

## Original Article

**Cite this article:** Busnardo de Oliveira F, Fortunato Silva J, Prado HS, Ferreira-Neto ML, and Balbi APC. (2023) Maternal high-fat diet consumption during pregnancy and lactation predisposes offspring to renal and metabolic injury later in life: comparative study of diets with different lipid contents. *Journal of Developmental Origins of Health and Disease* **14**: 33–41. doi: [10.1017/S2040174422000241](https://doi.org/10.1017/S2040174422000241)

Received: 12 July 2021

Revised: 8 January 2022

Accepted: 30 March 2022

First published online: 28 April 2022


### Keywords:

High-fat diet; renal function; offspring; Wistar rats

### Address for correspondence:

Ana Paula C. Balbi, Avenida Pará, 1720, Campus Umarama, Bloco 2A, CEP: 38400-902, Uberlândia, Minas Gerais, Brazil.  
Emails: [paulabalb@ufu.br](mailto:paulabalb@ufu.br); [paulabalb@yahoo.com.br](mailto:paulabalb@yahoo.com.br)

# Maternal high-fat diet consumption during pregnancy and lactation predisposes offspring to renal and metabolic injury later in life: comparative study of diets with different lipid contents

Fernanda Busnardo de Oliveira<sup>1</sup>, Jéssica Fortunato Silva<sup>1</sup>,  
Helena Severino do Prado<sup>2</sup>, Marcos Luiz Ferreira-Neto<sup>3</sup> and  
Ana Paula Coelho Balbi<sup>3</sup> 

<sup>1</sup>Applied Structural and Cellular Biology, Federal University of Uberlândia, Uberlândia, Brazil; <sup>2</sup>Federal University of Uberlândia, Uberlândia, Brazil and <sup>3</sup>Department of Physiology, Federal University of Uberlândia, Uberlândia, Brazil

## Abstract

Accumulating evidence suggests that maternal overnutrition can result in a higher development risk of obesity and renal disease in the offspring's adulthood. The present study tested different lipid levels in the maternal diet during pregnancy and lactation and its repercussions on the offspring of Wistar rats. Offspring of 1, 7, 30 and 90-d-old were divided into the following groups: Control (CNT) – offspring of dams that consumed a standard chow diet (3.5% of lipids); Experimental 1 (EXP1) – offspring of dams exposed to a high-fat diet (HFD) (28% of lipids); and Experimental 2 (EXP2) – offspring of dams exposed to a HFD (40% of lipids). Regarding maternal data, there was a decrease in the amount of diet ingested by EXP2. Daily caloric intake was higher in EXP1, while protein and carbohydrate intakes were lower in EXP2. While lipid intake was higher in the experimental groups, EXP1 consumed more lipids than EXP2, despite the body weight gain being higher in EXP2. Adult offspring from EXP1 presented higher blood glucose. Regarding morphometric analysis, in both experimental groups, there was an increase in the glomerular tuft and renal corpuscle areas, but an increase in the capsular space area only in EXP1. There was a decrease in the glomerular filtration rate (GFR) in EXP1, in contrast to an increase in GFR of EXP2, along with an increase in urinary protein excretion. In conclusion, the maternal HFDs caused significant kidney damage in offspring, but had different repercussions on the type and magnitude of recorded change.

## Introduction

Fetal exposure to a hostile intrauterine environment can lead to permanent structural and/or functional damage in tissues and organs. Emerging evidence suggests that significant alterations in maternal nutrition may cause severe injuries in fetal development.<sup>1</sup> Additionally, several studies have shown that high maternal caloric intake is one of the main factors that predispose to child obesity and metabolic syndrome.<sup>2</sup> According to a recent report of the World Health Organization,<sup>3</sup> the global prevalence of obesity among children and adolescents aged 5–19 years increased from 0.8% in 1975 to 6.8% in 2016. Other complications may also affect the offspring in adult life, such as altered glucose homeostasis, hypertension, dyslipidaemia, altered vascular endothelial function and impairment of renal function.<sup>4–6</sup>

Several animal models have been developed with the aim of studying obesity and metabolic syndrome. Studies indicate that prolonged treatment of rodents with a high-fat diet (HFD) can lead to an increase of 10%–20% in body weight of these animals, when compared to control groups,<sup>6–9</sup> and the lipid contents of these diets vary within the range of 30%–78%.<sup>6,10</sup> Curiously, the metabolic responses of these animals submitted to HFDs containing different amounts of lipids varies considerably,<sup>11–14</sup> including those that are fed with HFD during pregnancy.<sup>15</sup>

Evidences from human and animal studies shows that maternal obesity increases the risk of chronic kidney disease in the offspring.<sup>16–19</sup> Furthermore, a sudden weight gain in experimental models may result in glomerular hyperfiltration in dams and in the offspring.<sup>20,21</sup> In the long term, this problem can lead to a significant loss in the number of nephrons and a reduction of renal function.<sup>22</sup> Thus, the aim of this study was to investigate the renal and metabolic effects of a maternal diet with different lipid contents for the offspring of Wistar rats.

## Material and methods

Male (350 g) and female (>180 g) Wistar rats (90–120-d-old) were housed separated by sex in cages under standard conditions with a 12 h light/dark cycle, controlled temperature ( $22 \pm 2$  °C) and free access to water and standard show diet. The female rats were put in the same cage as males overnight for mating. Pregnancy was confirmed by the presence of sperm in the vaginal smear. Once pregnant, the females were housed in individual cages and allowed free access to water, standard chow diet or HFD (28% or 40% of lipids) during gestational and lactation periods. After birth, the female offspring of 1, 7, 30 and 90-d-old were divided into the following groups:

- 1) Control (CNT) – offspring of dams ( $n = 7$ ) that consumed a standard chow diet (3.5% of lipids) and water *ad libitum*;
- 2) Experimental 1 (EXP1) – offspring of dams ( $n = 6$ ) that were exposed to a HFD (28% of lipids) and water *ad libitum*;
- 3) Experimental 2 (EXP2) – offspring of dams ( $n = 9$ ) that were exposed to a HFD (40% of lipids) and water *ad libitum*.

During treatment, maternal parameters were evaluated such as food intake, daily caloric intake, estimated macronutrient intake (carbohydrates, proteins and lipids), weight gain, number of pups per litter and blood glucose. Regarding food intake, the chows were weighted over five consecutive days at the same hour. The difference between the amount of chow consumed in the first and second measure were considered as the daily dietary consumption. The body weight variation was obtained by the difference between the measures of the initial (day 1 of pregnancy) and final weight (day 20 of pregnancy). The blood glucose was verified with a glucometer (Accu-Check® Active, Santo André, SP, Brazil) by a blood sample collected from the animal tail on gestational day 20. After weaning (21-d-old), the offspring were allowed free access to water and standard chow diet. Offspring of 1, 7 and 30-d-old were submitted to evaluations such as body weight, and renal weight–body weight ratio. Before surgery for kidney removal, the adult offspring (90-d-old) were submitted to functional experiments such as blood glucose, total cholesterol (TC), triglycerides (TGL), systolic blood pressure (SBP), renal function evaluation and morphometric analysis.

### Dietary composition

The centesimal composition of the three diets and its total energy content are shown in Table 1. The HFD of the EXP1 dams consisted of the addition of hypercaloric food to 48 g of a standard chow diet in the following proportions: 12 g of roasted peanuts, 4 g of milk chocolate, 2 g of corn starch and 17 g of lard.<sup>23</sup> All of these ingredients were mixed and offered to the animals as pellets. The HFD of the EXP2 dams consisted of the addition of 18 g of roasted peanuts, 5 g of milk chocolate, 3 g of corn starch and 26 g of lard to 64.65 g of a standard chow diet.<sup>24</sup>

### Surgery and histology

The 1, 7, 30 and 90-d-old offspring of Control (1D = 10, 7D = 8, 30D = 11 and 90D = 11), Experimental 1 (1D = 6, 7D = 7, 30D = 7 and 90D = 8) and Experimental 2 (1D = 6, 7D = 12, 30D = 9 and 90D = 14) groups were anaesthetised with Halothane (Cristália, Produtos Químicos e Farmacêuticos Ltda; Itapira, SP, Brazil) and had their kidneys carefully removed and weighted. After surgery, the animals were euthanised with an anaesthesia overdose (Thiopental 2.5%, 150 mg/kg). Kidneys were placed in methacarn fixative solution for 24 h, followed by a 70%

**Table 1.** Diet composition of Control, Experimental 1 and Experimental 2 groups

|                     | Control<br>( $n = 7$ ) | Experimental 1<br>( $n = 6$ ) | Experimental 2<br>( $n = 9$ ) |
|---------------------|------------------------|-------------------------------|-------------------------------|
| Protein (%)         | 19                     | 15.5                          | 14                            |
| Carbohydrates (%)   | 56                     | 42                            | 35                            |
| Lipids (%)          | 3.5                    | 28                            | 40                            |
| Total energy (kJ/g) | 17.03                  | 25.18                         | 34.35                         |

Dietary composition of the Control (standard chow diet), Experimental 1 (high-fat diet – 28%) and Experimental 2 (high-fat diet – 40%) diets. Protein, carbohydrates and lipids content in percentage and total energy in kJ/g.

alcohol solution, until its paraffin inclusion. After obtaining the paraffin blocks, they were cut into 5  $\mu$ m thick sections using a microtome. Then, the sections were extended on glass slides, previously gelatinised and stored at 60 °C for 24 h to remove the excess of paraffin.

### Morphometric analysis

The kidney tissue sections were stained with Toluidine Blue and the images were obtained by a scanning optical microscope (Leica Biosystems, Wetzlar, Hessen, Germany) and evaluated using the s HL Image++ 97 software (Western Vision Software, Salt Lake City, Utah, USA). Twenty renal corpuscles were selected for analysis of the following parameters: renal corpuscle, glomerular tuft and capsular space areas of Control ( $n = 11$ ), Experimental 1 ( $n = 8$ ) and Experimental 2 ( $n = 14$ ) groups.

### Renal function evaluation: glomerular filtration rate (GFR) and urinary protein excretion (UPE)

These experiments were performed on the 90-d-old pups of Control ( $n = 11$ ), Experimental 1 ( $n = 8$ ) and Experimental 2 ( $n = 14$ ) groups. The analysis of GFR was performed by Creatinine Clearance, an enzyme-colorimetric method (Labtest Diagnóstica Ltda, Lagoa Santa, Minas Gerais, Brazil). The pups were housed in metabolic cages for 24 h for adaptation. Then, their urine samples were collected for the 24 h following the adaptation period. A tail blood sample was also collected for plasma creatinine evaluation. The UPE was obtained by a colorimetric method using pyrogallol red (Labtest Diagnóstica Ltda, Lagoa Santa, Minas Gerais, Brazil). Creatinine concentrations and UPE were measured spectrophotometrically through the specified protocol absorbances, as previously described by Roza *et al.*<sup>25</sup>

### TC and TGL

These experiments were performed on the 90-d-old pups of Control ( $n = 11$ ), Experimental 1 ( $n = 8$ ) and Experimental 2 ( $n = 14$ ) groups and were determined by the enzymatic-Trinder method (Labtest Diagnóstica Ltda, Lagoa Santa, Minas Gerais, Brazil). The concentrations were measured spectrophotometrically through the specified protocol absorbances, as previously described by Tanko *et al.*<sup>26</sup>

### SBP

The SBP was determined in 90-d-old pups of Control ( $n = 11$ ), Experimental 1 ( $n = 8$ ) and Experimental 2 ( $n = 14$ ) groups and performed by plethysmography. The signals were detected with

a pneumatic pulse transducer/amplifier coupled to the PowerLab software (ADInstruments, Dunedin, New Zealand) for amplification and converted by the LabChart 7 Pro software (ADInstruments, Dunedin, New Zealand) as previously described by Roza et al.<sup>25</sup> The values were obtained in mmHg. The measurements were performed for five consecutive days at the same hour, and the arithmetic mean of the values per day were considered. After the last day, a new arithmetic mean was obtained and this value was considered as definitive.

### Statistical analysis

The statistical analysis was performed using the GraphPad Prism software (version 5.00). First, it was verified whether the data presented a normal distribution or not using the Kolmogorov–Smirnov test. For data with a normal distribution, the ANOVA with a Newman–Keuls' post-test was used as a parametric test. For those that did not present this distribution pattern, a non-parametric test was used (Kruskal–Wallis with Dunn's post-test). Data are expressed as mean  $\pm$  SEM. The significance level was  $p < 0.05$ .

## Results

### Maternal outcomes

There was no significant difference in food intake between the CNT and EXP1 groups. However, EXP2 consumed less chow when compared to the other groups. Regarding daily caloric intake, we observed that EXP1 consumed more calories than the other groups. When we analysed the estimated consumption of macronutrients (proteins, carbohydrates and lipids), we observed a lower ingestion of carbohydrates and proteins in EXP2 when compared to the others. Both experimental groups ingested more lipids when compared to the CNT, moreover EXP1 consumed more lipids than EXP2. However, EXP2 showed a higher weight gain in comparison to EXP1. There was no significant difference in blood glucose levels and number of pups per litter among the studied groups (Table 2).

### Offspring outcomes

Offspring body weight and renal weight–body weight ratio were evaluated in the pups of all ages. Regarding offspring body weight, we observed weight gain in all groups, as the offspring were developing. However, when comparing groups of the same age, the 30-d-old pups of both experimental groups had a lower body weight in comparison to the CNT group (Fig. 1a), although there was no significant difference in renal weight–body weight ratio (Fig. 2a).

We observed an increase of blood glucose levels in EXP1 in comparison to the CNT group (Fig. 1b). However, we observed no significant difference in TC and TGL among the studied groups (Fig. 1c, d).

There was a decrease of GFR in EXP1 in comparison to the CNT group. In contrast, we observed an increase of GFR in EXP2, when compared to the CNT and EXP1 groups (Fig. 2b). In addition, UPE was also higher in EXP2, in comparison to the other groups (Fig. 2c). EXP1 showed an increase of SBP in comparison to the other groups (Fig. 2d).

Regarding morphometric studies, we observed an increase of renal corpuscle and glomerular tuft areas in both experimental groups in comparison to the CNT group (Fig. 3a, b). However, there was an increase in capsular space area only in EXP1, in comparison to the CNT group (Fig. 3c).

## Discussion

Recent studies have demonstrated that high maternal caloric intake can lead to important alterations in the intrauterine environment. This factor may cause negative impacts on fetal development as well as an increase in the risk of obesity in the offspring later in life.<sup>27,28</sup> The aim of this study was to study the impacts of maternal HFDs with different lipid contents (28% and 40%) during pregnancy and lactation on the female offspring. Recently, Ribaroff et al.<sup>29</sup> (2017) conducted a meta-analysis and meta-regression study including several experimental models of maternal HFD and its metabolic effects on female and male offspring. Some differences in sexual dimorphism were found in some metabolic parameters, such as blood glucose and insulin levels. Female offspring have shown hyperglycaemia, in contrast of hyperinsulinemia in the absence of hyperglycaemia in male offspring. Some hypotheses have been raised for these differences, such as the fact that males and females may respond differently to maternal hormones that cross the placental barrier. However, the authors highlight the importance of studies that assess the effects of maternal HFD in both male and female offspring, but also in separated sexes. Additionally, Hariri et al.<sup>10</sup> (2010), observed that male laboratory rats have constant weight gain until their last days of life, while female rats maintain constant body weight after reaching adulthood. As a result, female rats are a better choice for obesity models, since their weight gain in adulthood is similar to what is observed in humans. Based on this discussion, our study focused on the renal and metabolic consequences of the female offspring of dams treated with HFD.

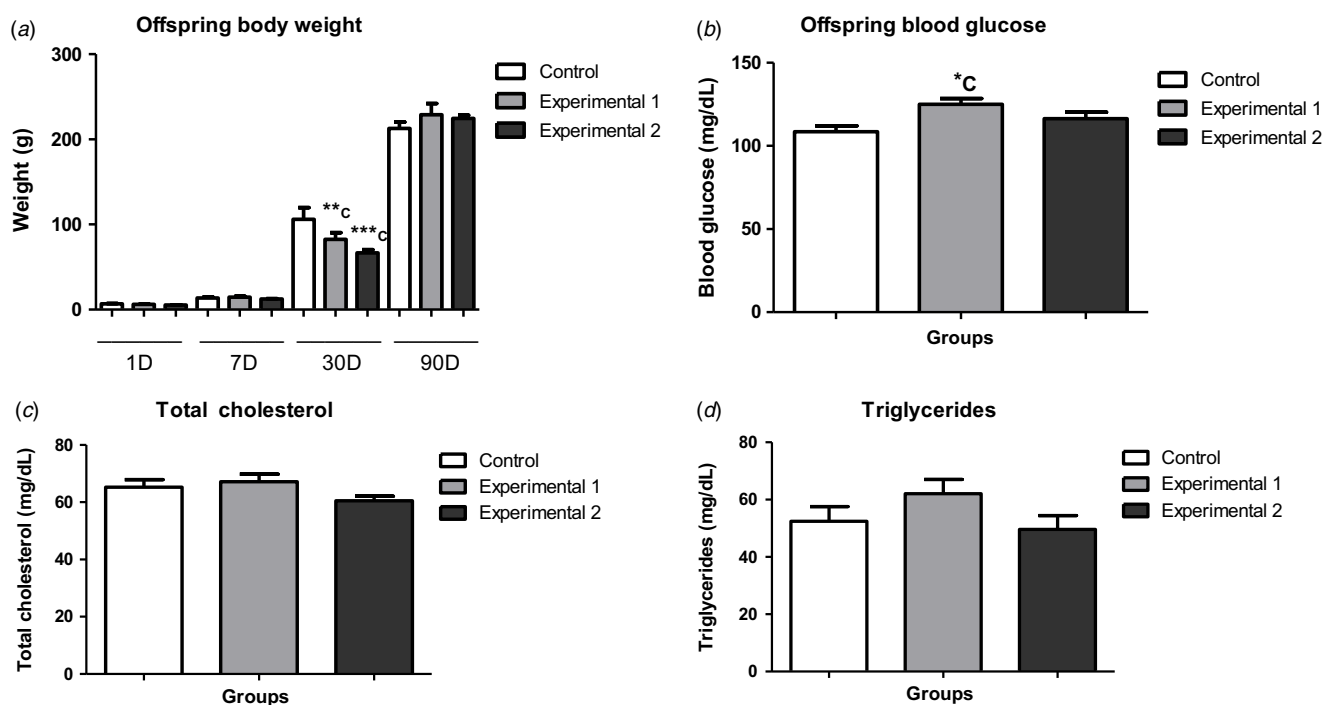
The literature shows many divergences in the parameters of body weight and daily chow intake in pregnant female rats treated with HFD. For example, Burgueño et al.<sup>30</sup> (2013), fed Wistar female rats with HFD (40% of lipids) for 15 d before mating and also during gestational and lactational period. There was no significant difference in body weight gain and daily food intake in dams, but important metabolic changes were observed on the offspring, such as insulin resistance and hepatic disorders. Another study, by Khan et al.<sup>31</sup> (2004), fed Sprague Dawley female rats with HFD (25.7%) during pregnancy and lactation, and they observed an increase in maternal weight gain despite of a decrease in daily dietary intake by the dams. In contrast with the maternal data observed by Burgueño<sup>30</sup> and Khan,<sup>31</sup> the present study observed a decrease in the amount of chow intake in dams from EXP2 and the daily caloric consumption was higher only in dams from EXP1, when compared to the other groups. Thus, to explain these results it is important to analyse the macronutrient consumption. The macronutrient consumption analysis showed a decrease of carbohydrate and protein intake in dams from EXP2 and an increase in lipid intake of this group, in comparison to the CNT group. Nevertheless, lipid intake of dams from EXP1 was higher than EXP2.

Carbohydrates, lipids and proteins are groups of macronutrients that play an essential role in the body's average daily energy intake. The dynamics of absorption of these components are very complex and depend on several factors. Any change in proportion of macronutrient intake can cause significant changes in weight gain, even if the energy content ingested remains constant.<sup>32,33</sup> Usually, an increase in lipid consumption results in a higher energy intake. However, high-fat and low-carbohydrate diets can reduce the appetite, leading to an increase of circulating ketonic bodies.<sup>34–37</sup> Additionally, studies in animals and humans suggests that homeostatic signals related to body weight, such as leptin, can

**Table 2.** Maternal food, daily caloric, carbohydrates, proteins and lipids intake, blood glucose, weight gain and number of pups per litter of Control, Experimental 1 and Experimental 2 groups

| Maternal parameters             | Control (n = 7) | Experimental 1 (n = 6)           | Experimental 2 (n = 9)           |
|---------------------------------|-----------------|----------------------------------|----------------------------------|
| Food intake (g)                 | 27.17 ± 0.85    | 32.76 ± 4.65                     | 15.90 ± 1.19 <sup>***E1</sup>    |
| Daily caloric intake (Kcal/day) | 110.50 ± 3.42   | 189.30 ± 26.89 <sup>**C*E2</sup> | 135.40 ± 9.66                    |
| Carbohydrates intake (g)        | 15.22 ± 0.47    | 13.79 ± 1.96                     | 5.39 ± 0.44 <sup>***C***E1</sup> |
| Proteins intake (g)             | 5.16 ± 0.16     | 5.07 ± 0.72                      | 2.10 ± 0.17 <sup>***C***E1</sup> |
| Lipids intake (g)               | 0.95 ± 0.03     | 9.08 ± 1.29 <sup>***C*E2</sup>   | 6.09 ± 0.50 <sup>***C</sup>      |
| Blood glucose (mg/dl)           | 69.50 ± 3.90    | 78.40 ± 2.66                     | 74.33 ± 5.30                     |
| Weight gain (g)                 | 93.00 ± 2.81    | 66.20 ± 7.96                     | 108.20 ± 12.98 <sup>E1</sup>     |
| Number of pups/litter           | 12.00 ± 0.87    | 10.17 ± 1.19                     | 12.22 ± 0.86                     |

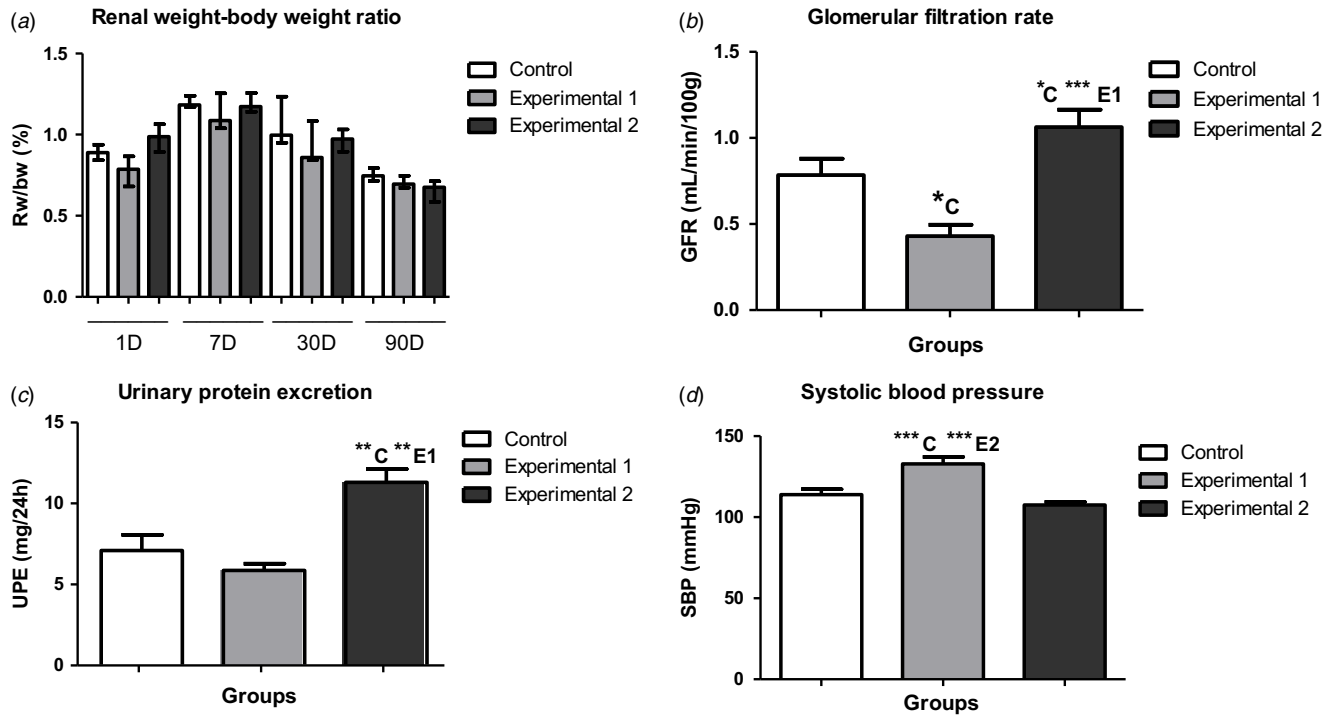
Maternal food, daily caloric, carbohydrates, proteins and lipids intake and blood glucose, weight gain and number of pups per litter: values are expressed as mean ± SEM (ANOVA with Newman-Keuls post-test). Significance level:  $p < 0.05$ . <sup>\*\*\*C</sup>:  $p < 0.001$  versus Control; <sup>\*\*C</sup>:  $p < 0.01$  versus Control; <sup>\*\*E2</sup>:  $p < 0.01$  versus Experimental 2; <sup>\*E2</sup>:  $p < 0.05$  versus Experimental 2; <sup>\*\*\*E1</sup>:  $p < 0.001$  versus Experimental 1; <sup>\*E1</sup>:  $p < 0.05$  versus Experimental 1.

**Figure 1.** Offspring body weight, blood glucose, total cholesterol and triglycerides of Control, Experimental 1 and Experimental 2 groups. Offspring body weight (a), blood glucose (b), total cholesterol (c) and triglycerides (d): values are expressed as mean ± SEM (ANOVA with Newman-Keuls pPost-tTest). Significance level:  $p < 0.05$ . <sup>\*\*\*C</sup>:  $p < 0.001$  versus Control 30D; <sup>\*\*C</sup>:  $p < 0.01$  versus Control 30D; <sup>\*C</sup>:  $p < 0.05$  versus Control. Total number of animals: Control (1D = 10, 7D = 8, 30D = 11 and 90D = 11), Experimental 1 (1D = 6, 7D = 7, 30D = 7 and 90D = 8) and Experimental 2 (1D = 6, 7D = 12, 30D = 9 and 90D = 14) groups.

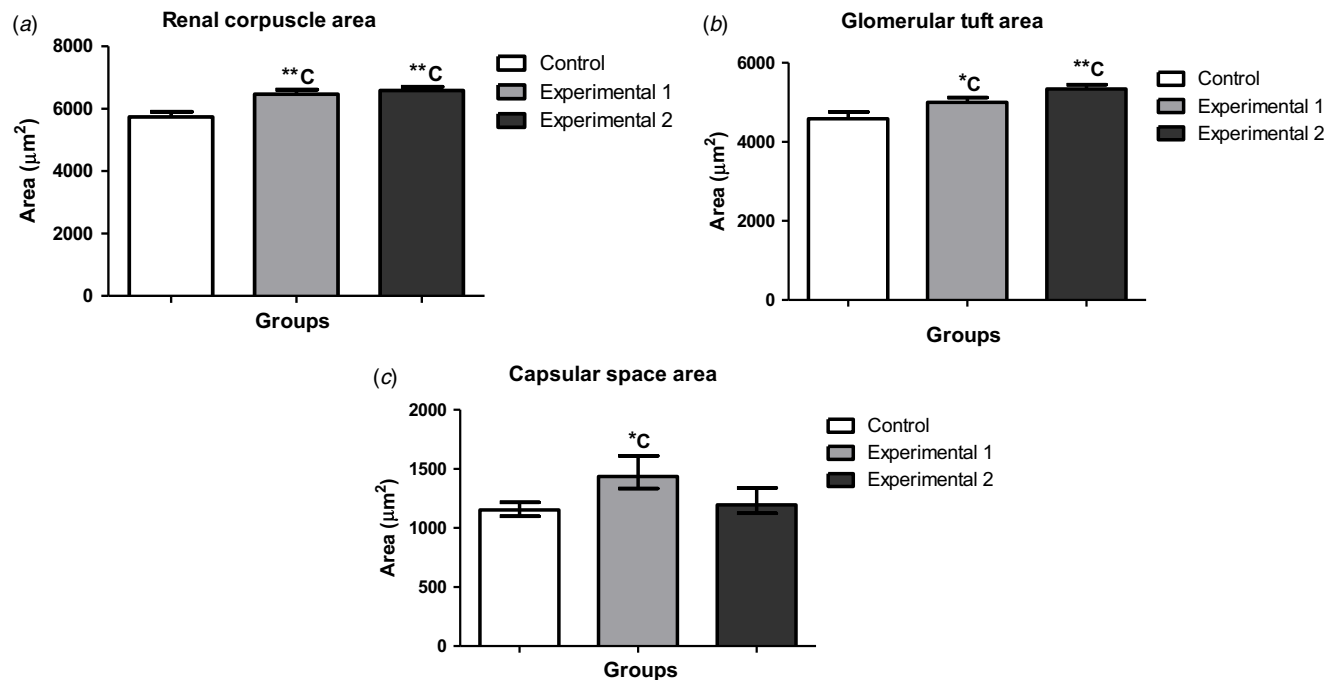
be also involved in appetite.<sup>10,38-40</sup> Studies indicate that obesity or high caloric intake can cause important changes in leptin levels.<sup>41,42</sup> Nevertheless, this mechanism by which this works remains unknown. In this study, dams from EXP2 may have reduced their consumption ratio because this diet (40% of lipids) presented a greater energetic content. Additionally, the reduction of diet intake led to a lower ingestion of carbohydrates and proteins in these animals. Furthermore, the hypothesis of a maternal imbalance in leptin production and/or secretion in EXP2 cannot be ruled out. In 2019, Schellong *et al.*<sup>43</sup> used a model of Wistar rats treated with HFD (30% lipids) for 6 weeks before pregnancy, and also during pregnancy and lactation. There was an increase in maternal serum leptin levels in comparison to control group. However, the

maternal hyperleptinemia was accompanied by an increase in body weight gain in these rats, which was not observed in this present study. Possibly, the HFD treatment period used in this study was not sufficient to observe an increase in body weight gain in EXP1. Furthermore, the amount of chow ingested by EXP2 was lower than EXP1.

In rodent models, obesity can be measured using some criteria. The most common parameters used in the literature are: (1) Lee's index; and (2) an increase in the amount of adipose tissue (adiposity).<sup>10</sup> The first parameter can be evaluated by the calculation performed using a formula standardised by Lee<sup>44</sup> in 1929. The formula consists on the cube root of body weight (g) divided by the naso-anal length (cm) and multiplied by 1000. According to Lee,<sup>44</sup> values



**Figure 2.** Offspring renal weight–body weight ratio, glomerular filtration rate, urinary protein excretion and systolic blood pressure of Control, Experimental 1 and Experimental 2 groups. Renal weight–body weight ratio (a): values are expressed as or median with percentiles 25 and 75. Glomerular filtration rate (b), Urinary protein excretion (c) and Systolic blood pressure (d): values are expressed as mean ± SEM (ANOVA with Newman–Keuls post-test). Significance level:  $p < 0.05$ . \*\*\*C:  $p < 0.001$  versus Control; \*\*C:  $p < 0.01$  versus Control; \*C:  $p < 0.05$  versus Control; \*\*\*E2:  $p < 0.001$  versus Experimental 2; \*\*\*E1:  $p < 0.001$  versus Experimental 1; \*\*E1:  $p < 0.01$  versus Experimental 1. Total number of animals: Control (1D = 10, 7D = 8, 30D = 11 and 90D = 11), Experimental 1 (1D = 6, 7D = 7, 30D = 7 and 90D = 8) and Experimental 2 (1D = 6, 7D = 12, 30D = 9 and 90D = 14) groups.



**Figure 3.** Morphometrical analyses: renal corpuscle, glomerular tuft and capsular space areas of Control, Experimental 1 and Experimental 2 groups. Renal corpuscle area (a) and glomerular tuft area (b): values are expressed as mean ± SEM (ANOVA with Newman–Keuls post-test). Significance level:  $p < 0.05$ . Capsular space area (c): values are expressed as median with percentiles 25 and 75 (Kruskal–Wallis with Dunn post-test). Significance level:  $p < 0.05$ . \*\*C:  $p < 0.01$  versus Control; \*C:  $p < 0.05$  versus Control. Total number of animals: Control (90D = 11), Experimental 1 (90D = 8) and Experimental 2 (90D = 14) groups.

greater than 310 are an indicator of obesity. The second parameter usually consists of the surgical removal of adipose tissue from some parts of the rodent's body, such as the retroperitoneal cavity, and its posterior evaluation of weight (g).<sup>10,44</sup> However, in this present study, although we recorded the variation in maternal and offspring's body weight, obesity was not assessed, since the exposure time of dams to HFD was only 42 d, that is, only in pregnancy and lactation.

In order to discard the hypothesis that the number of pups per litter may have influenced in the maternal body weight gain, we investigated this parameter in all experimental groups. Thus, there was no significant difference in this parameter.

Evidence shows that obesity is associated with an increase of energetic intake. When the amount of diet ingested is relatively low and even then, there is an increase in body weight, the energy density of the diet must be considered.<sup>10</sup> In this study, the dams from EXP2 had a low energetic consumption and a low dietary intake per day. However, the body weight increased significantly. Our hypothesis is that the animals learned how to compensate for the high energy density consumption by eating a lesser amount of chow. On the other hand, weight gain can also be associated with epigenetic changes in these rats during treatment. Several studies report that animal models exposed to a HFD can become obese easily, but in other cases, some animals may become resistant to weight gain.<sup>10,45,46</sup>

The literature suggests that epigenetic mechanisms are directly related to an individual's lifestyle, and epigenetic changes can occur at any stage of life.<sup>45,47</sup> Furthermore, some animals have a greater capacity to store energy or their fatty acid oxidation metabolism can be reduced.<sup>10,46–49</sup>

We observed no significant difference among the studied groups in blood glucose levels. Roza *et al.*<sup>25</sup> in 2006, evaluated the renal and metabolic impacts caused by the consumption of an HFD (60% of lipids) on non-pregnant Wistar rats for eight weeks. The study did not find an increase in body weight in the treated group and the consumption ratio was significantly lower in this group, when compared to the control. However, the treated rats were hyperglycaemic in comparison to the control group. Possibly, the longer treatment period applied by Roza *et al.*<sup>25</sup> associated with a higher lipid content in the diet may have influenced the blood glucose alteration, which was not observed in our study.

According to the literature, high maternal caloric intake is commonly associated with an increase in the offspring's body weight at birth and even in the first days of life. Similar data were found in animal models treated with a HFD, also in the gestational period.<sup>50–53</sup> On the other hand, there are studies reporting a decreased birth weight in offspring from HFD fed dams.<sup>54,55</sup> These variabilities of results may be related to the macronutrient composition of each HFD used in these studies, as well as the caloric content.<sup>56</sup> In addition, changes in hypothalamic appetite control and/or circulating leptin levels can also have an influence on these parameters.<sup>57</sup> In this study, the groups of offspring at different ages (1, 7, 30 and 90-d-old) had body weight gain during all the studied period. However, the 30-d-old pups of dams treated with a HFD (EXP1 and EXP2) presented a lower body weight when compared to the CNT group, despite the fact that maternal caloric intake was higher in dams from EXP1 and the maternal weight gain was higher in EXP2. Nevertheless, intrauterine overnutrition can reprogramme the offspring's metabolism, as well as its body weight gain.<sup>57</sup> Howie *et al.*<sup>56</sup> (2009) observed similar results in their study, where dams fed with a HFD (45% of lipids) during pregnancy and lactation gave birth to smaller offspring, when compared to the

control group. Other studies also observed a decrease in birth weight in this animal model,<sup>54,55,58</sup> while others observed no effects on the parameter.<sup>59–61</sup> However, these mechanisms remain unclear. In all of these cases, it would be important to assess, in addition to body weight, the adiposity of the rats. Recently, Chaves *et al.*<sup>62</sup> (2020) conducted a study with maternal HFD treatment and found no body weight gain on mothers, however, their adiposity was considerably increased. In addition, other factors should be investigated in order to clarify these results, such as milk leptin and lactational performance of the dams.

Regarding the glycaemic data of the adult offspring, there was an increase in blood glucose levels in EXP1, in comparison to the other groups. Similar data were found in Hokke's<sup>63</sup> study, in which females of C57B16 mice were treated with a HFD (21% of lipids) for six weeks before the gestational period and also during pregnancy and lactation. The offspring's body weight in all groups was very similar during the first days of life while the adult offspring showed a slight increase in their glycaemic levels, while body weight had no significant alteration when compared to the control group. The change in blood glucose showed by Hokke<sup>63</sup> and also in the present study may be related to fetal exposure to a hostile intra-uterine environment caused by high maternal caloric intake. The noted difference in body weight may be explained by the lipid contents of the diets or the species of the animal models used in these studies and their way of responding to an increase in lipid supply.

Obesity is associated with dyslipidaemia and alterations in glucose homeostasis.<sup>64</sup> Additionally, several studies indicate that diets containing high energy density can lead to obesity and its metabolic consequences.<sup>10,65–67</sup> In this study, we did not observe changes in plasma TC and TGL levels in the 90-d-old pups. In contrast, Bouanane *et al.*<sup>68</sup> observed important impacts on the lipid profile of 12-week-old adult offspring of Wistar rats treated with a HFD (42% of lipids) during pregnancy and lactation. The difference between Bouanane<sup>68</sup> and the present study can be explained by the type of diet used and/or its caloric content. Although the diets obtained similar lipid concentration, the HFD used by Bouanane<sup>68</sup> contained bacon, chocolate, cheese, among other lipid sources, resulting in total energy value of 420 kJ/100g. The HFD used in EXP1 and EXP2 had lower energy density values, 241,8 kJ/100 g and 343,5 kJ/100 g, respectively. Therefore, the total energy density of the diets may explain the differences observed on the offspring's lipid profile.

Evidence shows that obesity can cause intraglomerular haemodynamic changes, leading to an increase in renal plasma flow and GFR, which can also cause albuminuria.<sup>69–71</sup> In this study, EXP1 had a lower GFR than the CNT group. In contrast, EXP2 demonstrated higher values of this parameter in relation to the other groups, revealing a glomerular hyperfiltration case. This group also presented an elevated UPE, when compared to the CNT and EXP1 groups. Jackson *et al.*<sup>72</sup> evaluated metabolic and renal parameters in Sprague Dawley rat offspring treated with a HFD (45% of lipids) and fructose rich diet (0.1 g/ml) for six months prior to pregnancy and also during gestational and lactation periods. The adult pups treated with a standard chow diet after weaning developed albuminuria and glomerulosclerosis. However, these animals did not demonstrate significant changes in GFR. These results suggest that albuminuria and glomerulosclerosis cases found in these pups may be caused by renal haemodynamic alterations. Furthermore, with advancing age, changes in GFR could be found, as well as in the experimental groups of the present study. The glomerular hyperfiltration observed in EXP2 can be directly related to the increase in UPE and it could also be a result of the increase in glomerular tuft

area that was also observed in this group. However, the decrease in GFR in EXP1 may be associated with an increase in SBP observed in this study. It is worth mentioning that the dams from EXP2 ate lower protein and carbohydrate content in relation to the other groups. Given that maternal obesity may programme hypertension in the offspring, further investigation is necessary to ensure that the SBP alteration observed in this study was a predictor of the lower GFR noticed in EXP1. Evidence shows that chronic exposure to high blood pressure may cause structural changes to the kidney, which may lead to nephron loss and a reduction of renal plasma flow.<sup>5,73</sup> Additionally, it is important to consider that fetal exposure to maternal overnutrition *per se* can impair fetal kidney development, altering its structure and physiology. Black et al.<sup>74</sup> investigated renal consequences of hypertension in spontaneously hypertensive rats and observed no change in nephron number, despite the altered blood pressure of the animals. Therefore, these findings suggest that hypertension may not influence nephron number, which is an important predictor of changes in GFR and renal function.

According to the literature, animal models of low-protein diets have been using a percentage of 3.5%–10% of protein, while standard protein diets contain approximately 12%–20% of protein.<sup>75,76</sup> Despite the lower protein intake observed in dams from EXP2, the rats were not protein deficient, however, we cannot exclude the fact that a reduction in maternal protein intake might have adverse effects on offspring development. Accumulating evidence shows that low-protein maternal diets may have negative impacts on pups later in life, such as a decrease in nephron number, growth restriction, hypertension, increased fasting insulin and blood lipid levels.<sup>76–78</sup> Several studies have reported similar data in Wistar rats treated with low-protein diets containing about 6%–8% of protein in contrast with 17%–20% of this macronutrient in the normal-protein diets.<sup>79,80</sup> Nwagwu et al.<sup>81</sup> evaluated renal and metabolic effects in the offspring of Wistar rats treated with low-protein diet during pregnancy. The low-protein diet used in this study consisted of 9 g of casein/100 g of chow, and the standard diet consisted of 18 g of casein/100 g of chow. The four-week-old pups presented higher blood pressure, decreased GFR and increased UPE. In the present study, UPE was observed in adult pups from EXP2 group associated with an increased GFR. The results can be explained by the association of lower protein and carbohydrate content ingested by the dams of this group. Additionally, it shows that changes in macronutrients may result in severe kidney injury in the offspring.

Regarding the morphometric analysis, the following parameters were evaluated: renal corpuscle, glomerular tuft and capsular space areas. We found that both renal corpuscle and glomerular tuft areas were increased in the experimental groups. The capsular space area was only increased in EXP1, when compared to the others. Tobar et al.<sup>82</sup> evaluated morphological aspects of renal biopsies of non-diabetic obese patients with proteinuria and glomerular hyperfiltration. The study found an increase in capsular space volume in these individuals, when compared to a control group. These data, along with other studies, support a relationship between glomerulomegaly and glomerular hyperfiltration.<sup>83–85</sup>

Recent studies showed that maternal HFD treatment can cause cardiovascular problems in offspring, such as cardiac hypertrophy and hypertension.<sup>86,87</sup> One of the hypotheses that explain hypertension in these cases is the reduction in the number of nephrons in the offspring, which initiates during nephrogenesis, and can lead to a decrease in GFR later in life.<sup>21,88</sup> In the present study, EXP1 animals presented higher pressure values when compared to the

other groups, which agrees with the findings in the literature<sup>1,20,25,85,86</sup> and can be related to the reduction of GFR that was also observed in this group. In contrast, EXP2 showed no significant changes in SBP. In addition, its renal repercussions were different from those observed in EXP1. However, this shows us that maternal HFD treatment containing different lipid contents can cause renal and blood pressure changes in the offspring in different magnitudes.

## Conclusion

The maternal consumption of the HFD *per se*, during gestation and lactation, resulted in important renal and metabolic changes in the offspring of Wistar rats, such as increased glycaemia, changes in renal structure and function, and increased UPE and SBP. However, the lipid content (28% or 40%) had different repercussions on the type and/or magnitude of changes in the offspring.

**Acknowledgements.** We are grateful to the Federal University of Uberlândia (UFU), that allowed the development of this work and to REBIR-UFU (Rodent Bioterries Network from UFU) for supply and maintenance of the experimental animals.

**Financial Support.** This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Conflicts of Interest.** None.

**Ethical standards.** The authors assert that all procedures contributing to this work comply with the ethical standards of relevant national guides on the care and use of laboratory animals and has been approved by the institutional committee of Federal University of Uberlândia (057/14).

## References

1. Langle-Evans SC. Nutrition in early life and the programming of adult disease: a review. *J Hum Nutr Diet.* 2015; 28(Suppl 1), 1–14.
2. Parrino C, Vinciguerra F, La Spina N, et al. Influence of early life and parental factors on childhood overweight and obesity. *J Endocrinol Invest.* 2016; 39(11), 1315–1321.
3. Who (World Health Organization) Obesity and Overweight. Available <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (2022, accessed April 2022).
4. Armitage JA, Taylor PD, Poston L. Experimental models of fetal programming: consequences of exposure to an energy rich diet during development. *J Physiol.* 2005; 565(1), 3–8.
5. Glastras S, The NL, Wong MG, Saad S. Maternal obesity and offspring risk of chronic kidney disease. *Nephrology.* 2018; 23, 84–87.
6. Buettner R, Schölmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity.* 2007; 15(4), 15–808.
7. Peckham SC, Entenmann C, Carroll HW. The influence of a hypercaloric diet on gross body and adipose tissue composition in the rat. *J Nutr.* 1962; 77(2), 187–197.
8. Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. *Curr Opin Lipidol.* 2002; 13(2), 155–164.
9. Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O. High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism.* 1996; 45(12), 1539–1546.
10. Hariri M, Thibault L. High-fat diet-induced obesity in animal models. *Nutr Res Rev.* 2010; 23(2), 270–299.
11. Schemmel R, Mickelsen O, Tolgay Z. Dietary obesity in rats: influence of diet, weight, age, and sex on body composition. *Am J Physiol.* 1969; 216(2), 373–379.
12. Yaqoob P, Sherrington EJ, Jeffery NM, et al. Comparison of the effects of a range of dietary lipids upon serum and tissue lipid composition in the rat. *Int J Biochem Cell Biol.* 1995; 27(3), 297–310.

13. Smith BK, West DB, York DA. Carbohydrate versus fat intake: differing patterns of macronutrient selection in two inbred mouse strains. *Am J Physiology*. 1997; 272 (1 Pt 2), R357–R362.
14. Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. *Am J Physiol Endocrinol Metab*. 2002; 282(6), 1231–1238.
15. Kereliuk SM, Brawerman GM, Dolinsky VW. Maternal macronutrient consumption and the developmental origins of metabolic disease in the offspring. *Int J Mol Sci*. 2017; 18(7), 18.
16. Hsu C-N, Hou C-Y, Lee C-T, Chan JYH, Tain Y-L. The interplay between maternal and post-weaning high-fat diet and gut microbiota in the developmental programming of hypertension. *Nutrients*. 2019; 11(9), 1982.
17. Lee KK, Edwin AR, Lee AJ, *et al.* Maternal obesity during pregnancy associates with premature mortality and major cardiovascular events in later life. *Hypertension*. 2015; 66(5), 938–944.
18. Glastras SJ, Chen H, Teh R, *et al.* Mouse models of diabetes, obesity and related kidney disease. *Plos One*. 2016; 11(8), 1–15.
19. Glastras SJ, Chen H, Teh R, *et al.* The renal consequences of maternal obesity in offspring are overwhelmed by postnatal high fat diet. *Plos One*. 2017; 12(2), 1–17.
20. Hall JE. The kidney, hypertension, and obesity. *Hypertension*. 2003; 41(3), 625–633.
21. Paixão AD, Alexander BT. How the kidney is impacted by the perinatal maternal environment to develop hypertension. *Biol Reprod*. 2013; 89, 1–10.
22. Hall ME, Carmo JM, Silva AA, Juncos LA, Wang Z, Hall JE. Obesity, hypertension, and chronic disease. *Int J Nephrol Renovasc Dis*. 2014; 7, 75–88.
23. Duarte ACGdO, Fonseca DF, Manzoni MSJ, *et al.* Hyperlipidic diet and insulin secretory capacity in rats. *Rev Nutr*. 2006; 19(3), 341–348.
24. Correia-Santos AM, Suzuki A, Anjos JS, *et al.* Induction of type 2 diabetes by low dose of streptozotocin and high-fat diet in wistar rats. *Medicina (Ribeirão Preto)*. 2012; 45(4), 436–444.
25. Roza NAV, Possignolo LF, Palanch AC. Effect of long-term high-fat diet intake on peripheral insulin sensibility, blood pressure, and renal function in female rats. *Food Nutr Res*. 2016; 60(1), 60.
26. Tanko Y, Eze ED, Daja HS, Jimoh A, Mohammed KA, Musa KY. Ameliorative effects of vitamin C and E on serum lipid profiles and liver enzymes on fructose-induced hyperglycemia in wistar rats. *J Appl Pharm Sci*. 2013; 3, 80–84.
27. Hillier TA, Pedula KL, Schimidt MM, Mullen JA, Charles MA, Pettit DJ. Childhood obesity and metabolic imprinting – the ongoing effects of maternal hyperglycemia. *Diabetes Care*. 2007; 30(9), 2287–2292.
28. Ainge H, Thompson C, Ozanne SE, Rooney KB. A systematic review on animal models of maternal high fat feeding and offspring glycaemic control. *Int J Obes*. 2011; 35(3), 325–335.
29. Ribaroff GA, Wastnedge E, Drake AJ, Sharpe RM, Chambers TJG. Animal models of maternal high fat diet exposure and effects on metabolism in offspring: a meta-regression analysis. *Obes Rev*. 2017; 18(6), 673–686.
30. Burgueño AL, Cabrerizo R, Gonzales Mansilla N, Sookoian S, Pirola CJ. Maternal high-fat intake during pregnancy programs metabolic-syndrome-related phenotypes through liver mitochondrial DNA copy number and transcriptional activity of liver PPARGC1A. *J Nutr Biochem*. 2013; 24(1), 6–13.
31. Khan YI, Dekou V, Douglas G, *et al.* A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288(1), 127–133.
32. Hall KD, Guo J. Obesity energetics: body weight regulation and the effects of diet composition. *Gastroenterology*. 2017; 152(7), 1718–1727.
33. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. *Am J Clin Nutr*. 2012; 95(4), 989–989.
34. Rolls BJ, Bell EA, Castellanos VH, Chow M, Pelkman CL, Thorwart ML. Energy density but not fat content of foods affected energy intake in lean and obese women. *Am J Clin Nutr*. 1999; 69(5), 863–871.
35. Williams L, Seki Y, Vuguin PM, Charron MJ. Animal models of in utero exposure to a high fat diet: a review. *Biochim Biophys Acta*. 2014; 1842(3), 507–519.
36. Gibson AA, Seimon RV, Lee CMY, *et al.* Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev*. 2015; 16(1), 64–76.
37. Paoli A, Bosco G, Camporesi EM, Mangar D. Ketosis, ketogenic diet and food intake control: a complex relationship. *Front Psychol*. 2015; 6, 6.
38. Dirlwanger M, Di Vetta V, Guenat E, *et al.* Effects of short-term carbohydrate or fat overfeeding on energy expenditure and plasma leptin concentrations in healthy female subjects. *Int J Obes Relat Metab Disord*. 2000; 24(11), 1413–1418.
39. Horton T J, Drougas H, Brachey A, Reed G W, Peters J C, Hill J O. Fat and carbohydrate overfeeding in humans: different effects on energy storage. *Am J Clin Nutr*. 1995; 62(1), 19–29.
40. Aizawa-Abe M, Ogawa Y, Mazuzaki H, *et al.* Pathophysiological role of leptin in obesity related hypertension. *J Clin Invest*. 2000; 105(9), 1243–1252.
41. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57BL/6J mice. *Int J Obes Relat Metab Disord*. 2000; 24(5), 639–646.
42. Fam BC, Morris MJ, Hansen MJ, *et al.* Modulation of central leptin sensitivity and energy balance in a rat model of diet-induced obesity. *Diabetes Obes Metab*. 2007; 6(6), 840–852.
43. Schellong K, Melchior K, Ziska T, Rancourt RC, Henrich W, Plagemann A. Maternal but not paternal high-fat diet (HFD) exposure at conception predisposes for ‘diabesity’ in offspring generations. *Int J Environ*. 2019; 17(12), 4229, 1–15.
44. Lee MO. Determination of the surface area of the white rat with its application to the expression of metabolic results. *Am J Physiol*. 1929; 89(1), 24–33.
45. Levin BE, Dunn-Meynell AA, Ricci MR, Cummings DE. Abnormalities of leptin and ghrelin regulation in obesity-prone juvenile rats. *Am J Physiol Endocrinol Metab*. 2003; 285(5), 949–957.
46. Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol*. 2002; 283(4), 941–948.
47. Sapienza C, Issa J-P. Diet, nutrition, and cancer epigenetics. *Annu Rev Nutr*. 2016; 36(1), 665–681.
48. Chang S, Graham B, Yakubu F. Metabolic differences between obesity-prone and obesity resistant rats. *Am J Physiol*. 1990; 259(6), 1103–1110.
49. Hassanain M, Levin BE. Dysregulation of hypothalamic serotonin turnover in diet-induced obese rats. *Brain Res*. 2002; 929(2), 175–180.
50. Jackman MR, Kramer RE, MacLean PS. Trafficking of dietary fat in obesity-prone and obesity resistant rats. *Am J Physiol-Endocrinol Metab*. 2006; 291(5), 1083–1091.
51. Srinivasan M, Katewa SD, Palaniyappan A, Pandya JD, Patel MS. 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*. 2006; 291(4), 792–799.
52. Jones HN, Woollett LA, Barbour N. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J*. 2009; 23(1), 271–278.
53. Nivoit P, Jansen E, Remacle C. Established diet-induced obesity in female rats leads to offspring hyperphagia, adiposity and insulin resistance. *Diabetologia*. 2009; 52(6), 1133–1142.
54. Hausman DB, McCloskey HM, Martin RJ. Maternal dietary fat type influences the growth and fatty acid composition of newborn and weanling rats. *J Nutr*. 1991; 121(12), 1917–1923.
55. Taylor PD, Khan IY, Lakasing L, *et al.* Uterine artery function in pregnant rats fed a diet supplemented with animal lard. *Exp Physiol*. 2003; 88(3), 389–398.
56. Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *J Physiol*. 2009; 587, 4(4), 905–915.
57. Gardner DS, Rhodes P. Developmental origins of obesity: programming of food intake or physical activity? *Adv Exp Med Biol*. 2009; 646, 83–93.
58. Rasmussen KM. Effects of under- and overnutrition on lactation in laboratory rats. *J Nutr*. 1998; 128(2), 390–393.
59. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294(2), 528–538.



60. Khan IY, Taylor PD, Dekou V, *et al.* Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension*. 2003; 41(1), 168–175.
61. Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291(3), 768–778.
62. Chaves WF, Pinheiro IL, Silva LO, *et al.* Neonatal administration of kaempferol does not alter satiety but increases somatic growth and reduces adiposity in offspring of high-fat diet dams. *Life Sci*. 2020; 259(1), 118–224.
63. Hokke S, Puelles VG, Armitage JA, Fong K, Bertram JF, Cullen-McEwen LA. Maternal fat feeding augments offspring nephron endowment in mice. *PLoS One*. 2016; 11(8), e0161578, 1–19.
64. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics*. 2005; 115(3), 290–296.
65. Oscai LB. Dietary-induced severe obesity – a rat model. *Am J Physiol*. 1982; 242(3), 212–215.
66. Ainslie DA, Proietto J, Fam BC, Thorburn AW. Short-term, high-fat diets lower circulating leptin concentrations in rats. *Am J Clin Nutr*. 2000; 71(2), 438–442.
67. Ghibaudi L, Cook J, Farley C, van Heek M, Hwa JJ. Fat intake affects adiposity, comorbidity factors, and energy metabolism of Sprague-Dawley rats. *Obes Res*. 2002; 10(9), 956–963.
68. Bouanane S, Merzouk H, Benkafalt NB. Hepatic and very low-density lipoprotein fatty acids in obese offspring of overfed dams. *Metab: Clin Exp*. 2010; 59(12), 1701–1709.
69. Reisin E, Messerli FG, Ventura HO, Frohlich ED. Renal hemodynamic studies in obesity hypertension. *J Hypertens*. 1987; 5, 397–400.
70. Chagnac A, Weinstein T, Korzets A. Glomerular hemodynamics in severe obesity. *J Hypertens*. 2000; 278(5), 817–822.
71. Whaley-Cornell A, Sowers JR. Obesity and kidney disease: from population to basic science and the search for new therapeutic targets. *Kidney Int*. 2016; 92(2), 313–333.
72. Jackson CM, Alexander BT, Roach L, *et al.* Exposure to maternal overnutrition and a high-fat diet during early postnatal development increases susceptibility to renal and metabolic injury later in life. *Am J Physiol Renal Physiol*. 2011; 302(6), 774–783.
73. Wood-Bradley R, Barrand S, Giot A, Armitage J. Understanding the role of maternal diet on kidney development, an opportunity to improve cardiovascular and renal health for future generations. *Nutrients*. 2015; 7(3), 1881–1905.
74. Black MJ, Briscoe TA, Constantinou M, Kett MM, Bertram JF. Is there an association between level of adult blood pressure and nephron number or renal filtration surface area? *Kidney Int*. 2004; 65(2), 582–588.
75. Langle-Evans SC. Critical differences between two low protein diet protocols in the programming of hypertension in the rat. *Int. J. Food Sci. Nutr*. 2000; 51(1), 11–17.
76. Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci*. 1999; 11(11), 965–974.
77. Merlet-Bénichou C, Gilbert T, Muffat-Joly M, Lelièvre-Pégorier M, Leroy B. Intrauterine growth retardation leads to a permanent nephron deficit in the rat. *Pediatr Nephrol*. 1994; 2(2), 175–180.
78. Jahan-Mihan A, Rodriguez J, Christie C, Sadeghi M, Zerbe T. The role of maternal dietary proteins in development of metabolic syndrome in offspring. *Nutrients*. 2015; 7(11), 9185–9217.
79. Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE. Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288(2), 368–373.
80. Martin-Gronert MS, Fernandez-Twinn DS, Bushell M, Siddle K, Ozanne SE. Cell-autonomous programming of rat adipose tissue insulin signalling proteins by maternal nutrition. *Diabetologia*. 2016; 59(6), 1266–1275.
81. Nwagwu MO, Cook A, Langley-Evans SC. Evidence of progressive deterioration of renal function in rats exposed to maternal low-protein diet in utero. *Br J Nutr*. 2000; 83(1), 79–85.
82. Tobar A, Ori Y, Benchetrit S, *et al.* Proximal tubular hypertrophy and enlarged glomerular and proximal tubular urinary space in obese subjects with proteinuria. *Plos One*. 2013; 8(9), e75547, 1–9.
83. Rea DJ, Heimbach JK, Grande JP, *et al.* Glomerular volume and renal histology in obese and non-obese living kidney donors. *Kidney Int*. 2006; 70(9), 1636–1641.
84. Goumenos D, Kavar B, Nahas ME, *et al.* Early histological changes in the kidney of people with morbid obesity. *Nephrol Dial Transplant*. 2009; 24(12), 3732–3738.
85. Khan IY, Dekou V, Douglas G, *et al.* A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am J Physiol Regul Integr Comp Physiol*. 2005; 288(1), 127–133.
86. Chen F, Cao K, Zhang H, Yu H, Liu Y, Xue Q. Maternal high-fat diet increases vascular contractility in adult offspring in a sex-dependent manner. *Hypertens Res*. 2020; 44(1), 36–46.
87. Loche E, Blackmore HL, Carpenter AA, *et al.* Maternal diet-induced obesity programmes cardiac dysfunction in male mice independently of post-weaning diet. *Cardiovasc Res*. 2018; 114(10), 1372–1384.
88. Menendez-Castro C, Nitz D, Cordasic N, *et al.* Neonatal nephron loss during active nephrogenesis – detrimental impact with long-term renal consequences. *Sci Rep*. 2018; 8, 4542, 1–11.