). CON: control; HF: high-fat. † P<0.05, †† P<0.01, ††† P<0.001 vs. respective control group

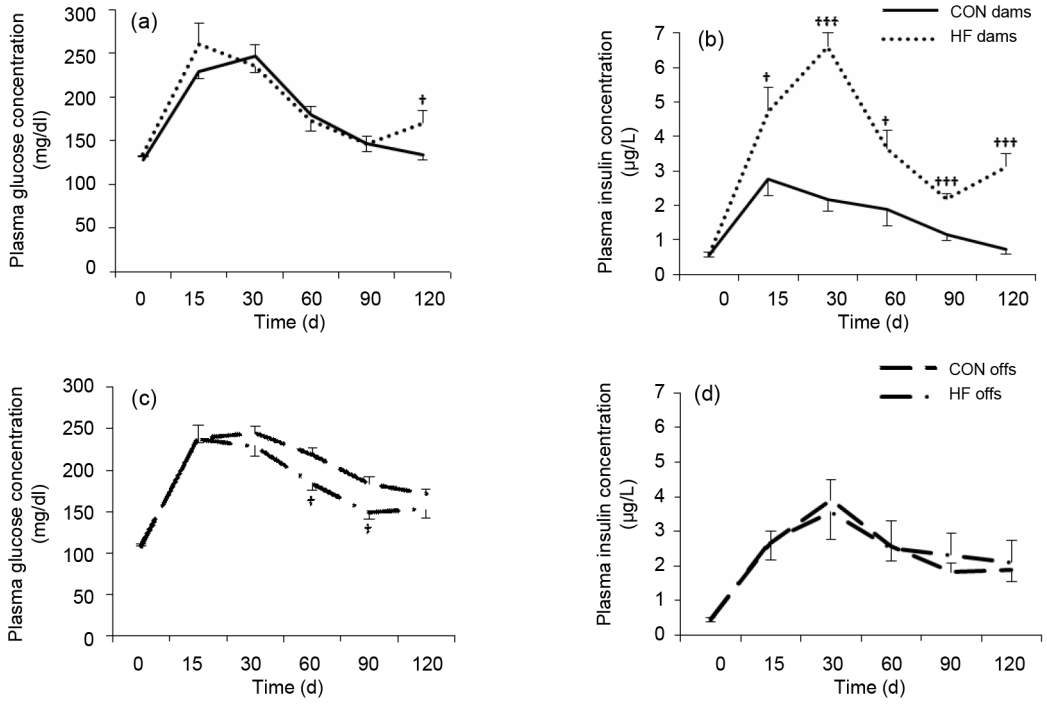

Fig. 1 Changes in plasma glucose (a, c) and insulin (b, d) levels during IPGTT in dams (a, b) and offspring (c, d)
Each point represents mean±SEM (n=10, group for dams; n=8, group for offspring). CON: control; HF: high-fat; offs: offspring. † P<0.05, ††† P<0.001 vs. control group

Also the elevated corticosterone level of HF dams could be related to the stimulatory effect of the HF diet on hypothalamic-pituitary-adrenal (HPA) axis (Tannenbaum *et al.*, 1997). Despite the lack of difference in HOMA-IR index between HF and control groups of dams and offspring, the increment of plasma insulin levels during IPGTT, which was observed in HF dams, represents insulin resistance, whereas it is expected that the physiological state of insulin resistance in pregnancy dissipates during lactation (Stuebe and Rich-Edwards, 2009), and therefore the lack of dissipation could be the result of HF feeding.

In offspring most of the studies have shown that prenatal exposure to an HF diet increases birth weight and weight gain in postnatal days. In this study, a decrease in food and energy intakes as well as body weight, which was observed in HF offspring, could be the result of changes in feeding regulatory centers programming or switching from the HF to a control diet (Miotto *et al.*, 2013). Since HF offspring are exposed to high level of dams' corticosterone during critical period of life, their HPA axis programmings might be affected and cause a lower level of plasma corticosterone in adulthood.

In HF offspring during IPGTT, a faster decline of plasma glucose level from peak to base line shows a degree of insulin sensitivity. Exposure to high levels of fatty acids in fetal and neonatal periods (Innis, 2005) could affect metabolic programming of HF offspring including their neuroendocrine system and responsiveness of cells to insulin (Kabaran and Besler, 2015). In addition, following maternal HF feeding we observed several limitations including decreased number of pups per litter and increased mortality rate of the pups as a result of cannibalistic events which have been reported in other studies (Bellisario *et al.*, 2014).

It seems that long-term maternal high-fat feeding may act as a stressor and induce insulin resistance in dams and alter neuroendocrine system programming in offspring.

Compliance with ethics guidelines

Roxana KARBASCHI, Forouzan SADEGHIMAHALLI, and Homeira ZARDOOZ declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Bellisario, V., Berry, A., Capoccia, S., *et al.*, 2014. Gender-dependent resiliency to stressful and metabolic challenges following prenatal exposure to high-fat diet in the $p66^{Shc-/-}$ mouse. *Behav. Neurosci.*, **8**:285. http://dx.doi.org/10.3389/fnbeh.2014.00285

Cerf, M.E., Chapman, C.S., Louw, J., 2012. High-fat programming of hyperglycemia, hyperinsulinemia, insulin resistance, hyperleptinemia, and altered islet architecture in 3-month-old Wistar rats. *ISRN Endocrinol.*, **2012**: 627270. http://dx.doi.org/10.5402/2012/627270

Clegg, D.J., Gotoh, K., Kemp, C., *et al.*, 2011. Consumption of a high-fat diet induces central insulin resistance independent of adiposity. *Physiol. Behav.*, **103**(1):10-16. http://dx.doi.org/10.1016/j.physbeh.2011.01.010

Desai, M., Jellyman, J.K., Han, G., *et al.*, 2014. Rat maternal obesity and high fat diet program offspring metabolic syndrome. *Am. J. Obstet. Gynecol.*, **211**(3):237.e1-237.e13. http://dx.doi.org/10.1016/j.ajog.2014.03.025

Innis, S.M., 2005. Essential fatty acid transfer and fetal development. *Placenta*, **26**(Suppl. A):S70-S75. http://dx.doi.org/10.1016/j.placenta.2005.01.005

Jacobs, S., Teixeira, D.S., Guilherme, C., *et al.*, 2014. The impact of maternal consumption of cafeteria diet on reproductive function in the offspring. *Physiol. Behav.*, **129**(22):280-286. http://dx.doi.org/10.1016/j.physbeh.2014.03.003

Kabaran, S., Besler, H.T., 2015. Do fatty acids affect fetal programming? *J. Health Popul. Nutr.*, **33**:14. http://dx.doi.org/10.1186/s41043-015-0018-9

Miotto, P.M., Castelli, L.M., Amoye, F., *et al.*, 2013. Maternal high fat feeding does not have long-lasting effects on body composition and bone health in female and male Wistar rat offspring at young adulthood. *Molecules*, **18**(12):15094-15109. http://dx.doi.org/10.3390/molecules181215094

Mueller, W.M., Gregoire, F.M., Stanhope, K.L., *et al.*, 1998. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology*, **139**(2): 551-558. http://dx.doi.org/10.1210/endo.139.2.5716

Rostamkhani, F., Zardooz, H., Zahediasl, S., *et al.*, 2012. Comparison of the effects of acute and chronic psychological stress on metabolic features in rats. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **13**(11):904-912. http://dx.doi.org/10.1631/jzus.B1100383

Srinivasan, M., Katewa, S.D., Palaniyappan, A., *et al.*, 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**(4):E792-E799. http://dx.doi.org/10.1152/ajpendo.00078.2006

Stuebe, A.M., Rich-Edwards, J.W., 2009. The reset hypothesis: lactation and maternal metabolism. *Am. J. Perinatol.*,

26(1):81-88. http://dx.doi.org/10.1055/s-0028-1103034

Sullivan, E.L., Riper, K.M., Lockard, R., *et al.*, 2015. Maternal high-fat diet programming of the neuroendocrine system and behavior. *Horm. Behav.*, **76**:153-161. http://dx.doi.org/10.1016/j.yhbeh.2015.04.008

Tannenbaum, B.M., Brindley, D.N., Tannenbaum, G.S., 1997. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. *Am. J. Physiol. Endocrinol. Metab.*, **273**(6):E1168-E1177.

Tuohetimulati, G., Uchida, T., Toyofuku, Y., *et al.*, 2012. Effect of maternal high-fat diet on pancreatic beta cells of the offspring. *Diabetol. Int.*, **3**(4):217-223. http://dx.doi.org/10.1007/s13340-012-0081-z

中文概要

题　目：母代高脂饮食反向影响母鼠和子代成年雄性大鼠的胰岛素敏感度

目　的：进一步明确母代高脂肪饮食对母鼠和子代成年雄鼠的葡萄糖稳态的潜在影响。

方　法：将母代大鼠及其子代雄鼠（断奶后 70 天）分为对照组和高脂组，检测其血液代谢参数并进行腹腔内葡萄糖耐受试验。

结　论：长期母代高脂肪饮食可能诱导母鼠的胰岛素抵抗，并改变子代的神经内分泌系统编程。

关键词：母代高脂饮食；葡萄糖耐量试验；胰岛素；稳态模型胰岛素抵抗指数；肾上腺酮