

## **Early type 2 diabetes and obesity does not affect eicosanoids level and renal morphology in a rat model**

### **Diabetes tipo 2 no estágio inicial e obesidade não afetam o nível de eicosanóides e a morfologia renal em um modelo de rato**

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**ABSTRACT**

This study evaluated the effects of the early development of Diabetes Mellitus 2 (T2D) and diet-induced Obesity in the eicosanoid pathways and its effects on renal tissue. Thirty male Wistar rats were fed with a high-fat or standard diet and were divided into 3 groups: The Control group received a standard diet, the T2D group received a high-fat diet and a single dose of streptozotocin (25mg/Kg) and the Obesity group received high-fat diet. Caloric intake, feed efficiency, body weight gain, visceral fat, blood glucose, plasma levels of 14,15 EET/DHET, 20-HETE, and kidneys' morphology were analyzed. Total caloric intake and feed efficiency were higher in the animals of the Obesity group than in Control. Body weight gain, visceral fat, and blood glucose were higher in Obesity and T2D induced groups than in Control. Body weight gain, visceral fat, and feed efficiency associated positively with blood glucose. However, there was no difference in 14,15 EET/DHET, 20-HETE levels, or kidney injury between groups. In conclusion, we were unable to assess whether changes in eicosanoids are due to obesity or diabetes induction. So, this study suggests that longer periods of homeostatic disturbance caused by these protocols seem to be necessary to induce complications related to the disruption of the eicosanoid's pathway and its effects on renal tissue.

**Keywords:** 20- Hydroxyeicosatetraenoic acids, dihydroxyeicosatrienoic acids, epoxyeicosatrienoic acids, high-fat diet, insulin resistance.

**RESUMO**

Este estudo avaliou os efeitos do Diabetes Mellitus 2 (T2D) em estágio inicial e da Obesidade induzidos por dieta hiperlipídica nas vias dos eicosanóides e seus efeitos no tecido renal. Trinta ratos Wistar machos foram alimentados com dieta rica em gordura ou padrão e divididos em 3 grupos: o grupo controle recebeu dieta padrão, o grupo T2D recebeu dieta rica em gordura e dose única de estreptozotocina (25mg / Kg) e o grupo Obesidade recebeu dieta rica em gordura. Ingestão calórica, eficiência alimentar, ganho de peso corporal, gordura visceral, glicose no sangue, níveis plasmáticos de 14,15 EET / DHET, 20-HETE e morfologia dos rins foram analisados. A ingestão calórica total e a eficiência alimentar foram maiores nos animais do grupo Obesidade do que no Controle. O ganho de massa corporal, gordura visceral e glicose no sangue foram maiores nos grupos induzidos à obesidade e ao DM2 do que no controle. Ganho de massa corporal,

gordura visceral e eficiência alimentar associaram-se positivamente à glicemia. No entanto, não houve diferença em 14,15 EET / DHET, níveis de 20-HETE ou lesão renal entre os grupos. Em conclusão, não foi possível avaliar se a obesidade ou o Diabetes Mellitus tipo 2 afetam as vias dos eicosanóides. Portanto, este estudo sugere que períodos mais longos de distúrbio homeostático causados por esses protocolos parecem ser necessários para induzir complicações relacionadas à via dos eicosanóides e seus efeitos no tecido renal.

**Palavras-chave:** ácidos 20-hidroxitetraenóicos, ácidos di-hidroxi-heteratrienóicos, ácidos epoxyeicosatrienóicos, dieta hiperlipídica, resistência à insulina.

## 1 INTRODUCTION

The maintenance of body mass is a function of the balance between caloric intake and energy expenditure (Andrich et al., 2018; Evert et al., 2014; Lichtman et al., 1992) with the combination of the genetic component (Martínez et al., 2014). The increase in the intake of industrialized, extremely caloric and high-fat foods in western populations of developed and developing countries has increased significantly in recent decades, which could drive the high incidence of overweight and obesity. Obesity is an increasingly important public health issue (WHO, 2016) and obese individuals exhibit a higher risk of chronic diseases including cardiovascular diseases and type 2 diabetes mellitus (T2D) (American Diabetes Association, 2017). T2D is an important chronic metabolic disease that affects approximately 425 million people worldwide and is responsible for 4 million deaths per year (International Diabetes Federation (IDF), 2017). T2D is characterized by blood glucose resistance or the inability of target tissues to uptake glucose (Ahlqvist et al., 2018; International Diabetes Federation (IDF), 2017). The relevance of this chronic disease as a public health issue has been recognized by the high worldwide prevalence and by the secondary complications, such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy which could contribute to the collapse of public health systems (International Diabetes Federation (IDF), 2017).

Glucose metabolism in pancreatic Beta cells leads to the closure of ATP-dependent  $K^+$  channels and open voltage-dependent  $Ca^{2+}$  channels. These changes to the permeability of ions result in cell membrane depolarization and consequently, the activation of cytosolic phospholipase A2 (PLA2). PLA2 hydrolyzes cell membrane lipids releasing arachidonic acid (AA) which is the precursor of the eicosanoids

epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (DHETs), and 20-hydroxyeicosatetraenoic acids (20-HETE) (Tunaru et al., 2018). EETs are produced from arachidonic acid (AA) by cytochrome-P450-epoxigenic enzymes while DHETs are synthesized from EETs by soluble epoxide hydrolase (sEH). EETs are angiogenic and anti-inflammatory signaling molecules which the receptors have not been characterized yet (Campbell et al., 1996). EETs agonist has been reported to prevent vascular damages (Sodhi et al., 2012), and to reduce insulin resistance due to its effect on improving vasculature of pancreatic islets (Luria et al., 2011). However, EETs are converted to DHET when sEH is overactivated, lowering EET's metabolic effects on the vasculature (Campbell et al., 1996) and being rapidly conjugated and excreted (Yu et al., 2000). Additionally, the activation of cytochrome P450-hydroxylase leads to 20-HETE production from AA. This product has vasoconstrictor and pro-inflammatory functions (Fan et al., 2016). Cytochrome-P450 enzymes family are mainly synthesized in the liver, however, extrahepatic tissues (e.g. kidney) have been shown to have considerable amounts of these enzymes (Laniado-Schwartzman & Abraham, 1992).

As previously stated, DHETs are biologically less potent than EETs and, therefore, therapies that could inhibit or block sEH activity have called the attention of several researchers. Previously, studies have demonstrated that alterations in activity and expression of sEH and cytochrome P450-hydroxylase in targeted tissues are directly correlated to insulin resistance, high blood pressure, increased inflammation process and renal diseases (De Taeye et al., 2010; He et al., 2016; Zha et al., 2014). Moreover, several studies have shown that obesity, which often arises before T2D, can cause changes in the eicosanoids pathway, promoting vascular dysfunction that can progress to deleterious effects of reduced EET and increased 20-HETE (Theken et al., 2012; Wang et al., 2003; Zhou et al., 2005). Nevertheless, few studies have evaluated the changes in eicosanoids resulting from the development of early T2D.

The usual T2D-induced model involves the combination of a high-fat diet with the administration of STZ, a  $\beta$ -cell toxin. These two stressors are designed to mimic the pathology of T2D (hyperinsulinemia, insulin resistance and/or glucose intolerance) in a short timeline. As previously reported, short-time feeding (two weeks) with high-fat diets tend to induce insulin resistance and/or glucose intolerance (Skovsø, 2014). Additionally, although the metabolic effects caused by a high-fat diet seemed to be more pronounced

in Wistar Rat, only the use of this protocol has been demonstrated to be sufficient for inducing obesity in Wistar and Sprague-Dawley rats (Marques et al., 2016).

Therefore, we designed a trial to test whether these protocols (high-fat diet with and without STZ administration) were able to affect the plasma levels of specific eicosanoids (14,15-EET, 14,15-EET, and 20-HETE). Additionally, we assessed the ability of the diet to induce obesity and the pathobiology of T2D by evaluating renal cells morphology.

## 2 SUBJECTS AND METHODS

### 2.1 ANIMALS

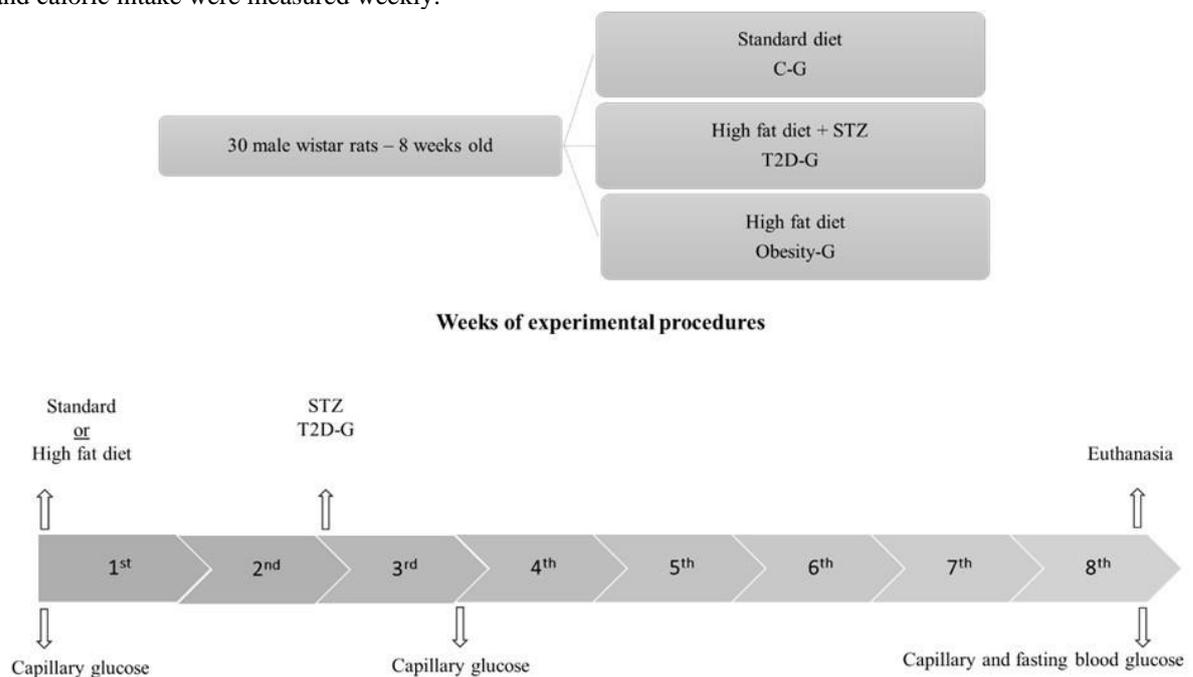
The Ethics Committee on the Use of Animals (CEUA) of Federal University of Jataí approved the experimental protocol according to guidelines and regulations from the National Council for Animal Experimentation Control in Brazil (CONCEA) (protocol n° 034/14/CEUA/UFG). We used 30 eight-week-old male Wistar rats (*Rattus norvegicus*) housed in pairs, in polypropylene cages provided with drinkers and wood shavings substrates located in animal housing cabinets (Insight Ltda, Ribeirão Preto, São Paulo State, Brazil). The environmental conditions were standardized and comprised an inverted circadian cycle of 12h light/dark and an ambient temperature of  $22 \pm 2^\circ\text{C}$ . Feed and water were offered *ad libitum*.

### 2.2 EXPERIMENTAL PROTOCOL

The experimental protocol is presented in figure 1. The animals were fed a high-fat diet throughout the experiment (8 weeks) to induce T2D. Pragsoluções Biociências (Jaú, São Paulo State, Brazil) produced the animal feed containing 5.35 kcal/g and composed of 15.2% kcal protein, 26.9% kcal carbohydrate, and 57.2% kcal fat. Eleven animals were randomly selected to comprise the induced-diabetes group by the administration of 25mg/kg streptozotocin (STZ, Sigma Aldrich) (T2D-G) on the 14<sup>th</sup> day as previously done in our laboratory (dos Santos et al., 2018). Eight animals were only fed a high-fat diet throughout the experiment to comprise the obesity-induced group (Obesity-G). The animals of the control group (C-G; n = 11) were fed a balanced diet for rats containing 3.87 kcal/g and composed of 24.8% kcal protein, 63% kcal carbohydrate, and 12% kcal fat (Pragsoluções Biociências, Jaú, São Paulo State, Brazil). The amounts

of ingested food (g) and body weight (g) were recorded at the beginning and every week until the end of the experiment. Blood glucose was analyzed weekly using a glucometer (Accu-Chek®, Santo André, State of São Paulo, Brazil) after blood sampling by a caudal puncture. All these parameters were assessed without previous fasting. All animals were anesthetized with inhaled isoflurane and euthanized by exsanguination followed by cervical dislocation at the end of the experiment.

**Figure 1.** Design and timeline of experimental procedures, STZ= streptozotocin, C= Control. Body weight and caloric intake were measured weekly.



### 2.3 BODY WEIGHT GAIN, CALORIC INTAKE, AND FEED EFFICIENCY

Body weight gain was calculated by the difference between the final and initial body weight of the experiment. The mean calorie intake was calculated by multiplying the feed intake (in grams) by the rat pair in each cage by the caloric value of balanced (3.87 kcal/g) and high-fat diet (5.35 kcal/g). The caloric intake was calculated by the consumption of calories ingested (kcal/g) during the week by the rat pair in each cage divided by the body weight of the pair (in grams) on that week. The calculation of the feed efficiency followed the relationship between the body weight gain (g) and the feed intake by the animals during the week (kcal/g), so it was calculated by dividing the body weight gain by the caloric intake of each week.

## 2.4 BLOOD AND FAT TISSUE COLLECTION

Blood was collected with a syringe containing a glycolysis-inhibiting anticoagulant solution (6 g/dL EDTA and 12 g/dL potassium fluoride from the Labtest® Glistab Kit, Lagoa Santa, State of Minas Gerais, Brazil) at the end of the experiment. The visceral fat was surgically removed from the abdominal region and weighed on a high-precision digital scale.

## 2.5 RENAL TISSUE AND HISTOLOGICAL ANALYSIS

Longitudinal kidney fragments were fixed in a buffered Karnovsky (4% paraformaldehyde and 4% glutaraldehyde) solution for 24 hours. Thereafter, tissue was dehydrated, diaphanized, and embedded in paraffin. Five  $\mu\text{m}$  thick sections (Leica RM2235, Biosystems®) were placed into histological slides and routinely stained with hematoxylin and eosin. Images were acquired under an optical microscope (Leica ICC50, Biosystems®) and slides were blinded-evaluated for a veterinary pathologist.

## 2.6 BIOCHEMICAL ANALYSIS

Blood glucose was determined by a glucometer (Accu-Chek®, Santo André / SP). Extraction and quantification of plasma 14,15-EET, 14,15-DHET and 20-HETE using commercial kits based on ELISA reactions (14,15-EET/DHET ELISA Kit and 20-HETE ELISA kit) according to manufacturer protocols (Detroit R&D Inc, Detroit / MI).

## 2.7 STATISTICAL ANALYSIS

Data are expressed as mean  $\pm$  standard deviation ( $M \pm SD$ ). Data normality distribution was checked by the Shapiro-Wilk test. We used the One-Way RM ANOVA test with Tukey post-hoc for the comparison of body weight between weeks within the same group. Data on caloric intake, feed efficiency, body weight gain, visceral fat, blood glucose and plasma levels of eicosanoids (14,15-EET, 14,15-EET and 20-HETE) were submitted to One-Way ANOVA followed by the Tukey post-hoc or Kruskal-Wallis test with Dunn post-hoc, depending on the data normality distribution. This analytical procedure was used to compare differences between groups. The associations of variables caloric intake, feed efficiency, body weight gain, visceral fat, and blood glucose were analyzed by linear regression. Statistical differences were established at  $P < 0.05$ .

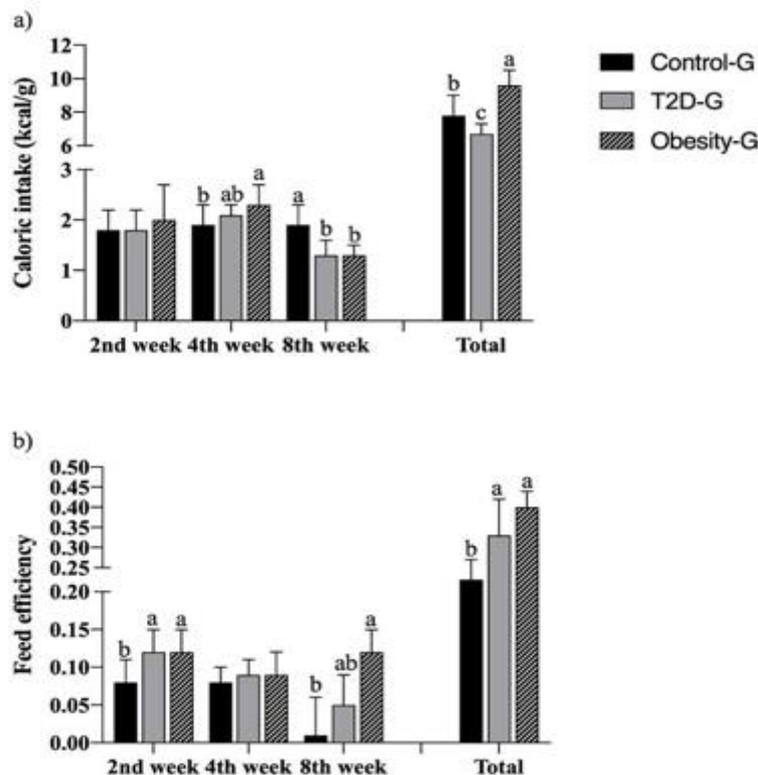
### 3 RESULTS

#### 3.1 CALORIC INTAKE AND FEED EFFICIENCY

No difference between groups in the second experimental week was found (Figure 2a). A higher caloric intake in the Obesity-G compared to the C-G was observed in the 4<sup>th</sup> week, while in the 8<sup>th</sup> week the control group showed the highest caloric intake (Figure 2a). However, the obesity group showed the highest cumulative caloric intake followed by the control group while the T2D-G had the lowest caloric intake (Figure 2a).

The protocols to induce T2D affected feed efficiency. In the 2<sup>nd</sup> week, feed efficiency was higher for the groups that received a high-fat diet (T2D-G and Obesity-G) when compared to the C-G. There was no difference between groups in the 4<sup>th</sup> week. In the 7<sup>th</sup> week, the feed efficiency was higher in the T2D-G than in the control group (C-G). Finally, in the 8<sup>th</sup> week, the feed efficiency was higher in the Obesity-G than in the C-G. Total feed efficiency was higher in the T2D-G and Obesity-G groups than in the animals of the C-G (Figure 2b).

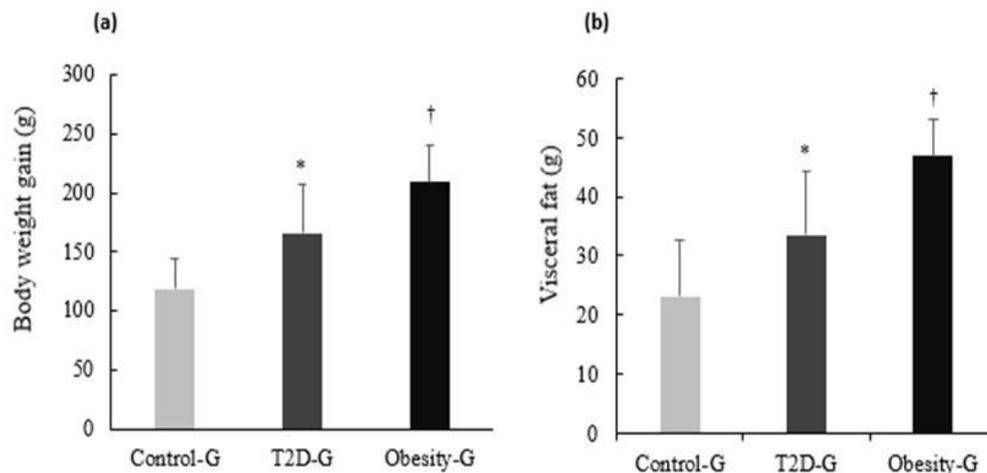
**Figure 2.** Caloric intake (a) and feed efficiency (b) at different times in the experiment (2nd, 4th, 8th weeks and total cumulative values). Values are expressed as mean ± standard deviation. C-G = control (n = 11), T2D-G = high-fat diet + streptozotocin (n = 11), Obesity-G = high-fat diet (n = 8), Different letters above the columns are significantly different by Tukey post-hoc test at P<0.05 within each week.



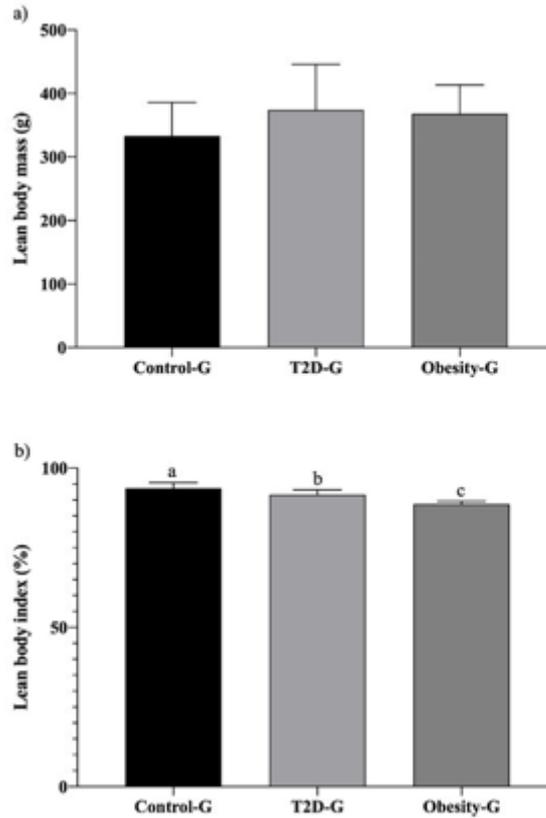
### 3.2 EFFECT OF T2D INDUCED PROTOCOLS ON BODY WEIGHT GAIN, VISCERAL FAT AND LEAN BODY INDICES

Body weight gain (Figure 3a) and visceral fat (Figure 3b) were higher in the Obesity-G than in the T2D-G and C-G. It was also observed that body weight gain and visceral fat of the T2D-G animals were higher when compared to the C-G. The lean body mass was similar between the groups ( $P > 0.05$ , Figure 4a). On the other hand, lean body index (LBI) was significantly reduced in the Obesity-G and T2D-G groups with the obesity group showing the lowest LBI ( $P < 0.05$ , Figure 4b).

**Figure 3.** Body weight gain (a) and visceral fat (b). Values are expressed as mean  $\pm$  standard deviation. C-G = control (n = 11), T2D-G = high-fat diet + streptozotocin (n = 11), Obesity-G = high-fat diet (n = 8). One-Way ANOVA with Tukey post-hoc \*  $p < 0.05$ , T2D-G vs. C-G, †  $p < 0.05$ , Obesity-G vs. T2D-G and C-G



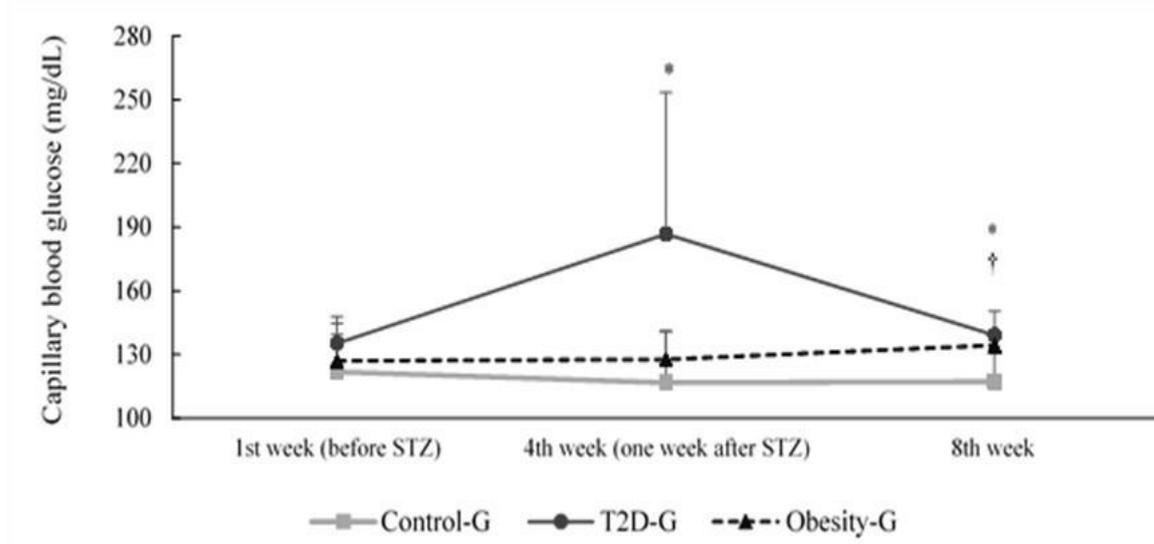
**Figure 4.** Lean body mass (a) and lean body index (b) of the experimental groups after 8 weeks. Values are expressed as mean  $\pm$  standard deviation. C-G = control (n = 11), T2D-G = high-fat diet + streptozotocin (n = 11), Obesity-G = high-fat diet (n = 8). Different letters above the columns are significantly different by Tukey post-hoc test at  $P < 0.05$  within each week.



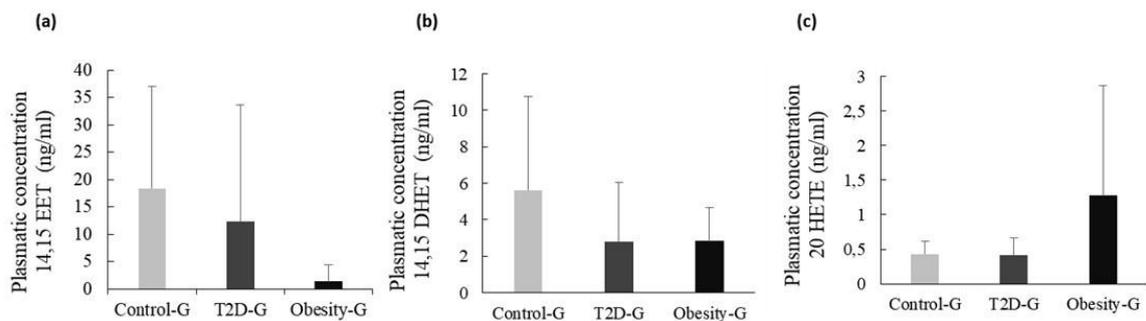
### 3.3 BIOCHEMICAL ANALYSIS

Blood glucose values measured at different times in the experiment (1<sup>st</sup>, 4<sup>th</sup>, and 8<sup>th</sup> weeks) are presented in Figure 5. There was no difference between groups in the 1<sup>st</sup> week of the experiment. In the 4<sup>th</sup> week of the experiment (soon after the injection of STZ in the T2D-G), blood glucose was higher in the T2D-G than in the C-G and Obesity-G groups. In the 8<sup>th</sup> week (end of the experiment), blood glucose was higher in T2D-G and Obesity-G than in the C-G. There were no differences between groups in the plasma levels of eicosanoids derived from arachidonic acid, shown in figures 6a-14,15-EET; 6b-14,15DHET and 6c-20-HETE (figure 6).

**Figure 5.** Blood glucose at different times in the experiment (1st, 4th, and 8th weeks). Values are expressed as mean  $\pm$  standard deviation. C-G = control (n = 10), T2D-G = high-fat diet + streptozotocin (n = 9), Obesity-G = high-fat diet (n = 8). Kruskal-Wallis with Dunn's post-hoc \*  $p < 0.05$ , T2D-G vs. C-G, and Obesity-G. One-Way ANOVA with Tukey post-hoc: \*  $p < 0.05$ , T2D-G vs. C-G; †  $p < 0.05$ , Obesity-G vs. C-G.



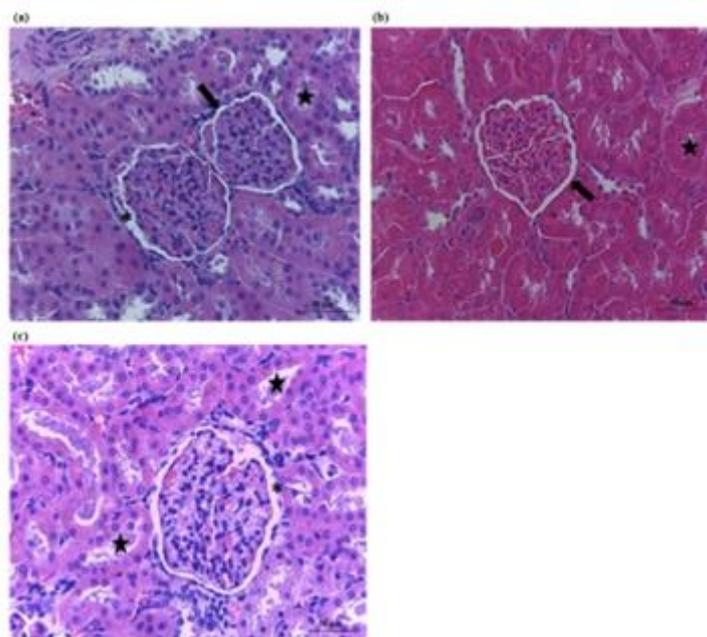
**Figure 6.** Plasma concentrations of eicosanoid 14,15-EET (a), 14,15-DHET (b) and 20 HETE (c). Values are expressed as mean  $\pm$  standard deviation. C-G= control (n = 10), T2D-G = high-fat diet + streptozotocin (n = 9), Obesity-G = high-fat diet (n = 7). The One-Way ANOVA test showed no significant difference between groups.



### 3.4 EFFECT OF T2D INDUCED PROTOCOLS ON RENAL TISSUE

Figure 7 shows the renal photomicrography of all experimental groups. The results demonstrate that the protocol was not efficient to cause injury and impairment of the glomeruli (arrows), renal tubules (stars), and Bowman's capsule (\*).

**Figure 7.** Photomicrograph of the normal kidneys from: (a) C-G = control; (b) T2D-G = high-fat diet + streptozotocin; and (c) Obesity-G = high-fat diet. Note the histological normal pattern of glomeruli (arrows), proximal renal tubules (stars), and Bowman’s capsule (\*). Hematoxylin-eosin, 400x (Scale bar: 50  $\mu$ m).



### 3.5 ASSOCIATIONS BETWEEN VARIABLES

There were positive associations between visceral fat and body weight gain and feed efficiency. Blood glucose was also positively associated with visceral fat, body weight gain, and feeding efficiency (Table 1).

**Table 1.** Association between variables caloric intake, feed efficiency, body weight gain, visceral fat, and blood glucose.

|                                | Body weight gain |                |        | Visceral fat |                |        | Total caloric intake |                |       | Feed efficiency |                |        |
|--------------------------------|------------------|----------------|--------|--------------|----------------|--------|----------------------|----------------|-------|-----------------|----------------|--------|
|                                | R                | R <sup>2</sup> | p      | r            | R <sup>2</sup> | p      | r                    | R <sup>2</sup> | p     | r               | R <sup>2</sup> | p      |
| <b>Visceral fat</b>            | 0.88             | 0.77           | 0.000* | -            | -              | -      | 0.32                 | 0.10           | 0.090 | 0.80            | 0.65           | 0.000* |
| <b>Capillary blood glucose</b> | 0.44             | 0.19           | 0.018* | 0.53         | 0.29           | 0.003* | 0.28                 | 0.08           | 0.147 | 0.57            | 0.32           | 0.001* |

Linear regression. \*p<0.05 n=29

## 4 DISCUSSION

Our results did not support our hypothesis that biochemical and morphometric changes similar to T2D and obesity could affect the plasma levels of eicosanoids and the renal morphology, despite the effects of the protocols on metabolic and morphometric changes. Comparing the protocols used to induced obesity and T2D, the use of a high-fat

diet only without pharmacological destructions of  $\beta$ -cells induced characteristics that better mimic the development of T2D in humans (Marques et al., 2016). Both groups presented higher blood glucose; however, feed intake, weight gain and visceral fat, common characteristics observed on the development of T2D, were higher when the high-fat diet was administered without STZ.

STZ has been widely used by researchers aiming to investigate diabetes and its complications in laboratory animals (Skovsø, 2014). However, in high doses, this pharmacological agent destroys completely pancreatic  $\beta$ -cells that is a common characteristic of type 1 diabetes. Moreover, STZ promotes a decrease in body weight, which is the opposite of what is observed in the initial phase of the development of T2D (Skovsø, 2014). Therefore a methodology of using a low dose of STZ in combination with a high-fat diet was tested and standardized by different laboratories (dos Santos et al., 2018; Mansor et al., 2013; Marques et al., 2016; Skovsø, 2014). Here morphometrics measures, visceral fat, and body weight gain, as well as blood glucose, were higher in T2D-G and Obesity-G than the animals of C-G supporting the efficiency of both obesity and T2D-induced protocols. However, important characteristics observed before the diagnosis of T2D such as visceral fat and body weight gain was higher when STZ was not administered, indicating the development of obesity. Besides, it was observed a positive association between body weight gain, feed efficiency, and visceral fat. The increase in body weight in the Obesity-G and T2D-G groups was driven by the increase in body fat. This was demonstrated by the similar lean body mass among the groups and the reduction in LBI of the rats fed the high-fat diets. This effect has been consistently demonstrated in previous studies (Skovsø, 2014).

It has been described that the deposition of fat in adipose and ectopic tissues results in an increased release of diacylglycerol (DAG) into the bloodstream. DAG is a dangerous lipid that can lead to lipotoxicity and insulin resistance (Ertunc & Hotamisligil, 2016; Guilherme et al., 2008). DAG activates novel protein kinase C (PKC) that stimulates the phosphorylation of serine from substrate 1 of the insulin receptor (IRS-1), which in turn prevents phosphorylation of tyrosine and impairs insulin signal transmission, which is one of the steps for insulin resistance and the development of T2D (Brøns & Grunnet, 2017; Drosatos, 2016; Ertunc & Hotamisligil, 2016; Morales et al.,

2017). In agreement, in the present study positive associations of visceral fat deposition, weight gain, and feed efficiency with blood glucose were observed.

Although metabolic and morphometric changes associated with early T2D development were obtained in both experimental protocols, T2D-G and Obesity-G, there were no changes on levels 14,15-EET and 14,15-EET, and their ratio or renal morphology alterations in animals. It is widely accepted that EETs are important in regulating blood pressure, inflammatory cascades, and glucose homeostasis. The diabetes-induced experiment in sixteen-week-old mice by 70 mg/kg of STZ, was observed significantly decreased EET/DHET ratio when compared to the group control (Elmarakby et al., 2011). Furthermore, changes in the EET pathways are observed in experimental models aiming to induce arterial hypertension (Wang et al., 2003) or renal condition (Roche et al., 2015). Previous studies have demonstrated that the increase in EETs due to the blockage of sEH in obese and T2D rats caused the decrease in plasma glucose, renal vascular resistance, glomerular infiltration and sodium retention, but increasing the insulin receptor phosphorylation. These effects clearly indicated an improvement in insulin sensitivity and signaling leading to a beneficial effect on renal function and blood pressure (Guglielmino et al., 2012; Huang et al., 2007; Luria et al., 2011).

In this way, we presume that the protocols used were efficient in promoting the early stage of T2D, but it was not long enough to promoting vascular changes associated with comorbidities that occur in more advanced stages of T2D.

Apart from EET, AA acid is converted to 20-HETE which is associated with vascular complications induced by diabetes (Eid et al., 2013). In this study, there was no difference in the levels of 20-HETE in plasma between groups. It is important to reinforce that the protocol might be too short to induce a disturbance in the AA pathway, hypertension, pro-inflammatory effects on kidneys, and the onset of T2D complications. Changes in the 20-HETE and cytochrome P450 pathway were reported in diabetic-induced nephropathy protocol where chronic exposure to high glucose contributed to an increase in the extracellular matrix and production of reactive oxygen species (ROS) in tubular cells and was associated with altered expression cytochrome P450 (Eid et al., 2013). Indeed, in our previous study the plasma level of ROS, evaluated through plasma 8-isoprostane, was not increased in animals submitted to a similar T2D protocol (dos Santos et al., 2018). Since neither the T2D protocols induced disturbance in the AA

pathway, it can also be hypothesized that 14,15-EET, 14,15-DHET and 20-HETE are rather disturbed by long periods of chronic hyperglycemia or high-fat accumulation to cause homeostatic disturbances.

In summary, both tested protocols for obesity and T2D induction were efficient to promote metabolic and morphometric alterations similar to the early stage of T2D development. Moreover, both protocols failed to promote alteration of the AA pathway since there were no changes in levels of 14,15-EET, 14,15-DHET or 20-HETE. Therefore, this study suggests that long periods of chronic hyperglycemia might be necessary to induce complications related to comorbidity of T2D such as pro-inflammatory effects on the kidney, endothelial complications, and arterial hypertension. Furthermore, the results of this study support the idea that a high-fat diet without pharmacological destruction of  $\beta$ -cells could be more suitable to induced obesity and further T2D in laboratory animals.

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#### **Author Contribution**

Kamila Lauany Lucas-Lima wrote the article, performed biochemical of the acids and statistical analyses. Julia Matzenbacher dos Santos has planned the project, wrote the article, performed, and interpreted data biochemical, and evaluated the results. Didier Quevedo Cagnini wrote the article, prepared slides, and performed histology analysis. Denise Silva de Oliveira performed and interpreted biochemical analyses. Igo Gomes Guimarães assisted in analyzing the data and prepared the graphs, Andréia Vitor Couto do Amaral evaluated the results and statistical analyses. Sandra Aparecida Benite-Ribeiro has planned and conducted the project, guided and wrote the article, evaluated the results and statistical analyses. All authors reviewed the manuscript.



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