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Hypothalamic endoplasmic reticulum stress and insulin resistance in offspring of mice dams fed high-fat diet during pregnancy and lactation

Arine M. Melo[a], *Rafaela O. Benatti*[a], *Leticia M. Ignacio-Souza*[b], *Caroline Okino*[a], *Adriana S. Torsoni*[a,d], *Marciane Milanski*[a,d], *Licio A. Velloso*[c], *Marcio Alberto Torsoni*[a,d,*]

[a] *Laboratory of Metabolic Disorders, Faculty of Applied Sciences, University of Campinas -UNICAMP, Limeira, São Paulo, Brasil*
[b] *Faculty of Nutrition, Federal University of Mato Grosso-UFMT, Cuiabá, Mato Grosso, Brasil*
[c] *Laboratoty of Cell Signaling, Department of Internal Medicine, Faculty of Medical Sciences, University of Campinas-UNICAMP, Campinas, São Paulo, Brasil*
[d] *Center for Studies of lipid in Nutrigenomic, Faculty of Applied Sciences, University of Campinas -UNICAMP, Limeira, São Paulo, Brasil*

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ABSTRACT

Objective. The goal of this study was to determine the presence early of markers of endoplasmic reticulum stress (ERS) and insulin resistance in the offspring from dams fed HFD (HFD-O) or standard chow diet (SC-O) during pregnancy and lactation.

Materials/Methods. To address this question, we evaluated the hypothalamic and hepatic tissues in recently weaned mice (d28) and the hypothalamus of newborn mice (d0) from dams fed HFD or SC during pregnancy and lactation.

Results. Body weight, adipose tissue mass, and food intake were more accentuated in HFD-O mice than in SC-O mice. In addition, intolerance to glucose and insulin was higher in HFD-O mice than in SC-O mice. Compared with SC-O mice, levels of hypothalamic IL1-β mRNA, NFκB protein, and p-JNK were increased in HFD-O mice. Furthermore, compared with SC-O mice, hypothalamic AKT phosphorylation after insulin challenge was reduced, while markers of ERS (p-PERK, p-eIF2α, XBP1s, GRP78, and GRP94) and p-AMPK were increased in the hypothalamic tissue of HFD-O at d28 but not at d0. These damages to hypothalamic signaling were accompanied by increased triglyceride deposits, activation of NFκB, p-JNK, p-PERK and p-eIF2α.

Conclusion. These point out lactation period as maternal trigger for metabolic changes in the offspring. These changes may occur early and quietly contribute to obesity and associated pathologies in adulthood. Although in rodents the establishment of ARC

Abbreviations: ACC, acetyl-CoA carboxylase; AGRP, agouti-related polypeptide; AKT, protein kinase B; AMPK, 5' AMP-activated protein kinase; ATF6, activating transcription factor 6; DIO, diet-induced obesity; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERS, endoplasmic reticulum stress; FFA, free fatty acids; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPR, glucose-regulated protein; GTT, glucose tolerance test; HFD, high-fat diet; HFD-O, offspring from HFD dams; ICV, intracerebroventricular; IKK, inhibitor of kappa B kinase; IL1β, interleukin 1 beta; IRE1, inositol requiring-1; ITT, insulin tolerance test; JNK, c-Jun N-terminal kinase; NFκB, nuclear factor-κB; NPY, neuropeptide Y; PERK, PKR-like ER kinase; SC, standard chow diet; SC-O, offspring from SC dams; TG, triglycerides; TNFα, tumoral necrosis factor alpha; UPR, unfolded protein response; XBP1, x-box binding protein 1.

* *Corresponding author at:* Faculdade de Ciências Aplicadas, Universidade de Campinas, Rua Pedro Zaccaria, 1300, Jardim Santa Luiza, Limeira-São Paulo, CEP 13484350. Tel.: +55 19 3701 6705; fax: +55 19 3701 6680.
E-mail address: marcio.torsoni@fca.unicamp.br (M.A. Torsoni).

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neuronal projections occurs during the lactation period, in humans it occurs during the third trimester. Gestational diabetes and obesity in this period may contribute to impairment of energy homeostasis.

1. Introduction

Obesity is associated with the activation of inflammatory pathways and increased TNFα and IL1-β cytokine expression in different tissues. It is known that proinflammatory cytokines activate serine kinases c-Jun N-terminal kinases (JNK) and inhibitor of kappa B kinase (IKK) [1,2], and the association between inflammatory pathway activation and insulin and leptin resistance has been extensively reviewed [3]. In models of diet-induced obesity (DIO), the consumption of a high-fat diet (HFD) induces an increase in cytokine expression, JNK and IKK activation in the liver and the hypothalamus [4–6], hepatic glucose production, and insulin resistance [7,8]. Furthermore, diet-induced inflammation can lead to the activation of apoptotic signaling pathways and to increased cell apoptotic death in hypothalamic neurons [9,10].

Maternal consumption of HFD during pregnancy and lactation clearly affects the brain. Offspring from HFD dams have increased mRNA levels of neuropeptide Y and agouti-related polypeptide in the hypothalamus, indicating that orexigenic neuropeptides in HFD progeny are upregulated. In addition, serum leptin and insulin levels were higher in HFD offspring than in control rats [11]. Finally, adult offspring from HFD dams showed hepatic insulin resistance, hepatic fat deposition, and JNK and IKK activation [12]. Recently Shankar et al. showed that inflammatory signals were present in the uterus and the blastocyst, suggesting the early activation of the inflammatory pathways in offspring from HFD dams [13].

The endoplasmic reticulum (ER) is the major site in the cell for protein folding and trafficking. The increase in the cellular demand for new molecules results in unfolded protein and ER stress (ERS) [14]. Initially chaperone proteins, like 78 kDa glucose-regulated protein (GRP78) and 94 kDa glucose-regulated protein (GRP94), are recruited to support the folding of new proteins. Next, three major transducers for sensing ER stress are activated: inositol requiring-1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). They promote the molecular adaptation to stress conditions through activation of additional proteins, like X-box binding protein 1 (XBP1) and eukaryotic translation initiation factor 2α (eIF2α), so-called the unfolded protein response (UPR), which has intersection with the inflammatory signaling pathway [14,15].

Hypothalamic inflammation and ERS can reportedly impair pancreatic islet function, lipid metabolism, central and peripheral insulin/leptin action, and blood pressure [16–19]. On the other hand, the inhibition of hypothalamic inflammation reverses diet-induced insulin resistance in the liver, leading to reduced steatosis and reduced gluconeogenesis; however, these were abrogated by vagotomy [20].

Although many studies have investigated the effects of maternal consumption of HFD on metabolic disorders in offspring, hypothalamic UPR activation in recently weaned mice remains unknown. To address this, 4-week-old (d28) and newborn pups (d0) from HFD and control dams were evaluated.

2. Material and methods

2.1. Animals and diets

This study was conducted in strict accordance with the recommendations of the COBEA (Brazilian College of Animal Experimentation) guidelines, approved by the Ethical Committee for Animal Use (ECAU) (ID protocol: 2758–1) of the Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil. Ten virgin female Swiss mice were taken from the university's central breeding colony. Before mating, the females were randomly divided into 2 groups and fed either HFD or standard chow (SC) (Table 1) *ad libitum* for 3 weeks to allow adaptation. Mating was performed by housing females with adult males overnight, and pregnancy was confirmed by examining vaginal smears for the presence of sperm. Pregnant females were maintained in individual cages in a room at 24 °C ± 1 °C with lights on from 6:00 a.m. to 6:00 p.m. They were fed a specific diet (HFD or SC) and water *ad libitum* during the pregnancy and lactation periods. On the first day (d) after birth (d0), the litter size of both the groups was adjusted to 6 animals per litter. After weaning, only male offspring from HFD dams (HFD-O) and offspring from SC dams (SC-O) were fed with the SC diet until 28 days (d28).

2.2. Glucose tolerance test (GTT) and insulin tolerance test (ITT)

The intraperitoneal GTT was performed after fasting the mice overnight (d28). After collection of an unchallenged sample (time 0), 2.0 g of glucose/body weigh mass was administered into the peritoneal cavity, and tail blood samples were collected at 15, 30, 60, 90, and 120 min after glucose administration. For ITT, insulin (0.5 U/body weight mass)

Table 1 – Nutritional composition of experimental and standard chow fed to mice during gestation and lactation.

	Chow Diet [1]	High-Fat Diet
Net protein (g %)	22.5	20.8
Ether Extract (Fat content) (g %)	4.5	23.6
Carbohydrates (g %)	55.0	41.2
Fibrous matter (g %)	8.0	5.8
Ash matter (g %)	10.0	8.6
Total	100.0	100.0
kcal/g	3.5	4.6

[1] (NUVILAB® Cr-1, Nuvital, PR, Brasil).