Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(11): 312
https://doi.org/10.31083/j.fbl2811312

Original Research

Effect of Low-Dose Progesterone on Glycemic Metabolism, Morphology and Function of Adipose Tissue and Pancreatic Islets in Diet-Induced Obese Female Mice

Matheus P. Santos[1,†], Leonardo F. R. Cauduro[1,†], Marilia Marcondes Ferreira[2], Luiz Felipe Martucci[2,3], Bruno Vecchiatto[2,3], Eloisa Aparecida Vilas-Boas[4], Anna Laura V. Américo[5], Renata O. Pereira[6], Marcelo Macedo Rogero[7], Patrícia Fiorino[8], Fabiana S. Evangelista[2], Anna Karenina Azevedo-Martins[1,*]

[1]Biosciences Studies Group, School of Arts, Sciences and Humanities, University of São Paulo, 03828-000 São Paulo, Brazil
[2]Sport Biology Research Group, School of Arts, Sciences and Humanities, University of São Paulo, 03828-000 São Paulo, Brazil
[3]Department of Experimental Pathophysiology, Faculty of Medicine, University of São Paulo, 01246-903 São Paulo, Brazil
[4]Institute of Chemistry, University of São Paulo, 05508-900 São Paulo, Brazil
[5]Heart Institute, University of São Paulo, 05403-900 São Paulo, Brazil
[6]Translational Medicine Division, Department of Medicine, Federal University of São Paulo, 04023-062 São Paulo, Brazil
[7]Nutritional Genomics and Inflammation Laboratory, School of Public Health, University of São Paulo, 01246-904 São Paulo, Brazil
[8]Renal, Cardiovascular and Metabolic Physiopharmacology Laboratory, Health and Biological Science Center, Mackenzie Presbyterian University, 01303-060 São Paulo, Brazil
*Correspondence: karenina@usp.br (Anna Karenina Azevedo-Martins)
[†]These authors contributed equally.

Academic Editor: Yuli Huang
Submitted: 17 February 2023 Revised: 30 May 2023 Accepted: 12 July 2023 Published: 28 November 2023

Abstract

Background: Obesity is a worldwide concern due to its global rapid expansion and remarkable impact on individual's health by predisposing to several other diseases. About twice as many women as men suffer from severe obesity and, in fact, there are stages in a woman's life when weight gain and adiposity can result in greater damage to health. For example, obesity triples the chance of a woman developing gestational diabetes. Many hormones promote the metabolic adaptations of pregnancy, including progesterone, whose role in female obesity is still not well known despite being involved in many physiological and pathological processes. **Methods**: Here we investigated whether progesterone treatment at low dose can worsen the glucose metabolism and the morpho functional aspects of adipose tissue and pancreas in obese females. Mice were assigned into four groups: normocaloric diet control (NO-CO), high-fat and -fructose diet control (HFF-CO), normocaloric diet plus progesterone (NO-PG) and high-fat and -fructose diet plus progesterone (HFF-PG) for 10 weeks. Infusion of progesterone (0.25 mg/kg/day) was done by osmotic minipump in the last 21 days of protocol. **Results**: Animals fed a hypercaloric diet exhibited obesity with increased body weight ($p < 0.0001$), adipocyte hypertrophy ($p < 0.0001$), hyperglycemia ($p = 0.03$), and glucose intolerance ($p = 0.001$). HFF-CO and HFF-PG groups showed lower adiponectin concentration ($p < 0.0001$) and glucose-stimulated insulin secretion ($p = 0.03$), without differences in islet size. Progesterone attenuated glucose intolerance in the HFF-PG group ($p = 0.03$), however, did not change morphology or endocrine function of adipose tissue and pancreatic islets. **Conclusions**: Taken together, our results showed that low dose of progesterone does not worsen the effects of hypercaloric diet in glycemic metabolism, morphology and function of adipose tissue and pancreatic islets in female animals. These results may improve the understanding of the mechanisms underlying the pathogenesis of obesity in women and eventually open new avenues for therapeutic strategies and better comprehension of the interactions between progesterone effects and obesity.

Keywords: obesity; hypercaloric diet; females; progesterone; adipose tissue; pancreatic islets

1. Introduction

Obesity is characterized by the increased fat accumulation in white adipose tissue (WAT) depots. While the number of cases has almost tripled since 1975 [1] around the world, life expectancy has decreased around 5 to 20 years in obese people [2]. About twice as many women as men suffer from severe obesity, which reveals sex differences in obesity prevalence [3]. The etiology of the disease is complex, involving factors such as increased physical inactivity and high caloric intake [1] that culminate in an imbalance between intake and expenditure of calories [2]. Besides the excessive calorie intake, the quality of calories consumed is also determinant [4] since there is a relationship between chronic consumption of a high-fat and -fructose diet with an increase in body weight, inflammatory status, and insulin resistance [5,6]. In several countries, the total sugar intake ranges between 14–25% in adults [7].

Table 1. Composition of the diets.

	Normocaloric diet	High-fat and -fructose diet
	g/kg of diet	
Cornstarch	397.5	20.0
Casein	200.0	200.0
Dextrinized cornstarch	132.0	20.0
Sucrose	100.0	62.5
Soybean oil	70.0	50.0
Fiber	50.0	50.0
Mineral mixture	35.0	35.0
Vitamin mixture	10.0	10.0
L-cystine	3.0	3.0
Choline bitartrate (41.1% choline)	2.5	2.5
Fructose	0.0	239.0
Lard	0.0	308.0
tert-Butylhydroquinone	0.014	0.014
Kcal/g	3.90	5.34

Individuals with obesity are more likely to be affected by cardiovascular diseases [8], cancer [9], dyslipidemia, diabetes, and insulin resistance [10]. The WAT expansion is accompanied by impaired vascularization, local hypoxia facilitating adipocyte necrosis, fibrosis, and reduced lipid storage capacity, which may result in ectopic fat accumulation in the pancreas, liver, or muscles, leading to insulin resistance [11]. In addition, increased production of inflammatory molecules and decreased secretion of insulin sensitizers such as adiponectin are observed in hypertrophied adipocytes of people with obesity, resulting in damage to the metabolic organs' functions and control of energy metabolism [12]. Obesity also increases the demand for insulin secretion leading to higher islet area/pancreas area ratio in mice [13] and impairs the islet function inducing apoptosis and disarray in its cells [14]. Chronically hyperglycemia and hyperlipidemia trigger beta cell dysfunction and death [15,16] and the excess of glucose and lipids in association (glucolipotoxicity) induces progressive beta cell failure in mice [15].

There are stages in a woman's life when weight gain and adiposity can result in greater damage to health. For example, obesity triples the chance of a woman developing gestational diabetes compared to non-obese women [17]. Pregnancy *per se* requires metabolic adaptations in the maternal organism, which are orchestrated by several hormones, including progesterone. Besides progesterone's roles in physiological adaptations of pregnancy [18], including insulin resistance during this period [19], this hormone regulates several cellular processes in other tissues. In that sense, the apoptotic effect of progesterone on beta cells and islets *in vitro* [20] and its action as an important counter regulator of lactogenic stimulating activity in islets [21] has been shown. In addition, progesterone has also been used as a pharmacological strategy to treat clinical conditions such as assisted reproduction, anovulatory menstrual cycles, contraception during lactation [22] and to prevent preterm birth [23]. In women receiving the oral contraceptive pill, the reduction in insulin sensitivity is attributed to progesterone [24]. On the other hand, estradiol but not progestin treatment, lower plasma very low-density lipoptrotein-triglyceride concentrations by increasing their plasma clearance in healthy postmenopausal women [25]. Although progesterone and adiposity are regulators of glycemic metabolism, the role of progesterone in female obesity is not yet clearly established. Thus, we investigated whether progesterone treatment at low dose can worsen the glucose metabolism and the morpho functional aspects of adipose tissue and pancreas in obese females.

2. Materials and Methods

2.1 Animals

Female post-weaned C57BL/6J mice, obtained from the Medical School of the University of São Paulo, were assigned into four groups, according to body weight: normocaloric diet control (NO-CO; n = 11), high-fat and -fructose diet control (HFF-CO; n = 10), normocaloric diet plus progesterone (NO-PG; n = 8) and high-fat and -fructose diet plus progesterone (HFF-PG; n = 13). The groups NO-CO and NO-PG were fed a AIN93G chow (Pragsoluções Biociências, Jaú, Brazil) and the groups HFF-CO and HFF-PG were fed a AIN93G chow added with fructose (24%) and lard (31%) (Table 1), that produces a high similarity of metabolic and hormonal alterations associated with human obesity [26]. The amount of casein in both diets did not differ, which is in accordance with AIN-93G rodent diets published [27]. Animals were kept under the same housing conditions (12 h light/12 h dark cycle, temperature 22 $\pm$ 2 °C) with food and water *ad libitum*. All procedures were approved by the Ethics Committee of the School of Arts,

Table 2. Tissues and organs weight.

	NO-CO	NO-PG	HFF-CO	HFF-PG
SC-WAT (mg/g)	19.41 ± 1.55	17.95 ± 1.95	37.56 ± 1.91 *	30.25 ± 3.33 *
RP-WAT (mg/g)	4.63 ± 0.58	4.55 ± 0.46	12.47 ± 1.01 *	12.79 ± 1.79 *
iBAT (mg/g)	2.91 ± 0.33	3.33 ± 0.42	2.58 ± 0.24	2.22 ± 0.24
Pancreas (mg/g)	20.14 ± 1.87	23.05 ± 1.99	20.40 ± 1.87	16.65 ± 1.35
Liver (mg/g)	49.04 ± 2.25	47.69 ± 0.38	35.97 ± 2.82 *	37.54 ± 1.46 *
Kidneys (mg/g)	9.96 ± 0.27	9.78 ± 0.20	7.91 ± 0.20 *	7.77 ± 0.50 *
Left Ventricle (mg/g)	3.16 ± 0.11	3.16 ± 0.08	2.56 ± 0.07*	2.77 ± 0.09 *

Data are presented as mean ± SEM. The results were compared by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG.

For SC-WAT, RP-WAT, iBAT, liver, kidneys and left ventricle: NO-CO (n = 11), NO-PG (n = 8), HFF-CO (n = 10), HFF-PG (n = 13). For pancreas: NO-CO (n = 7), NO-PG (n = 3), HFF-CO (n = 7), HFF-PG (n = 9).

NO-CO, normocaloric diet control; NO-PG, normocaloric diet plus progesterone; HFF-CO, high-fat and -fructose diet control; HFF-PG, high-fat and -fructose diet plus progesterone; SC-WAT, subscapular white adipose tissue; RP-WAT, subscapular white adipose tissue; iBAT, interscapular brown adipose tissue.

Sciences and Humanities of the University of São Paulo (003/2018). *In vivo* evaluations were performed during the non-ovulatory phase of the estrous cycle.

2.2 Body Weight and Food Intake

The animals were weighed weekly on a digital scale (Gehaka/model BG4001, São Paulo, Brazil), on the same day and time. In addition to body weight evolution, we calculated body weight gain by the difference between final body weight (week 10) and initial body weight (week 1). The 24 h food intake was determined weekly throughout the study in groups of mice that were housed in the same cage.

2.3 Progesterone Treatment

In the seventh week of protocol, NO-PG and HFF-PG animals received the subcutaneous implantation of osmotic minipump (model 1004; Alzet, Cupertino, CA, USA) for constant infusion of progesterone in a low dose (0.25 mg/kg/day), diluted in DMSO (100%), for 21 days [28]. For the implantation procedure, animals were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight). The SHAM groups (NO-CO and HFF-CO) underwent the same surgical procedure without the pump insertion. We followed manufacturer's instructions to measure residual volume inside the pump, thus assessing the correct infusion parameters.

2.4 Glycemic Metabolism

Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed at week 10 of protocol on awake animals after a 6 hour fast. For GTT, glycemia was determined at 15, 30, 60 and 90 min after intraperitoneal infusion of the glucose load (2 g/kg body weight). After 72 h from the GTT, the ITT was performed with insulin (0.75 U/kg body weight) injected as an intraperitoneal bolus, and glycemia was determined 5, 10, 15, 20, 25 and 30 min after injection. The values obtained between 5 and 30 min were used to calculate the rate constant for plasma glucose disappearance (kITT) according to the method proposed by Bonora *et al*. [29]. Fasting glycemia was determined from time point 0 of GTT, before glucose infusion. Glucose concentration was determined in tail blood samples using a glucometer (AccuChek Advantage Roche Diagnostics®; Rotkreuz, Zug, Switzerland).

2.5 Indirect Calorimetry at Rest and During Exercise

In the last week of the protocol, the animals were acclimated in the Oxylet Calorimetry System (Panlab, Barcelona, Spain) and measurements were made during rest. First, the animals were fasted (2 h) and then oxygen consumption volume (VO_2, mL/min/kg), carbon dioxide production (VCO_2, mL/min/kg), respiratory exchange ratio (RER) and energy expenditure (EE, kcal/day/kg) were measured during 30 min of rest. The rate of oxidation of carbohydrate (CHO) and lipid (LIP) were calculated using the formulas: $CHO = (4.55 \times VO_2) - (3.21 \times VCO_2)$ and $LIP = (1.67 \times VO_2) - (1.67 \times VCO_2)$. Data were expressed as mg/min/g [30].

After the rest measurement, the maximal exercise capacity was assessed by a graded progressive test without inclination until the animal reached exhaustion. Initial treadmill speed was 0.8 cm/s, increasing by 3 cm/s every 2 minutes, until the animal could not maintain the running pattern and keep continuous contact with the shock grid for 5 seconds [30]. VO_2, VCO_2, RER, and EE were continuously measured until the animal reached exhaustion. VO_2 maximal (VO_2 max, mL/min/kg) and VCO_2 maximal (VCO_2 max, mL/min/kg) were obtained from the mean values during the last phase of the test. The iVO_2 (cm/s) was defined

as the exercise intensity corresponding to VO_2 max as described by Machado *et al*. [31]. Results were expressed as mg/min/kg.

2.6 Tissue and Blood Collection

Forty-eight hours after the last glycemic test, the animals were killed with an intraperitoneal injection of thiopental sodium (3 mg/100 g body weight), followed by exsanguination. The animal was weighed and then the subcutaneous (inguinal) and visceral (retroperitoneal) white adipose tissue (WAT) pads (subscapular white adipose tissue (SC-WAT) and subscapular white adipose tissue (RP-WAT), respectively), interscapular brown adipose tissue (iBAT), pancreas, liver, kidneys and left ventricle were harvested and weighed. Then, the blood was maintained 30 minutes at room temperature for coagulation until the moment of its centrifugation at 4 °C and 12.000 rpm for 10 minutes. Aliquots of serum were stored in freezer –80 °C for adiponectin analysis.

2.7 Serum Concentration of Adiponectin

Serum adiponectin concentration was determined in duplicate using enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's guidelines (RAB1115 — Sigma, St Louis, MO, USA). Results were normalized by total serum protein determined by the BCATM protein assay kit (ThermoFischer Scientific, Waltham, Massachusetts, USA).

2.8 Histological Analysis

The morphology of adipocytes was measured on paraffin sections of SC-WAT and RP-WAT (3 µm) stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, Missouri, USA). Digital images from 50 adipocytes per animal were obtained using a light microscope (Axio. A1 Observer, Zeiss, Jena, Germany) at 400×. After scanning, adipocyte area (μm^2) was traced and calculated using a computerized morphometry analysis system (Image J software, V. 1.53k, National Institutes of Health, Bethesda, MD, USA). The following parameters were calculated: diameter in µm [32], volume in pL [33], mass in µg [34,35], and adipocyte number in RP-WAT [34]. The adipocytes size was distributed into quartiles: adipocytes in quartile 1 were classified as small; in the interquartile range 1 and 2, medium; in the interquartile range 2 and 3, large; and above quartile 3, very large. All WAT analyses were done by a single observer (Cauduro, L.) blinded to mice identification.

Additionally, three sequential sections (5 µm) of the splenic portion of pancreas were included in paraffin and subsequently stained with hematoxylin and eosin. The images of pancreatic islets and pancreas were acquired in a digital light microscopy (Axio observator. A1, Zeiss, Jena, Germany), at 400× and 40× magnification, respectively, using the Motic Images Plus 3.0 program. Measurements of the total islet area (μm^2) were taken by manually circling them in Image J software (V. 1.53k). Results were normalized dividing the islets by the total pancreas area, and data were expressed as islets/pancreas ratio [13]. The number of islets per mm^2, major axis, minor axis, aspect ratio (major axis/minor axis), perimeter and circularity ($4\pi \times$ area/perimeter^2) of the islets were also calculated. The analysis of the individual size of the islets was determined by the distribution of areas in quartiles, in the same way as the adipose tissue. All pancreas analysis was done by a single observer (Santos, M.) blinded to the identities of the mice.

2.9 Pancreatic Islets Isolation

Pancreatic islets were isolated by the collagenase digestion method, as previously described by Lacy and Kostianovsky [36]. In short, after distension via pancreatic duct injection of Hanks solution added with 0.07% collagenase (type V), pancreas was removed and digested in a shaking water bath at 37 °C. Pancreatic islets were isolated from animals of all experimental groups and used in the static insulin secretion.

2.10 Static Insulin Secretion

Groups of 10 isolated islets were pre-incubated for 30 min at 37 °C in 500 µL of Krebs-Henseleit solution (KH) containing 0.2% bovine serum albumin and 2.8 mM glucose. Subsequently, the islets were incubated for 60 min in KH (0.2% albumin) plus 5.6- or 16.7-mM glucose. After incubation, the solution was frozen at –20 °C for subsequent measurement of islet insulin secretion. For total insulin content, islets were disrupted in an alcohol-acid HCl aqueous solution (52 ethanol: 17 water; 1 hydrochloric acid v/v), sonicated (three pulses of 5 s) to release the insulin content inside the islets, and the islet lysate solution was also frozen at –20 °C [37]. These samples of insulin secretion and insulin content were measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Millipore Corporation, Billerica, USA). Results are expressed as the ratio of insulin secretion/insulin content

2.11 Statistical Analyses

All values are expressed as mean ± SEM. After Shapiro-Wilk's test to confirm normal distribution, data were analyzed by two-way analyses of variance (ANOVA). The Tukey's *post hoc* test was used for multiple comparisons. Pearson's correlation test was used to analyze the association between variables. A p value of 0.05 or less was considered statistically significant. The analyses were performed by GraphPad Prism, v.7.0 software (GraphPad Software, San Diego, CA, USA).

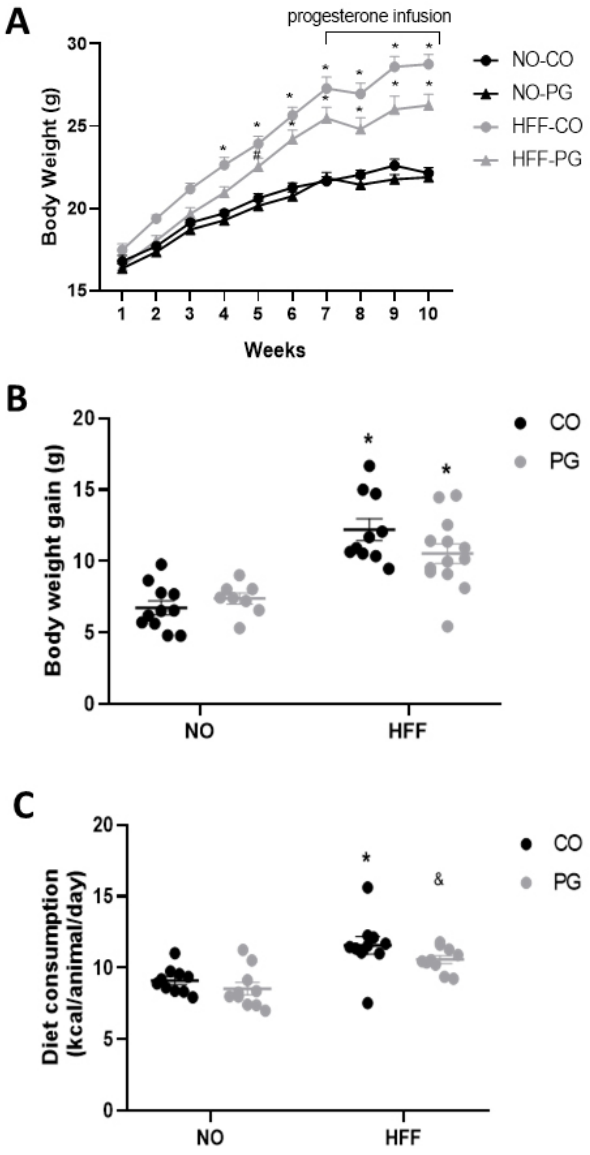

Fig. 1. Body weight and diet consumption. (A) Body weight evolution, (B) Body weight gain, and (C) Diet consumption (kcal/animal/day). Data are presented as means ± SEM. Results were analyzed by two-way analyses of variance (ANOVA) plus Tukey's post hoc test: * $p \leq 0.05$ vs. normocaloric diet control (NO-CO) and normocaloric diet plus progesterone (NO-PG), # $p \leq 0.05$ vs. NO-CO; & $p \leq 0.05$ vs. NO-PG. NO-CO (n = 11), NO-PG (n = 8), HFF-CO (high-fat and -fructose diet control) (n = 10), HFF-PG (high-fat and -fructose diet plus progesterone) (n = 13).

3. Results

3.1 Body and Tissues Weights, Food Intake and Metabolic Parameters

All animals started the protocol weighing around 16 g, without statistical difference. Hypercaloric diet efficiently increased body weight compared to normocaloric diet ($p <$ 0.0001). Body weight in the HFF-CO group was higher compared with NO-CO and NO-PG groups from the fourth week, while the difference between HFF-PG and NO-CO appeared only one week later. No difference in body weight was observed between HFF-CO and HFF-PG (Fig. 1A), keeping in mind that a possible influence of progesterone could be observed only after minipump implant at the 7th week. Additionally, the body weight gain in HFF groups (HFF-CO: 12.21 g and HFF-PG: 10.53 g) was significantly higher ($p < 0.0001$) than in NO groups (NO-CO: 6.74 g and NO-PG: 7.39 g) (Fig. 1B).The average daily food intake per animal in grams was not different among groups, but when expressed as kcal per animal, the caloric intake in HFF groups (HFF-CO: 11.56 ± 0.58 kcal/animal and HFF-PG: 10.58 ± 0.25 kcal/animal) groups was greater ($p <$ 0.0001) than NO groups (NO-CO: 9.11 ± 0.25 kcal/animal and NO-PG: 8.54 ± 0.41 kcal/animal) (Fig. 1C).

Table 2 shows the weights of tissues and organs corrected by the body weight measured at the time of death procedure. The weight of SC-WAT and RP-WAT were higher in HFF-CO and HFF-PG compared with NO-CO and NO-PG groups ($p < 0.0001$). On the other hand, the weight of liver, kidneys and left ventricle were lower in HFF groups compared with NO groups ($p < 0.0001$). No difference was observed between HFF-CO and HFF-PG. In addition, pancreas and iBAT did not show any significant difference in relation to diet or progesterone treatment.

Through the analysis of metabolic parameters at rest performed by indirect calorimetry, we observed that the values of VO_2, VCO_2, RER, EE, carbohydrate, and lipid oxidation were not different among groups (Table 3). Although the time until exhaustion and maximal velocity reached during the running test did not differ, HFF groups showed lower VO_2max ($p = 0.02$), VCO_2max ($p = 0.001$) and EE ($p = 0.03$) in relation to NO groups. No difference was observed between HFF-CO and HFF-PG. Carbohydrate and lipid oxidation assessed at the time of VO_2max, RER and iVO_2 did not differ among groups (Table 3).

3.2 Glycemic Metabolism

The HFF-CO and HFF-PG groups exhibited significantly higher fasting glycemia (HFF-CO: 143.10 ± 6.47 mg/dL and HFF-PG: 141.89 ± 4.68 mg/dL) compared with NO-CO and NO-PG groups (NO-CO: 131.55 ± 4.40 mg/dL and NO-PG: 127.88 ± 6.93 mg/dL; Fig. 2A; $p = 0.03$). In GTT, post-hoc tests at each time points showed that HFF-CO had higher glucose concentration than the NO-CO group at minute 15 (Fig. 2B; $p = 0.001$). In addition, the glucose concentration was higher in the HFF-CO group compared with NO-CO ($p < 0.0001$), NO-PG ($p < 0.0001$) and HFF-PG ($p = 0.03$) groups at minute 30 (Fig. 2B). Hypercaloric diet also statistically increased the area under the glycemic curve (AUC) compared to normocaloric diet ($p = 0.0005$; Fig. 2C). In the ITT, the HFF-CO and HFF-PG groups showed glucose concentration higher than the NO-

Table 3. Metabolic response at rest and at maximal running test.

Resting	NO-CO	NO-PG	HFF-CO	HFF-PG
VO_2 (mL/min/kg)	34.77 ± 5.59	35.16 ± 3.45	37.28 ± 3.32	32.59 ± 2.94
VCO_2 (mL/min/kg)	27.15 ± 3.48	28.33 ± 2.62	23.90 ± 2.09	27.01 ± 1.50
RER	0.78 ± 0.04	0.80 ± 0.05	0.71 ± 0.05	0.85 ± 0.03
EE (kcal/kg/day)	244.43 ± 36.54	246.80 ± 23.09	234.21 ± 27.42	226.38 ± 18.84
CHO oxidation (mg/min/kg)	75.61 ± 15.42	69.06 ± 9.78	110.01 ± 21.39	61.60 ± 8.88
Lipid oxidation (mg/min/kg)	14.40 ± 4.35	11.42 ± 3.17	22.35 ± 3.50	9.33 ± 2.62
Running test				
Exercise performance (min)	24.25 ± 1.25	27.25 ± 0.92	23 ± 1.71	23.43 ± 1.02
Max. velocity (cm/s)	31.25 ± 2.1	36.13 ± 1.6	29.75 ± 2.64	29.86 ± 1.56
VO_2 máx. (mL/min/kg)	53.03 ± 2.82	47.95 ± 4.40	43.57 ± 1.63*	42.83 ± 3.09*
VCO_2 máx. (mL/min/kg)	47.23 ± 3.35	47.20 ± 0.77	39.56 ± 2.26*	38.53 ± 1.86*
RER	0.89 ± 0.04	1.05 ± 0.10	0.93 ± 0.03	0.91 ± 0.05
iVO_2 (cm/s)	29.86 ± 1.87	34.44 ± 1.47	28.29 ± 2.50	29.46 ± 1.27
EE (kcal/kg/day)	375.12 ± 20.17	345.73 ± 23.57	326.4 ± 16.79*	303.67 ± 19.42*
CHO oxidation (mg/min/kg)	89.70 ± 9.20	66.66 ± 19.92	71.25 ± 6.49	71.21 ± 3.71
Lipid oxidation (mg/min/kg)	9.69 ± 4.01	1.25 ± 7.33	6.69 ± 3.03	7.18 ± 3.71

Data are presented as mean ± SEM. The results were compared by the two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG.

VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; EE, energy expenditure; CHO, carbohydrate; iVO_2, intensity at oxygen consumption maximum. NO-CO (n = 8), NO-PG (n = 8), HFF-CO (n = 8), HFF-PG (n = 7).

CO group at minute 5 ($p = 0.03$ and $p < 0.0001$, respectively), and the HFF-PG group maintained the difference ($p = 0.004$) from the NO-CO group at minute 10 (Fig. 2D). The kITT did not differ among groups (Fig. 2E).

3.3 Adipose Tissue Histological Analysis

Morphometric analysis of RP-WAT by histology slides stained with hematoxylin and eosin (Fig. 3A) showed that adipocytes area from the HFF-CO and HFF-PG groups were, respectively, 141.2% and 130.5% larger than NO-CO and NO-PG groups ($p < 0.0001$) (Fig. 3B). When we looked at the frequency of adipocyte size in RP-WAT (Fig. 3C), we observed a higher percentage of very large and medium size adipocytes in the HFF-CO compared with NO-CO and NO-PG groups, and in the HFF-PG compared with the NO-CO group. In the morphometric analysis of SC-WAT (Fig. 3D), the adipocytes area of the HFF-CO and HFF-PG groups were 90.4% and 60.1% larger than NO-CO and NO-PG groups, respectively ($p < 0.0001$) (Fig. 3E). Regarding the frequency of adipocyte size in SC-WAT, both HFF-CO and HFF-PG groups presented a higher percentage of very large adipocytes compared with NO groups (Fig. 3F). Additional data from RP-WAT and SC-WAT adipocytes are summarized in Table 4. In the RP-WAT, adipocytes diameter ($p < 0.0001$), volume ($p = 0.0006$) and mass ($p = 0.0003$) were greater in HFF groups than NO groups. These differences were also observed in SC-WAT adipocytes diameter ($p < 0.0001$) and mass ($p = 0.001$). The number of adipocytes estimated in the RP-WAT did not differ in any condition.

3.4 Serum Concentration of Adiponectin

Serum adiponectin concentration was lower in HFF groups than NO groups ($p < 0.0001$). Post-hoc test evidenced that HFF-CO group was significantly lower than NO-CO ($p = 0.002$) and NO-PG ($p = 0.0004$), while the HFF-PG group showed lower adiponectin concentration than NO-PG group ($p = 0.04$) (Fig. 4A). Furthermore, we correlated values from all groups and serum adiponectin was positively correlated to kITT (Fig. 4B) and negatively correlated to AUC (Fig. 4C).

3.5 Pancreas Histological Analysis

Representative pancreatic tissue of the four groups stained with hematoxylin and eosin for morphometric measurements of the islets are shown in Fig. 5A. The total area of the pancreatic islets was not statically different among groups (Fig. 5B), not even when we normalized this variable by the total area of the pancreas (islet/pancreas ratio) (Fig. 5C). Additionally, no significant difference was observed in the percentage of islets considered small, medium, large and very large in the pancreas of animals in each group (Fig. 5D). Table 5 summarizes some other morphometric parameters measured in pancreatic islets such as number of islets/mm^2 of pancreas, perimeter, major axis, minor axis, aspect ratio and circularity. Progesterone treatment statistically increased islets circularity ($p = 0.001$), and post-hoc test showed that islets from NO-PG ($p = 0.01$) and HFF-PG ($p = 0.01$) groups were closer to a perfect circle than the NO-CO group.

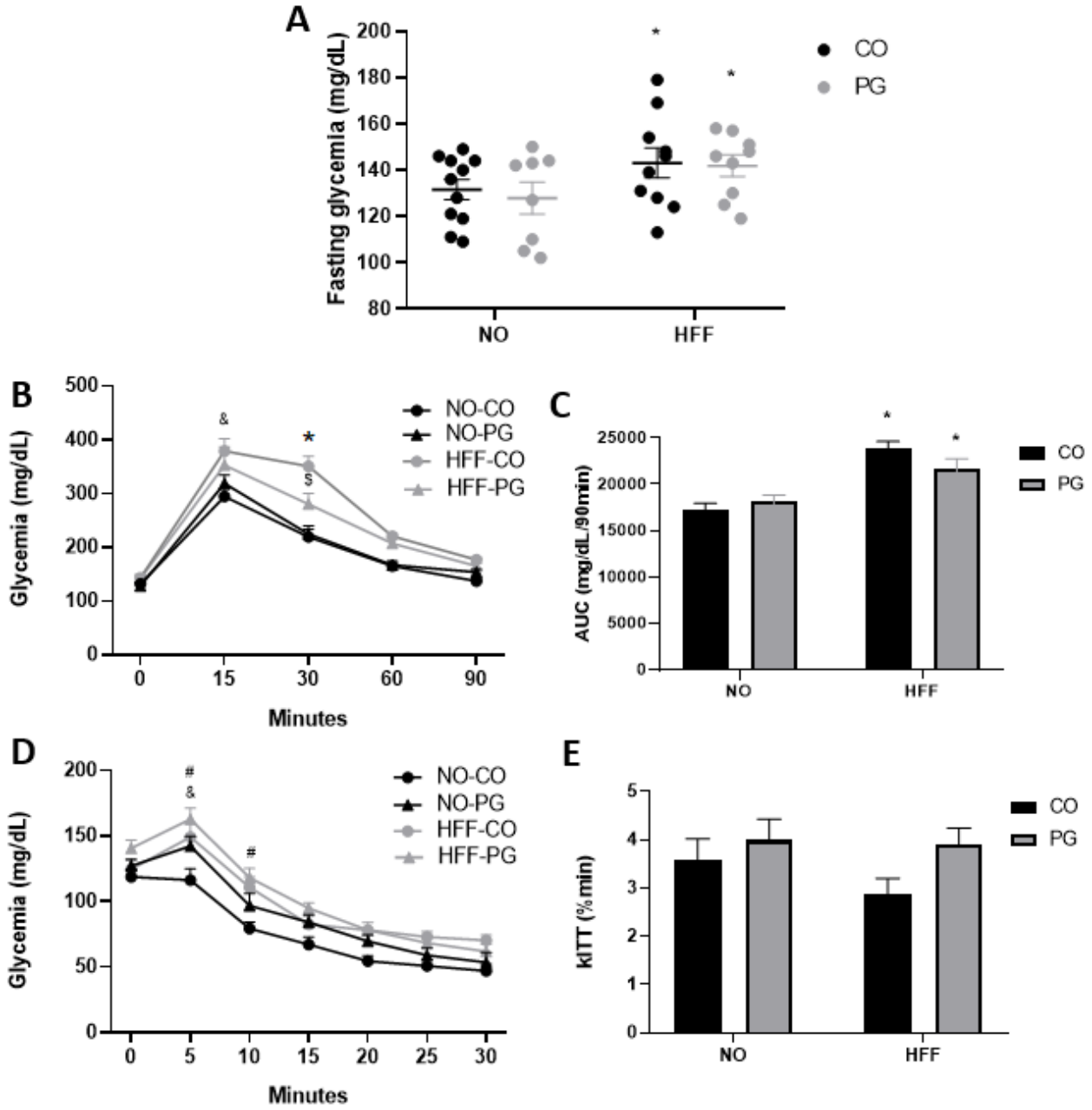

Fig. 2. Glycemic Metabolism. (A) Fasting glycemia. (B) Glycemic Curve obtained in glucose tolerance test (GTT). (C) Area under curve. (D) Glycemic Curve obtained in insulin tolerance test (ITT), and (E) kITT. Data are presented as means ± SEM. Results were analyzed by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG, & $p \leq 0.05$ HFF-CO vs. NO-CO, \$ $p \leq 0.05$ vs. HFF-PG, # $p \leq 0.05$ HFF-PG vs. NO-CO. NO-CO (n = 11), NO-PG (n = 8), HFF-CO (n = 10), HFF-PG (n = 9).

To obtain a clear picture of WAT and pancreatic islets dynamics, we correlated morphometric parameters from these tissues of the four groups and a positive correlation was found between islet/pancreas ratio to RP-WAT adipocytes area, RP-WAT adipocytes volume and RP-WAT adipocytes mass (Fig. 6). No correlation was found in SC-WAT and pancreatic islets parameters.

3.6. Insulin Secretion by Isolated Pancreatic Islets

Fig. 7A shows that islets incubated with basal glucose concentration (5.6 mM) did not present significant difference in insulin secretion. When islets were incubated at high glucose concentration (16.7 mM), the hypercaloric diet abolished glucose-stimulated insulin secretion, even in HFF-PG group ($p = 0.03$; Fig. 7B). In that scenario, we investigated which factors could be associated with this reduced insulin secretion, and the correlation analysis with all groups showed that insulin secretion/insulin content re-

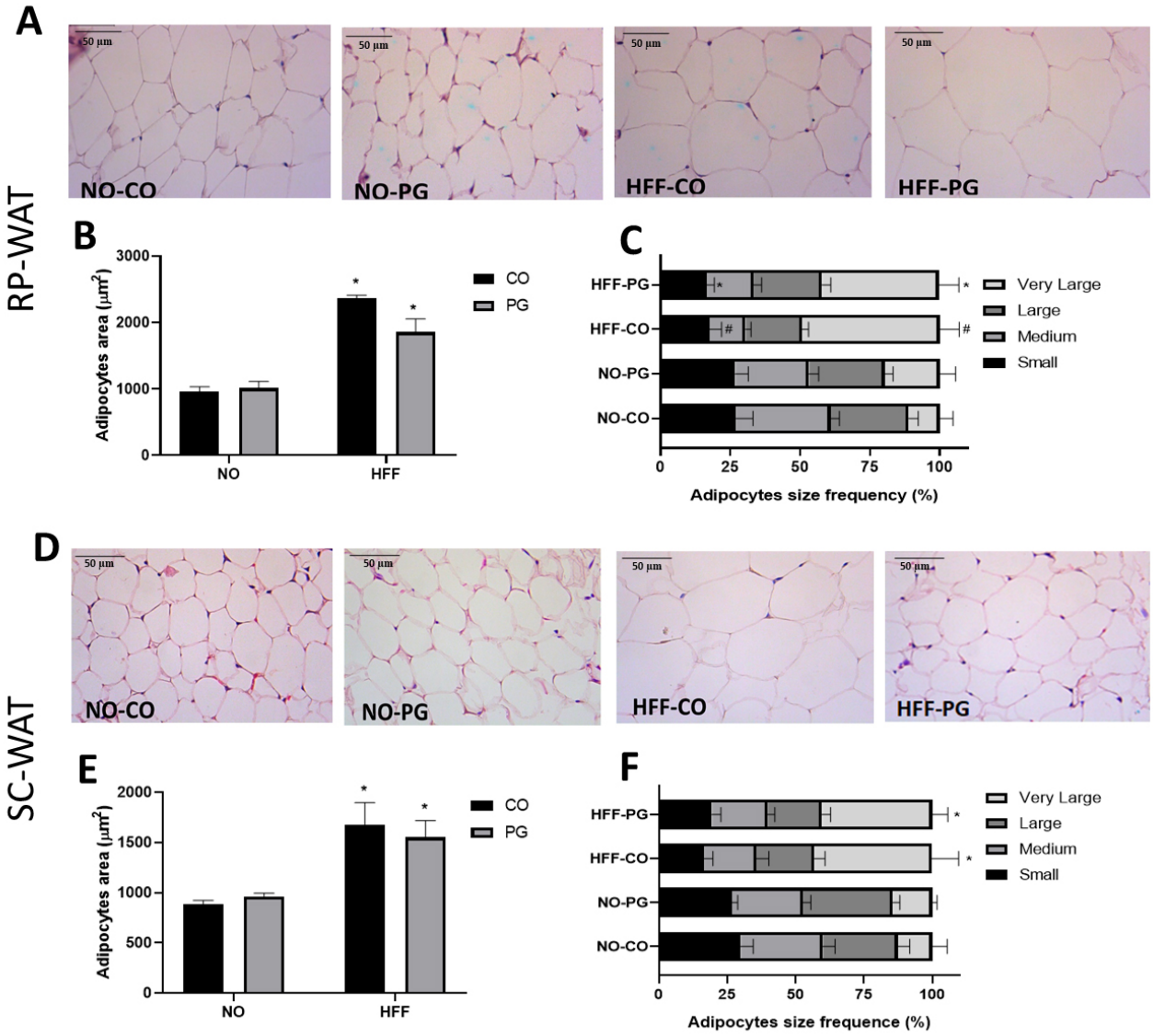

Fig. 3. Histological analysis of adipose tissue. (A) Representative photomicrographs of histological sections of RP-WAT stained with hematoxylin and eosin (400× magnification). (B) RP-WAT adipocytes area. (C) RP-WAT adipocytes size frequency. (D) Representative photomicrographs of histological sections of SC-WAT stained with hematoxylin and eosin (400× magnification). (E) SC-WAT adipocytes area and (F) SC-WAT adipocytes size frequency. Data are presented as means ± SEM. Results were analyzed by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG, # $p \leq 0.05$ vs. NO-CO. For RP-WAT: NO-CO (n = 7), NO-PG (n = 8), HFF-CO (n = 5), HFF-PG (n = 8). For SC-WAT: NO-CO (n = 8), NO-PG (n = 6), HFF-CO (n = 5), HFF-PG (n = 8).

duces according to SC-WAT adipocytes area (Fig. 7D). Although, in this study, we did not find a positive correlation between glucose-stimulated insulin secretion and serum adiponectin concentration ($p = 0.07$) (Fig. 7C), this correlation was obtained by Nakamura *et al.* [38] in a study with humans that illustrates the complex crosstalk between pancreatic β-cell and adipose tissue in obesity. The same correlation was not found in relation to RP-WAT adipocytes area.

4. Discussion

Obesity, increasingly prevalent in women of reproductive age, can be especially harmful at certain stages of a woman's life, such as pregnancy. We studied the morpho functional aspects of adipose tissue and pancreas of obese female mice treated with low dose of progesterone, and we did not observe any change at this dose in body weight gain, adiposity or adiponectin secretion, nor in morphometry of islets, except in circularity, that was improved in HFF-PG group. Although fasting glycemia did not change among HFF-CO and HFF-PG groups, the results from the

Table 4. Morphometric parameters of adipocytes.

RP-WAT	NO-CO	NO-PG	HFF-CO	HFF-PG
Diameter (µm)	36.36 ± 0.57	38.03 ± 3.38	50.77 ± 0.97*	48.99 ± 1.38*
Volume (pL)	35.27 ± 4.98	42.42 ± 19.21	102.81 ± 3.13$^{\#}$	95.28 ± 16.72$^{\#}$
Mass (µg)	0.03 ± 0.00	0.04 ± 0.01	0.09 ± 0.00$^{\#}$	0.09 ± 0.02*
Adipocyte number	$1.6 \times 10^6 \pm 0.17 \times 10^6$	$1.6 \times 10^6 \pm 0.4 \times 10^6$	$1.6 \times 10^6 \pm 0.2 \times 10^6$	$1.7 \times 10^6 \pm 0.1 \times 10^6$
SC-WAT	NO-CO	NO-PG	HFF-CO	HFF-PG
Diameter (µm)	31.51 ± 0.6	33.10 ± 0.42	43.60 ± 2.06*	41.85 ± 1.48*
Volume (pL)	22.91 ± 2.06	25.95 ± 1.78	52.29 ± 13.01	48.58 ± 9.61
Mass (µg)	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.01$^{\#}$	0.04 ± 0.01
Adipocyte number	$11 \times 10^6 \pm 1 \times 10^6$	$9 \times 10^6 \pm 1 \times 10^6$	$9 \times 10^6 \pm 1 \times 10^6$	$9 \times 10^6 \pm 1 \times 10^6$

Data are presented as mean ± SEM. The results were compared by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG, # $p \leq 0.05$ vs. NO-CO.
For RP-WAT: NO-CO (n = 7), NO-PG (n = 5), HFF-CO (n = 8), HFF-PG (n = 8). For SC-WAT: NO-CO (n = 8), NO-PG (n = 6), HFF-CO (n = 5), HFF-PG (n = 8).

Table 5. Morphometric parameters of pancreatic islets.

	NO-CO	NO-PG	HFF-CO	HFF-PG
Islets/mm^2	1.21 ± 0.15	1.04 ± 0.24	1.11 ± 0.14	1.08 ± 0.12
Perimeter (µm)	328.29 ± 27.10	276.98 ± 24.22	294 ± 11.70	293.84 ± 12.51
Minor Axis (µm)	75.67 ± 5.30	71.61 ± 6.71	70.48 ± 4.32	72.46 ± 3.20
Major Axis (µm)	114.95 ± 10.30	92.74 ± 9.19	100.09 ± 3.45	100.65 ± 4.34
Aspect Ratio	1.54 ± 0.05	1.34 ± 0.06	1.45 ± 0.05	1.40 ± 0.03
Circularity	0.79 ± 0.01	0.84 ± 0.01*	0.81 ± 0.01	0.83 ± 0.01*

Data are presented as mean ± SEM. The results were compared by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO. NO-CO (n = 8), NO-PG (n = 3), HFF-CO (n = 9), HFF-PG (n = 5).

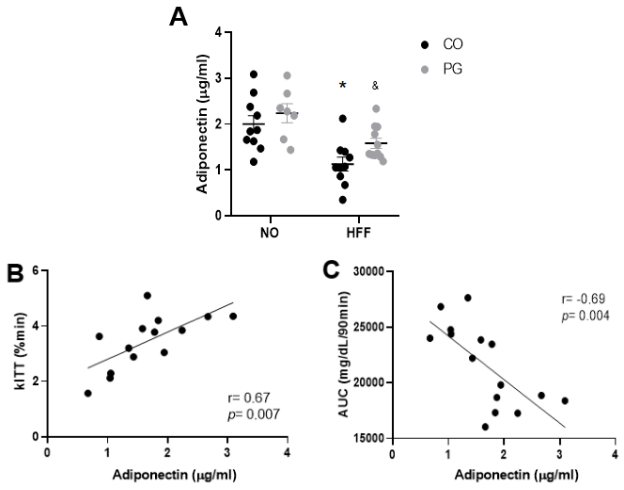

Fig. 4. Adiponectin and glucose metabolism. (A) Serum adiponectin concentration, (B) Correlation of adiponectin and kITT and (C) Correlation of adiponectin and AUC. Data are presented as means ± SEM, analyzed by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG, & $p \leq 0.05$ vs. NO-PG, and Pearson's correlation test. For adiponectin determination: NO-CO (n = 10), NO-PG (n = 7), HFF-CO (n = 10), HFF-PG (n = 11).

glycemic curve in GTT and AUC revealed that progesterone treatment attenuated glucose intolerance induced by hypercaloric diet in HFF-PG group.

Hervey and Hutchinson [39] showed that infusion of progesterone (5 mg/day) increased female rat body weight, with greater amounts of corporeal water and fat. In turn, Holmberg *et al.* [40] found that repeated exposure to 20 mg/kg of allopregnanolone (a progesterone metabolite that has been implicated in evoking hyperphagia), over 5 days induced body weight gain in rats fed on a high-fat diet. Here, progesterone (0.25 mg/kg/day) did not worsen body weight gain and adiposity induced by hypercaloric diet in HFF-PG group, possibly due to the lower concentration of the hormone used in the treatment. The body weight gain is a result of the energy balance, which is determined by the difference between energy intake and energy expenditure. Considering that HFF-CO and HFF-PG groups had higher daily caloric consumption, and that resting energy expenditure measured by indirect calorimetry (VO_2) did not change, the greater body weight gain shown by HFF-CO and HFF-PG groups was due to higher caloric intake. In addition, treatment with progesterone did not interfere with energy balance parameters, which can explain the unchanged body weight gain in both HFF-CO and HFF-PG groups.

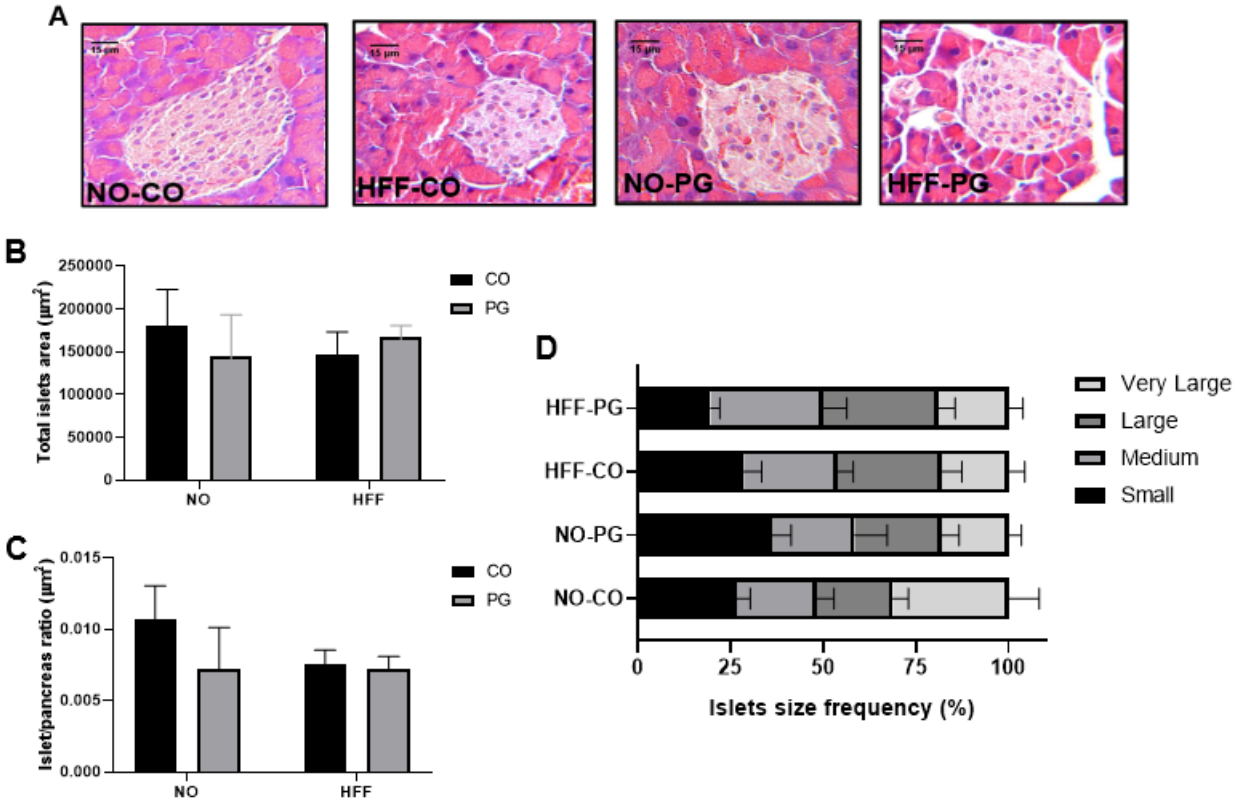

Fig. 5. Histological analysis of pancreas. (A) Representative photomicrographs of histological sections of pancreatic islets stained with hematoxylin and eosin (400× magnification). (B) Total islets area. (C) Pancreatic islet area/pancreas area ratio and (D) islet size frequency in pancreas. Data are presented as means ± SEM. Results were analyzed by two-way ANOVA. NO-CO (n = 7), NO-PG (n = 3), HFF-CO (n = 7), HFF-PG (n = 5).

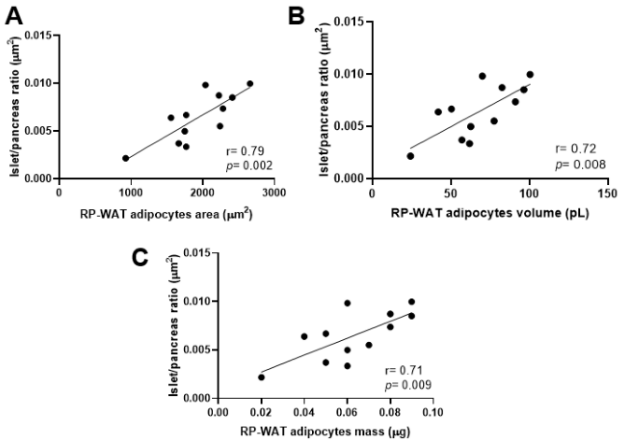

Fig. 6. Correlations of islet/pancreas ratio and RP-WAT morphometry. (A) Correlation of islets/pancreas ratio and adipocytes area. (B) Correlation of islets/pancreas ratio and RP-WAT adipocytes volume and (C) Correlation of islets/pancreas ratio and RP-WAT adipocytes mass. Data were analyzed by Pearson's correlation test.

Obesity is associated with chronic inflammatory state, metabolic dysfunction, and increased cardiovascular risk [12]. Low levels of cardiorespiratory fitness have been associated with higher risk of morbidity and mortality from chronic degenerative diseases, including coronary artery disease, systemic arterial hypertension, diabetes mellitus type 2 and some types of cancer [41]. In our study, we found that HFF-CO and HFF-PG groups showed reduction in aerobic capacity as measured by VO_2max during maximal exercise test. Although progesterone exerts effects on energy metabolism and cardiorespiratory function, and therefore directly influences aerobic capacity [42], the progesterone treatment did not modify the impairment of aerobic capacity associated with obesity in the HFF-PG animals.

The hypercaloric diet consumption impaired the glycemic metabolism as demonstrated by greater fasting glycemia in the HFF-CO and HFF-PG groups. However, contradicting previous evidence that progesterone induced insulin resistance [43,44], our results showed that progesterone treatment attenuated glucose intolerance induced by obesity in the HFF-PG group. A study with ovariectomized diabetic rats showed that the animals lose insulin sensitivity progressively, however, when these animals received progesterone their insulin sensitivity was higher than in the non-hormone-treated groups. However, at the end of the experiment, high doses of progesterone drastically reduced such sensitivity in these female diabetic rats [45], showing

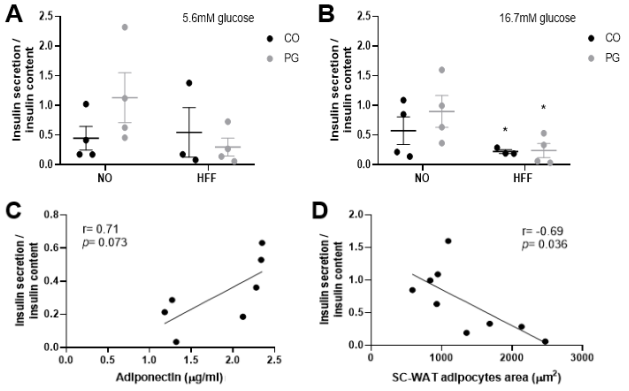

Fig. 7. Insulin secretion analysis. (A) Insulin secretion/insulin islets content after islets incubation with 5.6mM glucose. (B) Insulin secretion/insulin islets content after islets incubation with 16.7mM glucose. (C) Correlation of insulin secretion/insulin content in 16.7 mM and adiponectin and (D) Correlation of insulin secretion/insulin content in 16.7 mM and SC-WAT adipocytes area. Data are presented as means ± SEM, analyzed by two-way ANOVA plus Tukey's post hoc test: * $p \leq 0.05$ vs. NO-CO and NO-PG, and Pearson's correlation test. For insulin secretion and content: NO-CO (n = 4), NO-PG (n = 4), HFF-CO (n = 3), HFF-PG (n = 4).

a possible dose-dependent effect of progesterone. In the present study, despite we did not measured progesterone level after the treatment, we used progesterone infusion rate (0.25 mg/kg/day) based on previous study [28], which was considerably lower compared to dosages used in other studies such as 5, 10 or 20 mg/kg [40] and 3 mg/kg of progesterone [44]. Together, these results suggest that it is essential to consider the progesterone concentration when thinking about its effects on glycemic metabolism.

The increased RP-WAT and SC-WAT adipocytes area, diameter, and mass in the HFF-CO and HFF-PG groups, without change in adipocyte number, reveals that the higher adiposity was due to hypertrophy and not hyperplasia of adipocytes. In fact, when the adipocyte size was distributed into quartiles, the frequency of very large adipocytes was higher in RP-WAT and SC-WAT in both HFF-CO and HFF-PG groups. It has been shown that progesterone stimulates fat storage in females [46]. In addition, it is known that insulin and the adipokine acylation-stimulating protein (ASP) are the most potent fat storage hormones [47]. Thus, the non-worsening of adiposity in HFF-PG may be explained by the ASP resistance induced by obesity. This is supported by the fact that ASP levels follow the progesterone levels in eutrophic women [47]; that, in obese females ASP levels were higher than in obese males [48], and that high levels of ASP may also reflect ASP resistance as observed during pregnancy [47,49]. Further studies are still necessary to clarify this issue.

There is a remarkable predisposition to insulin resistance and β-cell dysfunction in obesity, which is mediated by mechanisms such as lipotoxicity in insulin-sensitive tissues, inflammatory response and adipokines action [50]. As a marker of adipocyte function, we measured adiponectin due to its role in improving insulin sensibility and insulin secretion [51]. We found lower adiponectin concentration in HFF-CO and HFF-PG groups, and a positive correlation between adiponectin and better response in glycemic tests. However, the progesterone treatment did not change these responses, which corroborates the magnitude of the damage to adipocyte morphometry caused by hypercaloric diet.

In face of insulin resistance, pancreatic β-cells must adapt and secrete enough insulin to avoid hyperglycemia [50]. Male mice exhibited glucose intolerance in response to diet-induced obesity, but a partial compensatory response was observed as an increase in pancreatic islet mass [52]. In our study with females, those compensatory responses were not observed in islets morphometry, although the ratio islet area/pancreas area was positively correlated to RP-WAT adipocytes area ($p = 0.002$), volume ($p = 0.008$) and mass ($p = 0.009$) reaffirming the greater demand that obesity imposes on the islet of these females. Compared to SC-WAT, visceral adipose tissue is more metabolically active, sensitive to lipolysis and insulin-resistant, containing a larger number of inflammatory cells and greater capacity to generate free fatty acids [53], which may play a role in islets adaptation. Progesterone did not interfere in islets morphometry, except in the circularity improvement in HFF-PG group. Although little is known about the importance of this morphometric aspect for islet functionality, it is shown that stellate cells presented in fibrotic islets of Goto-Kakizaki rats are associated with impaired β-cell function and proliferation [54], suggesting that islets with irregular shapes may be less functional.

Gonzalez *et al.* [55] studying female mice observed rises in β-cell mass and insulin secretion as a compensatory response to a 12-week high-fat diet. In contrast, our work showed an impairment in glucose-stimulate insulin secretion in the HFF-CO and HFF-PG groups after a 10-week high-fat and -fructose diet. Similar results were obtained in a study with male mice fed on a 6-week extremely high-fructose diet (60% fructose and 30% fructose supplementation in water) where an impaired glucose-stimulated insulin secretion by isolated islets was observed [56]. Here, progesterone treatment did not modulate islets secretion capacity of obese female mice, as observed by previous studies [57,58], in which progesterone infusion at high rates increased glucose-stimulate insulin secretion by isolated islets of non-obese female rats. However, we found an inverse correlation between insulin secretion and SC-WAT adipocytes area ($p = 0.036$). In obesity, the SC-WAT deposit, rather than visceral deposit, has been inversely associated also with adiponectin levels [59], probably because SC-WAT is more susceptible to endoplasmic reticu-

lum stress, which reduces the synthesis and secretion of this adipokine [60]. Although, we did not find a positive correlation between glucose-stimulated insulin secretion and serum adiponectin concentration (p = 0.07; Fig. 7C), this correlation was obtained by Nakamura *et al*. [38] in a study with humans that illustrates the complex crosstalk between pancreatic β-cell and adipose tissue in obesity.

5. Conclusions

Taken together, our results showed that a low dose of progesterone does not worsen the effects of hypercaloric diet in glycemic metabolism, morphology and function of adipose tissue and pancreatic islets in female animals. These results may improve the understanding of the mechanisms underlying the pathogenesis of obesity in women and eventually open new avenues for therapeutic strategies and better comprehension of the interactions between progesterone effects and obesity.

Availability of Data and Materials

Datasets used and/or analyzed for this study are available from the corresponding author upon appropriate request.

Author Contributions

MPS was responsible for the accompaniment and treatment of the animals, glycemic tests, calorimetry tests, analyses related to the pancreas, article writing and corrections to the publication. LFRC was responsible for the accompaniment and treatment of the animals, glycemic tests, calorimetry tests, analyses of adipose tissues, and article writing. MMF was responsible for the accompaniment and treatment of the animals, glycemic tests and calorimetry tests. LFM assisted with the glycemic tests, animal death procedure, and samples collection. BV performed calorimetry tests. EAVB performed islet isolation and insulin secretion assay. ALVA contributed to implantation of mini-pumps and dosage of insulin and adiponectin by ELISA. ROP collaborated to obtain the histology results, contributing with the methodological adjustments to each type of tissue and discussing the results obtained in the process. MMR contributed to the diet elaboration and data discussion. PF supported in histology analyses and discussion of the experimental design. FSE contributed in project elaboration, experimental design, data discussion, manuscript revision and corrections to the publication. AKAM was responsible for obtaining financial support, experimental design conception, data discussion, and writing and revision of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The animal study was reviewed and approved by Ethics Committee of School of Arts, Sciences and Humanities of University of São Paulo (protocol number 003/2018).

Acknowledgment

We thank Professors Angelo R. Carpinelli and José E. Krieger for providing laboratory support. Likewise, we would like to thank Isabel Marques and Fernanda Barrence for their technical support, and Cynthia Walser for the careful review of the English in this manuscript.

Funding

This study was supported by São Paulo Research Foundation (FAPESP) under the process number 18/22361-9.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] World Health Organization. Obesity and overweight. 2021. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (Accessed: 14 February 2023).

[2] Blüher M. Obesity: global epidemiology and pathogenesis. Nature Reviews. Endocrinology. 2019; 15: 288–298.

[3] Kautzky-Willer A, Harreiter J, Pacini G. Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. Endocrine Reviews. 2016; 37: 278–316.

[4] Mohan V, Unnikrishnan R, Shobana S, Malavika M, Anjana RM, Sudha V. Are excess carbohydrates the main link to diabetes & its complications in Asians? The Indian Journal of Medical Research. 2018; 148: 531–538.

[5] Pereira RM, Botezelli JD, da Cruz Rodrigues KC, Mekary RA, Cintra DE, Pauli JR, *et al*. Fructose Consumption in the Development of Obesity and the Effects of Different Protocols of Physical Exercise on the Hepatic Metabolism. Nutrients. 2017; 9: 405.

[6] Della Corte KW, Perrar I, Penczynski KJ, Schwingshackl L, Herder C, Buyken AE. Effect of Dietary Sugar Intake on Biomarkers of Subclinical Inflammation: A Systematic Review and Meta-Analysis of Intervention Studies. Nutrients. 2018; 10: 606.

[7] Newens KJ, Walton J. A review of sugar consumption from nationally representative dietary surveys across the world. Journal of Human Nutrition and Dietetics: the Official Journal of the British Dietetic Association. 2016; 29: 225–240.

[8] Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, *et al*. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021; 143: e984–e1010.

[9] Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism: Clinical and Experimental. 2019; 92: 121–135.

[10] Castro AVB, Kolka CM, Kim SP, Bergman RN. Obesity, insulin resistance and comorbidities? Mechanisms of association. Arquivos Brasileiros De Endocrinologia E Metabologia. 2014; 58: 600–609.

[11] Marcelin G, Gautier EL, Clément K. Adipose Tissue Fibrosis in

Obesity: Etiology and Challenges. Annual Review of Physiology. 2022; 84: 135–155.
[12] Kawai T, Autieri MV, Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. American Journal of Physiology. Cell Physiology. 2021; 320: C375–C391.
[13] Boschetti D, Muller CR, Américo ALV, Vecchiatto B, Martucci LF, Pereira RO, *et al*. Aerobic Physical Exercise Improves Exercise Tolerance and Fasting Glycemia Independent of Body Weight Change in Obese Females. Frontiers in Endocrinology. 2021; 12: 772914.
[14] Marinho TDS, Martins FF, Cardoso LEDM, Aguila MB, Mandarim-de-Lacerda CA. Pancreatic islet cells disarray, apoptosis, and proliferation in obese mice. The role of Semaglutide treatment. Biochimie. 2022; 193: 126–136.
[15] Cerf ME. Beta Cell Physiological Dynamics and Dysfunctional Transitions in Response to Islet Inflammation in Obesity and Diabetes. Metabolites. 2020; 10: 452.
[16] Azevedo-Martins AK, Monteiro AP, Lima CL, Lenzen S, Curi R. Fatty acid-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells. Toxicology *in Vitro*: an International Journal Published in Association with BIBRA. 2006; 20: 1106–1113.
[17] Šimják P, Cinkajzlová A, Anderlová K, Pařízek A, Mráz M, Kršek M, *et al*. The role of obesity and adipose tissue dysfunction in gestational diabetes mellitus. The Journal of Endocrinology. 2018; 238: R63–R77.
[18] Di Renzo GC, Giardina I, Clerici G, Brillo E, Gerli S. Progesterone in normal and pathological pregnancy. Hormone Molecular Biology and Clinical Investigation. 2016; 27: 35–48.
[19] Azeez JM, Susmi TR, Remadevi V, Ravindran V, Sasikumar Sujatha A, Ayswarya RNS, *et al*. New insights into the functions of progesterone receptor (PR) isoforms and progesterone signaling. American Journal of Cancer Research. 2021; 11: 5214–5232.
[20] Nunes VA, Portioli-Sanches EP, Rosim MP, Araujo MS, Praxedes-Garcia P, Valle MMR, *et al*. Progesterone induces apoptosis of insulin-secreting cells: insights into the molecular mechanism. The Journal of Endocrinology. 2014; 221: 273–284.
[21] Sorenson RL, Brelje TC, Roth C. Effects of steroid and lactogenic hormones on islets of Langerhans: a new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. Endocrinology. 1993; 133: 2227–2234.
[22] Sitruk-Ware R. Non-clinical studies of progesterone. Climacteric: the Journal of the International Menopause Society. 2018; 21: 315–320.
[23] EPPPIC Group. Evaluating Progestogens for Preventing Preterm birth International Collaborative (EPPPIC): meta-analysis of individual participant data from randomised controlled trials. Lancet (London, England). 2021; 397: 1183–1194.
[24] Shoupe D. Effects of desogestrel on carbohydrate metabolism. American Journal of Obstetrics and Gynecology. 1993; 168: 1041–1047.
[25] Smith GI, Reeds DN, Okunade AL, Patterson BW, Mittendorfer B. Systemic delivery of estradiol, but not testosterone or progesterone, alters very low density lipoprotein-triglyceride kinetics in postmenopausal women. The Journal of Clinical Endocrinology and Metabolism. 2014; 99: E1306–E1310.
[26] Lima TDR, Voltarelli FA, Freire LS, da Silva FA, de Almeida PC, Ávila ETP, *et al*. High-fat diet and fructose drink introduced after weaning rats, induces a better human obesity model than very high-fat diet. Journal of Food Biochemistry. 2021; 45: e13671.
[27] Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. The Journal of Nutrition. 1993; 123: 1939–1951.
[28] Rosen SF, Ham B, Drouin S, Boachie N, Chabot-Dore AJ, Austin JS, *et al*. T-Cell Mediation of Pregnancy Analgesia Affecting Chronic Pain in Mice. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2017; 37: 9819–9827.
[29] Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, *et al*. Estimates of *in vivo* insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. The Journal of Clinical Endocrinology and Metabolism. 1989; 68: 374–378.
[30] Petrosino JM, Heiss VJ, Maurya SK, Kalyanasundaram A, Periasamy M, LaFountain RA, *et al*. Graded Maximal Exercise Testing to Assess Mouse Cardio-Metabolic Phenotypes. PloS One. 2016; 11: e0148010.
[31] Machado CEP, Caputo F, Denadai BS. Exercise intensity corresponding to VO2max during cycling: analysis of different criteria in trained individuals. Brazilian Journal of Physical Education and Sport. 2004; 18: 333–341.
[32] Parlee SD, Lentz SI, Mori H, MacDougald OA. Quantifying size and number of adipocytes in adipose tissue. Methods in Enzymology. 2014; 537: 93–122.
[33] Di Girolamo M, Mendlinger S, Fertig JW. A simple method to determine fat cell size and number in four mammalian species. The American Journal of Physiology. 1971; 221: 850–858.
[34] Heinonen S, Saarinen L, Naukkarinen J, Rodríguez A, Frühbeck G, Hakkarainen A, *et al*. Adipocyte morphology and implications for metabolic derangements in acquired obesity. International Journal of Obesity (2005). 2014; 38: 1423–1431.
[35] Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, *et al*. Dynamics of fat cell turnover in humans. Nature. 2008; 453: 783–787.
[36] Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes. 1967; 16: 35–39.
[37] Oliveira CAMD, Paiva MF, Mota CAS, Ribeiro C, Leme JACDA, Luciano E, *et al*. Exercise at anaerobic threshold intensity and insulin secretion by isolated pancreatic islets of rats. Islets. 2010; 2: 240–246.
[38] Nakamura A, Miyoshi H, Ukawa S, Nakamura K, Nakagawa T, Terauchi Y, *et al*. Serum adiponectin and insulin secretion: A direct or inverse association? Journal of Diabetes Investigation. 2018; 9: 1106–1109.
[39] Hervey E, Hervey GR. The effects of progesterone on body weight and composition in the rat. The Journal of Endocrinology. 1967; 37: 361–381.
[40] Holmberg E, Johansson M, Bäckström T, Löfgren M, Haage D. Repeated allopregnanolone exposure induces weight gain in schedule fed rats on high fat diet. Physiology & Behavior. 2015; 140: 1–7.
[41] Neto G, Farinatti P. Non-exercise models for prediction of aerobic fitness and applicability on epidemiological studies: Descriptive review and analysis of the studies. Revista Brasileira de Medicina do Esporte. 2003; 9: 304–324.
[42] Constantini NW, Dubnov G, Lebrun CM. The menstrual cycle and sport performance. Clinics in Sports Medicine. 2005; 24: e51–e82, xiii–xiv.
[43] Kunju SM, Fousia L. Progesterone Induced Insulin Resistance-an Animal Study. Journal of Evidence-Based Medicine and Healthcare. 2019; 6: 922–928.
[44] Masuyama H, Hiramatsu Y. Potential role of estradiol and progesterone in insulin resistance through constitutive androstane receptor. Journal of Molecular Endocrinology. 2011; 47: 229–239.
[45] Ordóñez P, Moreno M, Alonso A, Fernández R, Díaz F,

González C. Insulin sensitivity in streptozotocin-induced diabetic rats treated with different doses of 17beta-oestradiol or progesterone. Experimental Physiology. 2007; 92: 241–249.
[46] Kalkhoff RK. Metabolic effects of progesterone. American Journal of Obstetrics and Gynecology. 1982; 142: 735–738.
[47] Saleh J, Al-Khanbashi M, Al-Maarof M, Al-Lawati M, Rizvi SG, Cianflone K. Acylation-stimulating protein increases and correlates with increased progesterone levels during the luteal phase of the menstrual cycle. European Journal of Endocrinology. 2009; 160: 301–307.
[48] Maslowska M, Vu H, Phelis S, Sniderman AD, Rhode BM, Blank D, *et al*. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. European Journal of Clinical Investigation. 1999; 29: 679–686.
[49] Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K. Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertility and Sterility. 2009; 91: 213–219.
[50] Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006; 444: 840–846.
[51] Nigro E, Scudiero O, Monaco ML, Palmieri A, Mazzarella G, Costagliola C, *et al*. New insight into adiponectin role in obesity and obesity-related diseases. BioMed Research International. 2014; 2014: 658913.
[52] Roat R, Rao V, Doliba NM, Matschinsky FM, Tobias JW, Garcia E, *et al*. Alterations of pancreatic islet structure, metabolism and gene expression in diet-induced obese C57BL/6J mice. PloS One. 2014; 9: e86815.
[53] Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity Reviews: an Official Journal of the International Association for the Study of Obesity. 2010; 11: 11–18.
[54] Li FF, Chen BJ, Li W, Li L, Zha M, Zhou S, *et al*. Islet Stellate Cells Isolated from Fibrotic Islet of Goto-Kakizaki Rats Affect Biological Behavior of Beta-Cell. Journal of Diabetes Research. 2016; 2016: 6924593.
[55] Gonzalez A, Merino B, Marroquí L, Ñeco P, Alonso-Magdalena P, Caballero-Garrido E, *et al*. Insulin hypersecretion in islets from diet-induced hyperinsulinemic obese female mice is associated with several functional adaptations in individual β-cells. Endocrinology. 2013; 154: 3515–3524.
[56] Asghar ZA, Cusumano A, Yan Z, Remedi MS, Moley KH. Reduced islet function contributes to impaired glucose homeostasis in fructose-fed mice. American Journal of Physiology. Endocrinology and Metabolism. 2017; 312: E109–E116.
[57] Howell SL, Tyhurst M, Green IC. Direct effects of progesterone on rat islets of Langerhans *in vivo* and in tissue culture. Diabetologia. 1977; 13: 579–583.
[58] Costrini NV, Kalkhoff RK. Relative effects of pregnancy, estradiol, and progesterone on plasma insulin and pancreatic islet insulin secretion. The Journal of Clinical Investigation. 1971; 50: 992–999.
[59] Frederiksen L, Nielsen TL, Wraae K, Hagen C, Frystyk J, Flyvbjerg A, *et al*. Subcutaneous rather than visceral adipose tissue is associated with adiponectin levels and insulin resistance in young men. The Journal of Clinical Endocrinology and Metabolism. 2009; 94: 4010–4015.
[60] Torre-Villalvazo I, Bunt AE, Alemán G, Marquez-Mota CC, Diaz-Villaseñor A, Noriega LG, *et al*. Adiponectin synthesis and secretion by subcutaneous adipose tissue is impaired during obesity by endoplasmic reticulum stress. Journal of Cellular Biochemistry. 2018; 119: 5970–5984.