Influence of maternal periuterine and periovarian fat on reproductive performance and fetal growth in rats

MARIA EDUARDA P. GOMES, LUIGI M.J. DIDOMIZIO, YURI K. SINZATO, VERÔNYCA G. PAULA, MAYSA R. SOUZA, FRANCIANE Q. GALLEGO, VINÍCIUS S. BARCO, GUSTAVO T. VOLPATO & DÉBORA CRISTINA DAMASCENO

Abstract: We aimed to evaluate how high-fat diet consumption can interfere with rat reproductive performance and fetal development. High-fat diet (HFD) was initiated in 30-day-old rats, distributed into two groups (n=7 animals/group): Rats receiving a standard diet and rats receiving HFD. At adulthood, the rats were mated, and on day 21 of pregnancy, the females were anesthetized, decapitated, and submitted to laparotomy to obtain visceral and periovarian adipose tissue. The uterine horns were exposed for analysis of maternal reproductive performance. The fetuses and placentas were weighed and analyzed. Pearson’s correlation test was used, and p<0.05 was considered significant. There was a significant positive correlation (HFD consumption x increased periovarian fat) and a negative correlation with the implantation, live fetus numbers and lower litter weight. Furthermore, the increased relative weight of periuterine fat was related to the lower number of live fetuses and litter weight. Regarding the fetal weight classification, there was a negative correlation between the relative weight of periovarian fat and the percentage of fetuses appropriate for gestational age and large for gestational age. Therefore, our findings show that HFD maternal intake negatively influenced on reproductive performance and fetal growth.

Key words: High-fat diet, fetus, rats, reproduction, pregnancy.

INTRODUCTION

In the last years, there is a change in lifestyle societies, with the adoption of a Westernized lifestyle characterized by high-fat diets, sedentary lifestyles, psychological stress, smoking, and environmental smoking (Carrera-Bastos et al. 2011). Excessive intake of high-fat diets causes several civilizational diseases caused by metabolic disorders, such as hyperinsulinemia, insulin resistance, dyslipidemia, low-grade systemic inflammation, increased production of reactive oxygen species (ROS) and oxidative stress (Kopp 2003, Corkey 2012, Nolan & Prentki 2019). These pathophysiological changes have been associated with obesity, type 2 diabetes, and dyslipidemia (Drews et al. 2010, Cohen & Leroith 2012).

Being overweight is a pre-existing condition in 40% of women who become pregnant (Kim et al. 2007) due to the consumption of high-fat diets (HFD), which leads to a risk for the mother and her pregnancy and increases the risk of preeclampsia and gestational Diabetes mellitus (Östlund et al. 2004). Exposure to an abnormal maternal intrauterine environment negatively influences fetal programming leading to lifelong effects and increasing the risk of developing chronic diseases; this concept is defined as
Developmental Origins of Health and Disease (DOHaD). This theory describes how exposure to environmental factors during intrauterine life and/or after birth causes developmental changes that result in long-term impacts such as illness in later life (Uauy et al. 2011).

High-fat diet (HFD) is widely used in experimental models for obesity induction to study its repercussions (Papáčková et al. 2012, Martinelli et al. 2020, Baiges-Gaya et al. 2021, Paula et al. 2022b). Compared to most genetically modified models, rodents fed by HFD can better reproduce the human obesity state. In addition, HFD may be the best choice for testing possible therapeutic alternatives (Lutz & Woods 2012). Studies show that a high-fat diet in pregnant rodents leads to pups with adiposity, hypertension, and elevated blood glucose and insulin levels over six months (Samuelsson et al. 2008). Our research group showed that postnatal consumption of HFD from weaning to 120 days of life was responsible for the increase in insulin synthesis and insulin resistance in adult rats (Paula et al. 2022a). Another study showed that rats that were submitted to HFD consumption before and during pregnancy also showed abnormal glucose metabolism and embryofetal losses during term pregnancy of these rats (Sinzato et al. 2022).

When we consider the excessive consumption of HFD and unhealthy foods in women of reproductive age and pregnant women, it is possible to verify the negative impact on reproductive functions and their fetuses. Then, there is a need to relate the maternal reproductive changes and the fetal outcomes involving a maternal HFD from weaning to the end of pregnancy. Herein, we hypothesized that rats fed HFD from weaning to the entire pregnancy negatively influenced embryo implantation and live fetus numbers and their offspring growth. This study aimed to relate maternal high-fat diet (HFD) consumption to maternal reproductive outcomes and offspring body growth.

**MATERIALS AND METHODS**

**Animals**

Female and male Sprague-Dawley rats were acquired from the Animal Facility of the State University of Campinas (CEMIB_UNICAMP) and kept in the vivarium of our Institution under controlled temperature conditions (22±2ºC), humidity (60±10%), and light/dark cycle (12 h). Filtered water and feed were offered ad libitum. For environmental enrichment, paper balls were used in animal cages (Simpson & Kelly 2011). National Council for the Control of Animal Experimentation (CONCEA) by the Ethics Committee for the Use of Animals (CEUA) of the Botucatu Medical School, UNESP, authorized all procedures and handling of animals performed by the guidelines provided by (Protocol Number 1875-2017).

**Treatment with a standard or high-fat diet**

The standard diet has 28.54% kcal of protein, 62.65% kcal of carbohydrate, and 8.7% kcal of fat (commercial feed for rats by Purina®, Brazil), and the HFD is composed of 23.43% kcal of protein, 46.63% kcal of carbohydrates, 30% kcal of fat (Paula et al. 2022a, Barco et al. 2022). The rats received a standard diet (SD) or a high-fat diet (HFD) according to the experimental group from the weaning up to the end of pregnancy. The HFD was prepared in our Institution. To prepare 10 kg of feed, the amounts described in Table I were used. The ingredients were ground, mixed, and offered as pellets. After preparation, the feed was refrigerated until the moment of consumption.
Experimental groups

Considering the two experimental groups and based on previous experiments performed in our laboratory with 90% power and 95% reliability, the sample size was seven rats per group. Immediately after weaning (approximately on day 30 of age), the rats were distributed into two experimental groups: SD-rats that received a standard diet from weaning to the end of pregnancy, and HFD-rats that received a high-fat diet (HFD) from weaning until the end of pregnancy.

Mating period and pregnancy

On day 120 of age, the rats were distributed three by three in polypropylene cages containing wood shavings in the presence of a male rat. The mating procedure had a maximum duration of 15 days for each animal, corresponding to at least three estrous cycles. On the following days, vaginal smears were performed, and in the presence of sperm, this was designated as day 0 of pregnancy (Damasceno et al. 2011). During pregnancy, the rats were kept in individual cages.

At-term pregnancy for sample collection

On day 21 of pregnancy, the rats SD and HFD were anesthetized with sodium thiopental (Thiopentax® - dose of 120 mg/kg of body weight) by intraperitoneal route (Faria-Neto & Santos 2008). Subsequently, these rats were killed by decapitation, and a laparotomy was performed. Visceral, periovarian, and periuterine adipose tissues were collected and weighed. Then, the uterine horns were exposed to count the number of implantations and live fetuses. The fetuses and their respective placentas were removed and weighed, as well as the litter. The placental weights were also used to calculate placental efficiency (fetal weight/placental weight) (Volpato et al. 2015). In addition, the fetuses were collected, and the classification of the fetal body weights according to the weights of the fetuses in the control group into small (SGA), adequate (AGA), or large (LGA) for gestational age (Souza et al. 2023).

Statistical analysis

The correlation of the data was analyzed by Pearson’s correlation Test, considering a minimum confidence limit of 95% (p<0.05).

RESULTS

The correlation analyses between relative visceral fat weight and the number of alive fetuses, and the weight of the same fat versus other variables, such as the number of embryo implantations, litter weight, and fetal weight, were performed, and no significant correlation was verified.

Periuterine fat versus maternal reproductive performance and fetal weight classification

Figure 1 shows the correlation between the relative weight of periuterine fat and maternal reproductive performance data. Periuterine fat

| Table I. High-fat diet ingredients for the preparation of 10 kg of feed. |
|---|---|
| ingredients | Amount for 10 kg of feed |
| Soybean meal | 3.443 kg |
| Sorghum | 0.800 kg |
| Soybean peel | 1.167 kg |
| Corn starch | 0.200 kg |
| Refined sugar | 1.500 kg |
| Lard | 1.400 kg |
| Iron | 50 mg/kg |
| Selenium | 0.05 mg/kg |
| Biotin | 0.05 mg/kg |
| Vitamin B12 | 20 μg/kg |
| Vitamin B6 | 7 mg/kg |
| Vitamin D3 | 1 800 IU/kg |
| Vitamin A | 12 000 IU/kg |
| Salt | 0.040 kg |

Legend: Kg = kilograms; mg = milligrams; μg = micrograms; IU = international unit.
weight showed a significant negative correlation with the number of live fetuses and litter weight. There was no significant correlation between the relative weight of this fat with the number of implantations and fetal weight classification (SGA, AGA, or LGA).

Periovarian fat versus maternal reproductive performance
The correlation analysis between the relative weight of periovarian fat and the data on maternal reproductive performance showed a significant negative correlation (Figure 2).

Periovarian fat versus classification of fetal body weight
Figure 3 shows the correlation analyses between the relative weight of periovarian fat and the classification of fetal body weight. The relative weight of periovarian fat was negatively correlated with AGA and LGA fetuses. There was no significant correlation between the evaluated variables regarding fetuses classified as SGA.

DISCUSSION
The study aimed to study the maternal lifestyle based on high-fat diets from birth to pregnancy. For this, the relationship between the data related to the relative weights of periuterine and periovarian fats with reproductive health and offspring growth was analyzed. A more significant amount of adipose tissue accumulated near the uterus and ovaries and a decreased number of alive fetuses, which caused a reduction in litter weight. In addition, it was verified that the increased relative weight of periovarian fat reduced the number of embryo implantations. This fact led to a lower number of live fetuses and a higher percentage of fetuses classified as small for gestational age (SGA). These findings contributed to the reduced litter weight. According to Sinzato et al. (2022), the rats that consumed the high-fat diet (HFD) had lower maternal weight gain (g) (Control SD= 126.3 ± 31.3g; HFD= 82.1 ± 24.5g), higher relative fat weight (g/100 g of body weight) (Control SD= 2.49 ± 0.58 g/100g; HFD= 4.14 ± 1.02g/100g), lower litter weight (g) (Control SD= 99.6 ± 12.8g; HFD= 79.0 ± 16.3g), and lower placental efficiency (Control SD= 10.74 ± 1.43; HFD= 9.89 ± 1.57), when compared with the rats that consumed standard diet (SD). These findings corroborate our results, which proved that a high-fat diet intake increased the weight of fat, both periuterine (Control SD= 0.469 ± 0.135g/100g; HFD= 0.876 ± 0.276g/100g) and periovarian (Control SD= 0.470 ± 0.218g/100g; HFD= 0.761 ± 0.242g/100g), which harmed the weight of the litter and maternal performance.

Periovarian fat in rodents plays an essential role in the secretion and release of reproductive
hormones and folliculogenesis (Li et al. 2015, Yang et al. 2018). Normal levels of white adipose tissue are essential to maintain the integrity of the hypothalamic-pituitary-gonadal axis. The increase in adipose tissue can interfere with the secretion of hormones [follicle stimulating hormone (FSH), luteinizing hormone (LH), and leptin] (Wang et al. 2017), which impairs the implantation process, in addition to harming the development of the ovary to produce healthy oocytes that will be fertilized (Bermejo-Alvarez et al. 2012), which confirms the decreased number of embryo implantation and alive fetuses when there was an increased relative weight of periovarian fat, as found in this study. These findings may be justified due to the local increase in inflammation indicated by the increased macrophage infiltration in the ovaries of HFD-fed rats (Skaznik-Wikiel et al. 2016). Ovarian macrophages can regulate cell proliferation, apoptosis (Benyo & Pate 1992), inflammation, and steroidogenesis through the secretion of cytokines in the ovary (Wu 2004). However, dysregulation of hormonal number and function can negatively affect ovarian function, which implies the appearance of diseases such as Polycystic Ovary Syndrome (PCOS) (Qiao & Feng 2011) and endometriosis (Carlberg et al. 2000).

In addition, the expansion of adipose tissue by hyperplasia and/or hypertrophy causes adipocytes to secrete high levels of inflammatory cytokines, which are associated with the higher generation of reactive oxygen species (ROS) and can impair fertility (Furat Rencber et al. 2018) that prevents embryo implantation and leads to a lower number of alive fetuses, as evidenced in our study. Thus, the correlation analysis showed that, regardless of the increased relative weight of periovarian or periuterine fat, the increase in total fat weight negatively influenced maternal reproductive performance, confirmed by the lower number of implantations and alive fetuses, causing lower litter weight.

The increase in the relative weight of periovarian fat is related to the decreased percentage of adequate for gestational age (AGA) and large for gestational age (LGA) fetuses. Several factors are involved in fetal growth, one of which is the ability of the placenta to maintain an adequate supply, verified by the ratio between the fetal body weight at birth and the placental weight (BWPW-ratio) (Wilson & Ford 2001). This relationship is described in the literature as “placental efficiency.” Rodent models have shown that maternal HFD ingestion has variable effects on offspring growth. Some studies showed no effect (Caluwaerts et al. 2007, Férezou-Viala et al. 2007, Shankar et al. 2008),

Figure 2. Correlation analyses between the relative weight of periovarian fat and data on maternal reproductive performance of rats given or not a high-fat diet (HFD) from weaning to the end of pregnancy. 2a- Number of alive fetuses, 2b- Embryo implantation sites, and 2c- Litter weight.

p<0.05- statistically significant (Pearson’s correlation test).
while others presented an increased fetal birth weight (Samuelsson et al. 2008) or fetuses with growth restriction (Taylor et al. 2003, Cerf et al. 2005). As evidenced in the present study, maternal HFD consumption caused a decreased percentage of AGA fetuses and an increased percentage of SGA fetuses, as seen in another laboratory study (Sinzato et al. 2022). The increased percentage of SGA fetuses due to intrauterine growth restriction may be related to functional or morphological placental changes, contributing to decreased fetal weight (Araujo-Silva et al. 2021). Due to intrauterine growth restriction and the increased number of SGA fetuses, there was a reduced litter weight.

We identified two limitations of the study: The non-dosage of proinflammatory cytokines in blood, ovary, and uterus samples at different ages of rats because we might be related to HFD-induced effects found in these rats, such as abnormal fat weights. The dosage of cytokines would be essential to relate to maternal data to understand intrauterine changes that impair embryofetal development and growth.

This study corroborates other studies that currently only work with periovarian fat weight. Studies are scarce comparing the relative weights of periovarian and periuterine fats with maternal reproduction and fetal growth. In conclusion, consuming a high-fat diet caused an increase in periovarian and periuterine fats, negatively influencing maternal reproductive performance and fetal development.

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MEPG - Analysis and interpretation; manuscript writing; LMID - Manuscript writing; VGP - Conception and design; data acquisition, analysis, and interpretation; manuscript writing; MRS - interpretation, manuscript writing; YKS - Data acquisition and analysis; FOQ - Data analysis and interpretation; VSB - Data analysis and interpretation; GTV - Manuscript writing; DCD - Conception and design; data acquisition, analysis, and interpretation; manuscript writing.