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Maternal vitamin D administration attenuates metabolic disturbances induced by prenatal exposure to dexamethasone in a sex-dependent manner

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ABSTRACT

Purpose: The overexposure to synthetic glucocorticoids (GC) during pregnancy can predispose to metabolic diseases during adulthood. Vitamin D is not only crucial for fetal development, but also exerts direct effects on the GC sensitivity and down-regulates GC receptors. Given the vitamin D effects on glucocorticoid-related parameters, we aimed to investigate a possible protective role of maternal vitamin D administration on the glucose homeostasis of rats exposed to dexamethasone in utero.

Methods: Pregnant rats received dexamethasone (0.1 mg/kg, Dex) daily between the 14th and 19th days of pregnancy. A subgroup of dexamethasone-treated dams received oral administration of vitamin D (500UI, DexVD) during the whole gestation. The corresponding control groups of dams were included (CTL and VD groups, respectively). Male and female offspring were evaluated at 3, 6 and 12 months of age.

Results: Prenatal exposure to dexamethasone caused metabolic disruption in an age and sex-dependent manner being the older male offspring more susceptible to insulin resistance, fatty liver and beta-cell mass expansion than females. Furthermore, we demonstrated that prenatal GC led to glucose intolerance in male and female offspring in an age-dependent manner. Maternal vitamin D administration did not influence glucose intolerance but attenuated the insulin resistance, liver lipid accumulation and prevented the beta-cell mass expansion caused by prenatal dexamethasone in the male offspring.

Conclusion: Maternal vitamin D administration mitigates metabolic disturbances that occur later in life in male rats exposed to GC in utero. Moreover, our data suggest vitamin D as an important nutritional supplement for pregnant overexposed to GC during gestation.

1. Introduction

Several lines of evidence indicate that stress experienced during critical windows of fetal development can lead to long-term effects on metabolic parameters during adulthood. A cohort study demonstrated that undernutrition in pregnant women during Dutch famine was associated with reduced offspring's glucose tolerance and raised insulin concentrations at age 50 and 58 [1]. In animal models, pharmacological stress induced by overexposure to glucocorticoids (GC) in utero led to hyperinsulinemia and hyperglycemia [2] as well as increased hepatic lipid accumulation [3].

Fetuses are usually protected from excessive levels of endogenous GC due to the placental enzyme 11 β-hydroxysteroid dehydrogenase 2, which inactivates maternal GC. However, synthetic GC like dexamethasone are poorly affected by this enzyme, thus, it crosses to the fetal circulation and consequently, leads to an excessive exposure of the fetus [4]. The long-term exposure to GC during gestation decreases placental and fetal growth and has been related to cardiovascular, neurological and metabolic disorders during adulthood, including metabolic syndrome and diabetes *mellitus* [5,6]. Insulin resistance, for example, can be found not only in humans [7] but also in rats [8] born from mothers treated with GC during pregnancy, a process known as fetal metabolic programming induced by GC.

The mechanisms underlying this fetal metabolic programming are

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unclear, but since prenatal use of GC impacts intrauterine growth [9], deficiencies in fetus nutrition could be part of these programming mechanisms. *Ex vivo* data indicate a role of prenatal undernutrition on the impairment in insulin synthesis and release [10]. It is also demonstrated that GC exposure during the late period of gestation increases the activity of phosphoenolpyruvate carboxykinase (PEPCK), a crucial enzyme in the process of gluconeogenesis, in the liver of adult rats [11], which favor the elevation in blood glucose values.

Few studies have investigated strategies to revert fetal programming induced by prenatal insults in both, humans and animal models. These strategies include gestational administration of folate and vitamin D [12, 13]. In this context, there is a crosstalk between vitamin D and GC: dexamethasone increases the transcription of vitamin D receptor (VDR) leading to an increased VDR protein content [14], which in turn increases vitamin D beneficial effects.

Vitamin D plays a role as a critical regulator of placental and fetal growth [15]. For instance, gestational vitamin D deficiency is associated with intrauterine growth restriction (IUGR) and placental insufficiency [16]. The impact of gestational vitamin D insufficiency or deficiency may be deleterious in the offspring adult lifespan. Several models of gestational vitamin D deficiency show that the adult offspring may present dyslipidemia, glucose intolerance, insulin resistance and adipogenesis leading the offspring to develop obesity [17,18]. Reduced insulin synthesis and secretion are also observed in studies related to low concentrations of vitamin D, both in animal models and humans [19, 20]. There is evidence that vitamin D supplementation, either under deficient states or not, improves the metabolic profile during gestation in humans [21] and in the male mice offspring [22].

Considering the evident impact of prenatal administration of GC to adult offspring metabolism and that vitamin D may counterbalancing adverse GC' effects, this study aimed to investigate whether maternal vitamin D administration during the entire pregnancy could have any protective effect on GC-induced metabolic programming. To achieve this goal, we followed both male and female offspring in three different periods of life (3, 6 and 12 months). We hypothesized that maternal vitamin D administration will prevent the metabolic disturbances induced by prenatal exposure to GC.

2. Methods

2.1. Animals

Experimental protocols were performed under the Guidelines for Ethical Care of Experimental Animals and approved by the Animal Research Ethics Committee of the Federal University of Santa Catarina (Florianópolis, SC, Brazil- protocol number 7174170417). All rats were housed with standard chow, not deficient in vitamin D, (protein: 22 %; lipids: 4%; calcium: 10 %; and vitamin D3: 500UI/100 g- BIOBASE®), and water *ad libitum* and maintained at a temperature of 21 °C ± 1 °C, on a 12 -h light-dark cycle (lights on 6 p.m.).

2.2. Gestational vitamin D administration and dexamethasone administration

Wistar female rats between 80–120 days-old were allowed to mate with males for 24 h. After that, the presence of spermatozoa in the vaginal smears was considered for confirmation of pregnancy and definition of the gestational day 0 (GD0). Then, female rats were placed individually in a standard home cage and randomly divided into prenatal vitamin D supplementation (VD, n = 19) and control groups (CTL, n = 20). The success rate of pregnancy was 78 % (39 of 50 rats became pregnant). Vitamin D3 (cholecalciferol-500 UI diluted in 0.4 mL of sunflower oil *per* animal) was administered daily by oral gavage during the entire gestational period. On the 13th day of gestation, half of the animals from CTL and VD groups were randomly selected and separated to receive dexamethasone (0.1 mg/kg of body mass in the drinking water) between the 14th and 19th days of pregnancy (Dex and DexVD groups, respectively). The vehicle groups received sunflower oil by oral gavage (0.4 mL *per* animal). Dexamethasone administration was regulated daily according to the water intake of the previous day and adjusted to the body mass of the current day. The chosen dexamethasone dosage and period of administration during gestation were based on previous studies in rats showing that both are enough to cause metabolic disturbances in the offspring in adulthood [9,23], without leading the newborns to death [24]. The final number of rats *per* group was n = 10 for CTL, n = 9 for VD, n = 10 for Dex, and n = 10 for DexVD.

2.3. Offspring experimental groups

The birthday was defined as postnatal day 0 (PND 0). Male and female offspring were weaned at PND 21 and allowed to free access to food and water. The medium-term programming effects were assessed at postnatal month 3 (PNM), and the long-term programming effects were assessed at PNM 6 and PNM 12. To apply the 3Rs concept, the same groups of animals were studied at PNM 3 and PNM 6 followed by euthanasia, while another group of rats was kept up to PNM 12 and then evaluated. To avoid nutritional differences between the dam, the litter size was cut off to 8 pups per dam (the male/female ratio was approximately 1:1). The remaining pups were euthanized by decapitation. To prevent any effect of the litter *per se*, only one male and one female pup per dam were used to compose the experimental groups. The offspring groups were composed of males and females according to the dams' nomination (CTL, VD, Dex and DexVD groups).

2.4. Intraperitoneal glucose tolerance test and insulin tolerance test measurements

Intraperitoneal glucose tolerance test (ipGTT) was performed in non-anesthetized fasted animals (8–10 am) at PNM 3, PNM 6 and PNM 12 four days before the day of euthanasia. For this, blood was collected from the tail at four time points: before the injection (time 0) and at 30, 60 and 120 min after the intraperitoneal injection of 50 % glucose solution [(2 g/kg, body weight (b.w.)]. The first blood drop was discarded, and the second was used for the glycemia determination using a glucometer (Accu-Check® Performa; Roche Diagnostics GmbH, Mannheim, Germany).

For the intraperitoneal insulin tolerance test (ipITT), an injection of regular insulin (Biohulin® 1 IU/kg, b.w.) was given to non-anesthetized fed rats 48 h after the ipGTT (8–10 am). Blood glucose concentrations were measured before and at 10, 20, 30 and 45 min after the insulin injection using a glucometer, as described above. The area under the curve (AUC) (normalized at baseline values) and glucose decay constant (linear phase of glucose decay) (*k*ITT) were calculated to evaluate the glucose tolerance and insulin sensitivity, respectively [25].

2.5. Murinometric measurements and biochemical analysis

Body weight was measured at PND 0, weekly during the whole lactation period, and monthly from PNM 2 to PNM 12. Rats were euthanized by intraperitoneal anesthetic overdose (ketamine- 1.5 mg/kg, b.w.) followed by decapitation at PNM 6 and PNM 12. Perigonadal, perirenal and omental adipose tissues were immediately removed and weighed and the sum of them corresponded to total visceral adipose tissue. Blood samples were collected by cardiac puncture before decapitation in a tube containing heparin. Plasma was stored at −20 °C for posterior measurements of plasma triglycerides and total cholesterol using commercial kits (K117−2 and K083−2, respectively; Bioclin, Brazil), according to the manufacturer's instructions.

2.6. Hepatic triacylglycerol content

Immediately after decapitation, livers fragments (100 mg, always

from the same lobe) were extracted, weighed, frozen in liquid nitrogen and stored at −80 °C. Samples were transferred into glass tubes containing 0.7 mL 1 M NaCl and homogenized with T18 UltraTurrax® (IKA®; Staufen, Germany). After homogenization, 2 mL methanol/chloroform solution (1:2, v/v) was added into the tubes and centrifuged for 30 min at 4, 000 rpm (Eppendorf 5810R). The methanolic phase was placed into another tube and then in a water bath at 98 °C for solvent evaporation. A methanol/Triton 100 (1:2, v/v) solution was added to the samples for the quantification of hepatic triglycerides content [26].

2.7. Immunohistochemistry analysis

Immediately after decapitation, the entire pancreas was excised, weighed, and the splenic portion was fixed in 4% (w/v) paraformaldehyde for 24 h and embedded in paraffin. The paraffin-embedded pancreas samples were sectioned (5 μm thickness- 250 μm apart from each other), dewaxed, rehydrated and washed in 0.01 M phosphate-buffered saline (PBS) (pH 7.4). Insulin-immunostaining was performed according to a previous publication [27]. Sections were then incubated with 10 mM sodium citrate solution (pH 6.0), preheated at 98 °C for 30 min for antigen retrieval. Endogenous peroxidase activity was blocked by incubation in 0.3 % hydrogen peroxide, before

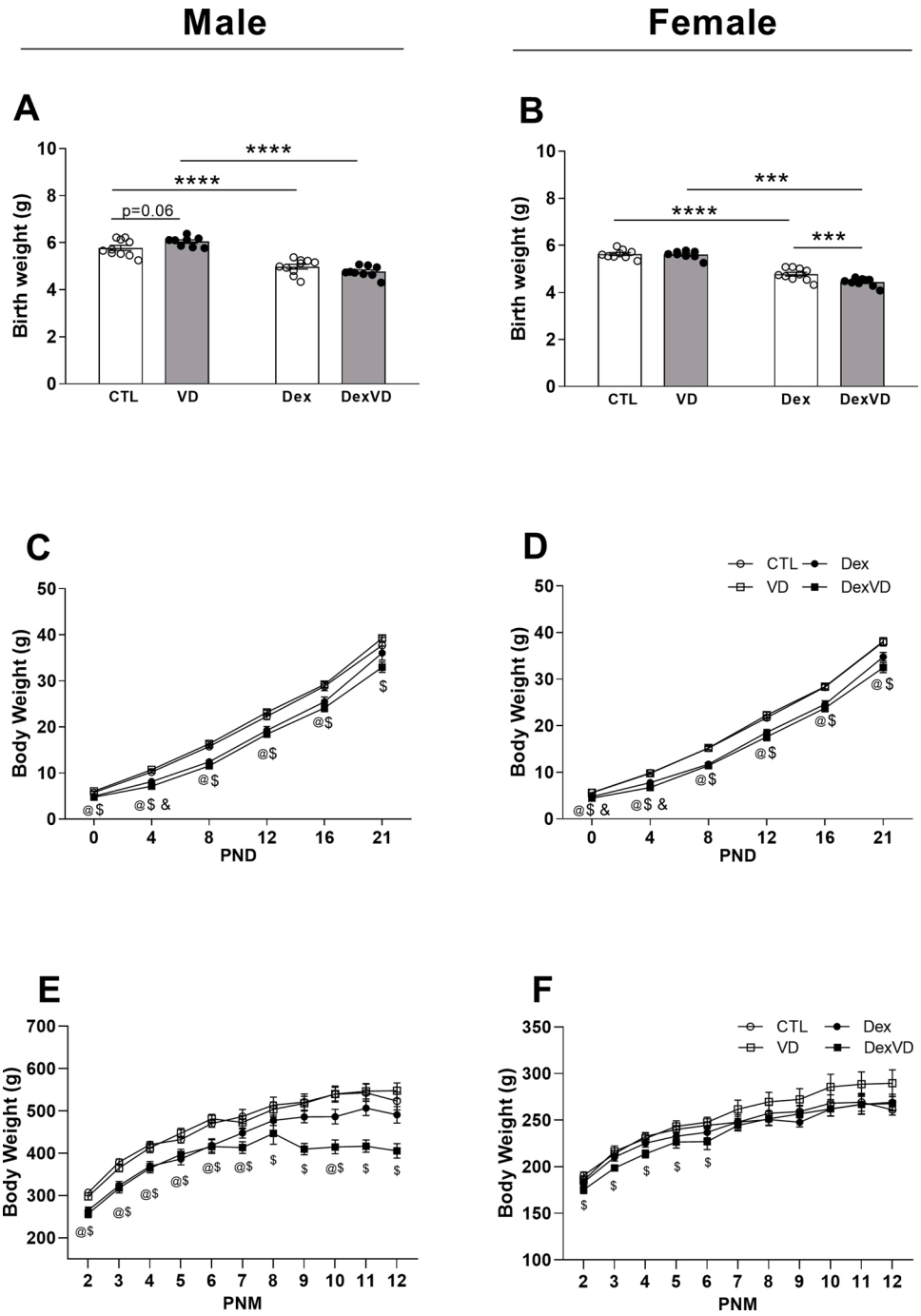

Fig. 1. Birth weight in male (A) and female (B); body weight during the lactation period in male (C) and female (D) and adult body weight in male (E) and female (F) offspring. Two-way ANOVA followed by Fisher's LSD posthoc test. Data are means ± SEM. $p < 0.05$; @ difference between CTL and Dex groups; $ difference between VD and DVD groups; & difference between Dex and DexVD groups; **** $p < 0.0001$, ***≤ 0.0009.

permeabilization with PBS containing 0.1 % Tween-20 and 5% bovine serum albumin (BSA) at room temperature. Then, sections were incubated with a primary guinea pig anti-insulin antibody (1:400; Dako North America, Inc.; cat. no. A0564) diluted in PBS containing 3% BSA overnight at 4 °C. Posteriorly, sections were washed in PBS and incubated with HRP-conjugated anti-guinea-pig antibody (1:1000; Invitrogen; cat. no. 614,620) for 2 h at room temperature. Insulin-positive cells were detected by incubation with 3,3′-diaminobenzidine (Sigma Chemical) solution. After stained with Ehrlich's hematoxylin, slides were scanned using the AxioScan automatic slide scanner (ZEISS, Oberkochen, Germany) for posterior analyses [28].

The total area of the pancreas and the pancreatic beta-cells area were calculated using the ZEN software (ZEISS) according to a previous publication [28]. To obtain the percentage of pancreatic beta-cells mass in each section, the summation of the areas corresponding to the insulin-immunostained cells was divided by the total pancreas area and multiplied by 100. The absolute beta-cell mass was obtained according to the following: the entire pancreas mass was multiplied by the percentage of beta cells in the section and expressed as mg. Representative images were taken in an OLYMPUS IX83 microscope (OLYMPUS; Tokyo, Japan) under 40× and 200× of magnification.

2.8. Statistical analysis

Data were analyzed by Two-Way ANOVA, using "dexamethasone" and "Vitamin D" as the two factors, followed by Fisher's LSD *post hoc* test. The outliers were removed considering the interval containing 95 % of data, by calculating the mean ± 2× standard deviation of the mean (SDM). Statistical analyses were performed using the Prism Statistical software 8.1 (GraphPad Software Inc., San Diego, Calif., USA). Differences were considered statistically significant if $p < 0.05$ and the results were presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Birth, lactation and adult weights

Prenatal exposure to dexamethasone resulted in a significant decrease in the birth weight of males ($p < 0.0001$; F1, 33 = 116.1; r2 = 76.55; Fig. 1A) and females ($p < 0.0001$; F1, 32 = 230.8; r2 = 84.39; Fig. 1B) compared to controls. In addition, there was an interaction between the factors in males ($p = 0.01$; F1, 33 = 6.16; r2 = 4.06; Fig. 1A). However, the *post hoc* test showed only a statistical tendency for the difference between the CTL and VD groups (p = 006) but not between Dex and DexVD. In the female offspring, the interaction between dexamethasone and vitamin D ($p = 0.02$; F1, 32 = 5.45; r2 = 1.99; Fig. 1B) led to an exacerbation of birth weight reduction in the DexVD group. However, at the end of lactation (PND 21), male ($p = 0.006$; F1, 32 = 8.50; r2 = 19.66; Fig. 1C) and female ($p < 0.0001$; F1, 32 = 21.78; r2 = 38.60; Fig. 1D) rats exposed to prenatal Dex showed a sustained reduction in body weight compared to control rats, with no influence of vitamin D.

Prenatal exposure to dexamethasone led to a reduction in the body weight of Dex adult males during the whole experimental trial (up to PNM 12). Moreover, from PNM 9 to PNM 12, the reduction in the body weight was greater in males from the DexVD group (Fig. 1E). Prenatal GC treatment led to a reduction in adult female body weight, but only until PNM 6, when it became similar between the groups during the remaining period of experimental trials (Fig. 1F).

3.2. Oral glucose tolerance test

At PNM 3, 6 and 12 we evaluated the glucose tolerance in male and female rats (Fig. 2). There was no significant difference between the male groups at PNM 3 and 6 (Fig. 2A and B; Fig. 2C and D, respectively). However, at PNM 12, males from the Dex and DexVD groups exhibited

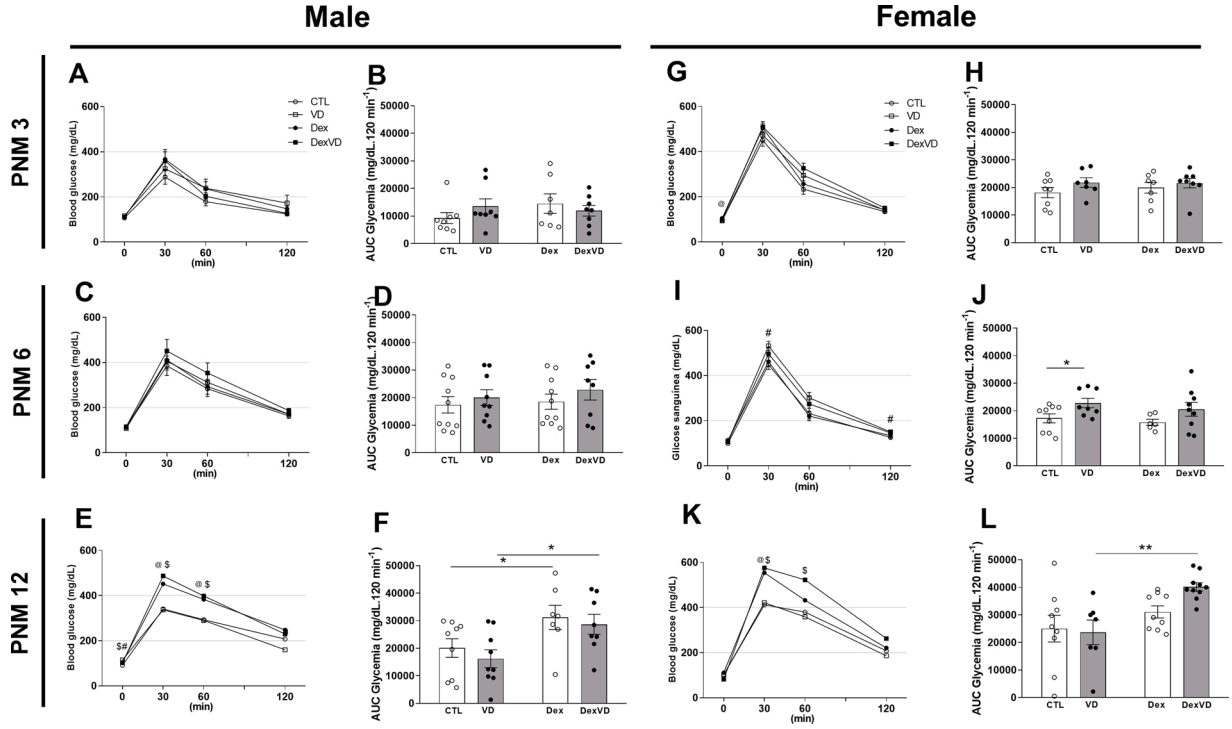

Fig. 2. Glycemia before and after an intraperitoneal glucose load described as blood concentration (A, C and E) and area under the curve (AUC) (B, D and F) in 3 (A, B), 6 (C,D) and 12 (E,F) month old male offspring and; Glycemia before and after an intraperitoneal glucose load described as blood concentration (G, I and K) and area under the curve (AUC) (H, J and L) in 3 (G,H), 6 (I,J) and 12 (K,L) month old female offspring. Two-way ANOVA followed by Fisher's LSD *posthoc* test. Data are means ± SEM. $p < 0.05$; $^{@}$ difference between CTL and Dex groups; $^{\$}$ difference between VD and DexVD groups; $^{\#}$ difference between CTL and VD groups; * $p < 0.05$, **≤ 0.009.

higher glycemia at minutes 30 and 120 that agreed with the corresponding AUC values compared to control groups ($p = 0.002$; F1, 29 = 10.54; r2 = 27.17, Fig. 2E and F). There were no effects of gestational vitamin D administration on the response to the glucose challenge in males neither at PNM 3, 6 or 12.

The glucose tolerance in the female offspring at PNM 3 was similar between the studied groups (Fig. 2G and H). However, at PNM 6 females from the VD group showed higher blood glucose concentrations 30 and 120 min after glucose administration and higher AUC value ($p = 0.01$; F1, 28 = 6.74; r2 = 19.08, Fig. 2I and J) compared to the CTL group. Nevertheless, these results appeared to be transient, since this glucose intolerance disappeared at PNM 12 (Fig. 2K and L). Yet, at this age, the DexVD female group presented a higher glycemia at 30 min and 60 min after glucose administration and higher AUC ($p = 0.002$; F1, 31 = 11.12; r2 = 23.87, Fig. 2K and L) compared to the VD group.

3.3. Intraperitoneal insulin tolerance test

In response to an exogenous insulin challenge, there was no significant difference in the glucose decay in the male offspring at PNM 3 (Fig. 3A and B). At PNM 6, rats from the Dex group exhibited reduced glucose decay compared to the CTL group ($p = 0.03$). Administration of vitamin D during gestation prevented this reduction in the insulin sensitivity at PNM 6 in the DexVD group ($p = 0.02$; F1, 22 = 6.25; r2 = 20.44; Fig. 3C and D). Similar results were found at PNM 12. Although the *posthoc* results did not show a difference between CTL and Dex ($p = 0.06$), there was an interaction between the factors ($p = 0.01$; F1, 21 = 7; r2 = 18.93), which showed an increase in *k*ITT in the DexVD group compared to the Dex group ($p = 0.006$, Fig. 3E and F). The female offspring groups exhibited similar insulin-induced glycemia decay at either PNM 3 (Fig. 3G and H), PNM 6 (Fig. 3I and J) or PNM 12 (Fig. 3K and L).

3.4. Adipose tissue depots

Prenatal GC promoted a significant decrease in the relative weights of the omental ($p = 0.007$; F1, 30 = 8090; r2 = 20.84; Table 1) and perigonadal adipose tissue ($p = 0.04$; F1, 33 = 4.51; r2 = 11.39; Table 1) in males at PNM 6, with no influence of gestational administration of vitamin D. The perirenal and total adipose tissue relative weights were similar among the male groups at PNM 6 (Table 1). At PNM 12, there was no difference in the relative weight of omental and perigonadal adipose tissue among the groups. However, there was a significant interaction, as observed in the DexVD group leading to a reduction in the relative weight of perirenal adipose tissue ($p = 0.02$; F1, 29 = 5.43; r2 = 12.54; Table 2) as well as in total adipose tissue ($p = 0.03$; F1, 29 = 4.71; r2 = 8.97; Table 2). At the same age, there was no difference in the relative weights of omental, perigonadal and total adipose tissue among

Table 1
Weight of omental adipose tissue (OAT), perigonadal adipose tissue (GAT), perirenal adipose tissue (RAT) and total adipose tissue (TAT) and plasma triglycerides (TAG) and total cholesterol (CT) concentrations at 6-months-old male and female offspring born from dams treated with DEX (black bars) or not (white bars) supplemented with vitamin D (VD) or vehicle oil (Vehicle). Two-way ANOVA followed by Fisher's LSD *pos hoc* test. Data are means ± SD. [@] difference between CTL and DEX groups; [$] difference between VD and DVD groups.

PNM 6	CTL ♂	VD ♂	Dex ♂	DexVD ♂	P value
Omental AT (g/100 g b.w.)	0.2 ± 0.02	0.11 ± 0.04	0.1 ± 0.02[@]	0.1 ± 0.04	*Interaction*: p = 0.39 *VD*: p = 0.96 *DEX*: p = 0.007
Perigonadal AT (g/100 g b.w.)	1.3 ± 0.3	1.6 ± 0.4	1.1 ± 0.4[@]	1.2 ± 0.3[$]	*Interaction*: p = 0.57 *VD*: p = 0.06 *DEX*: p = 0.0006
Perirenal AT (g/100 g b.w.)	1.9 ± 0.5	1.8 ± 0.5	1.7 ± 0,8	1.5 ± 0.6	NS
Total AT (g/100 g b.w.)	3.3 ± 0.8	3.6 ± 1.1	3.±1.2	3.2 ± 1.2	NS
Plasma TAG (mg/dL)	68.1 ± 31.7	59.4 ± 20.8	53.3 ± 18.4	51.2 ± 20.6	NS
Plasma CT (mg/dL)	70.5 ± 14.2	77.9 ± 16.1	79.2 ± 24.1	87.1 ± 19.2	NS

	CTL ♀	VD ♀	Dex ♀	DexVD ♀	P value
Omental AT (g/100 g b.w.)	0.2 ± 0.03	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.06	NS
Perigonadal AT (g/100 g b.w.)	1.4 ± 0.6	1.5 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	NS
Perirenal AT (g/100 g b.w.)	1.9 ± 0.30	2.1 ± 0.4	1.8 ± 0.8	2 ± 0.9	NS
Total AT (g/100 g b.w.)	3.4 ± 0.7	3.8 ± 0.9	3.5 ± 1.5	3 ± 1.1	NS
Plasma TAG (mg/dL)	44.5 ± 8.2	54.2 ± 6.2	45.2 ± 6	46.9 ± 12.7	NS
Plasma CT (mg/dL)	78 ± 15.1	82.7 ± 15.5	70.2 ± 16.6	68.7 ± 18.4	NS

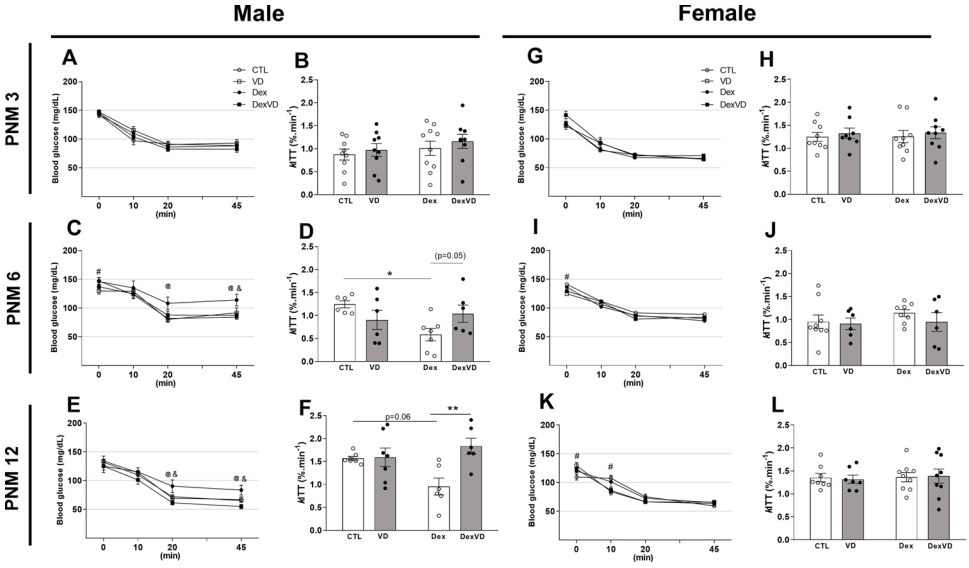

Fig. 3. Glycemia before and after an intraperitoneal insulin administration described as blood concentration (A, C and E) and glucose decay constant (kITT) (B, D and F) in 3 (A,B), 6 (C,D) and 12 (E,F) month old male offspring and; Glycemia before and after an intraperitoneal insulin administration described as blood concentration (A, C and E) and glucose decay constant (kITT) (B, D and F) in 3 (A,B), 6 (C,D) and 12 (E,F) month old female offspring. Two-way ANOVA followed by Fisher's LSD *posthoc* test. Data are means ± SEM. $p < 0.05$; [@] difference between CTL and Dex groups; [&] difference between Dex and DexVD groups; [#] difference between CTL and VD groups; * $p < 0.05$; ** ≤ 0.009.

Table 2
Weight of omental adipose tissue (OAT), perigonadal adipose tissue (GAT), perirenal adipose tissue (RAT) and total adipose tissue (TAT) and plasma triglycerides (TAG) and total cholesterol (CT) concentrations at 12-months-old male and female offspring born from dams treated with DEX (black bars) or not (white bars) supplemented with vitamin D (VD) or vehicle oil (Vehicle). Two-way ANOVA followed by Fisher's LSD *pos hoc* test. Data are means ± SD. $^{\#}$ difference between CTL and VD groups; $^{\$}$ difference between VD and DVD groups; $^{\&}$ difference between DEX and DVD groups.

PNM 12	CTL ♂	VD ♂	Dex ♂	DexVD ♂	P value
Omental AT (g/100 g b.w.)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	NS
Perigonadal AT (g/100 g b.w.)	1.4 ± 0.5	1.6 ± 0.6	1.2 ± 0.4	1.4 ± 0.4	NS
Perirenal AT (g/100 g b.w.)	2.5 ± 0.5	2.6 ± 0.5	2.5 ± 1.2	1.4 ± 0.4$^{\$\&}$	*Interaction*: p = 0.02 *VD*: p = 0.06 *DEX*: p = 0.02
Total AT (g/100 g b.w.)	5.2 ± 0.7	5.1 ± 0.6	4.6 ± 1.9	2.7 ± 1.2$^{\$\&}$	*Interaction*: 0.03 *VD*: p = 0.02 *DEX*: p = 0.001
Plasma TAG (mg/dL)	89 ± 19.9	82 ± 26.4	100.1 ± 54.5	95.3 ± 53.7	NS
Plasma CT (mg/dL)	93.6 ± 20.5	91 ± 19.9	90.1 ± 15.6	121.5 ± 37.1	NS
	CTL ♀	VD ♀	Dex ♀	DexVD ♀	P value
Omental AT (g/100 g b.w.)	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	NS
Perigonadal AT (g/100 g b.w.)	1.4 ± 0.1	1.2 ± 0.4	1.4 ± 0.5	1.6 ± 0.6	NS
Perirenal AT (g/100 g b.w.)	2.7 ± 1.6	2.8 ± 0.9	2.6 ± 0.8	2.6 ± 0.9	NS
Total AT (g/100 g b.w.)	4 ± 2.7	4.3 ± 0.9	4.3 ± 1.4	4.6 ± 1.4	NS
Plasma TAG (mg/dL)	67.3 ± 26.3	43.4 ± 8.1$^{\#}$	84 ± 36.4	45.2 ± 12$^{\&}$	*Interaction*: p = 0.34 *VD*: p = 0.0002 *DEX*: p = 0.28
Plasma CT (mg/dL)	78.7 ± 26.9	78.1 ± 24.9	85.7 ± 14.5	94.1 ± 16.4	NS

all the studied groups (Table 1). The same result was found at PNM 12, where the relative weights of omental, perigonadal, perirenal and total adipose tissue were similar between the experimental groups (Table 2).

3.5. Plasma and hepatic lipids

Males of the Dex group had an increased hepatic triglycerides' content at PNM 6 ($p = 0.01$; F1, 28 = 7.33; r2 = 15.79; Fig. 4A) and PNM 12 ($p = 0.0003$; F1, 27 = 17.69; r2 = 27.91; Fig. 4C) compared to CTL group. In contrast, the significant interaction between the factors showed that gestational administration of vitamin D prevented this hepatic lipid accumulation in both PNM 6 ($p = 0.02$; F1, 28 = 5.49; r2 = 11.82; Fig. 4A) and PNM 12 ($p = 0.02$; F1, 27 = 5.48; r2 = 8.66; Fig. 4C). The female offspring exhibited similar hepatic triglyceride content among the experimental groups at PNM6 and PNM12 (Fig. 4B and D).

The plasma concentration of triglycerides was similar between the experimental groups of males at PNM 6 (Table 1) and PNM 12 (Table 2). The female offspring born from dams that received vitamin D during gestation, independently of the exposure to dexamethasone, presented lower concentrations of plasma triglycerides at PNM 12 compared to CTL and Dex groups ($p = 0.0008$; F1, 30 = 13.94; r2 = 30.25; Table 2). The plasma concentrations of total cholesterol in the male and female offspring were not affected by either dexamethasone or vitamin D exposure during gestation, independently of age (Tables 1 and 2).

3.6. Immunohistochemistry

Male offspring rats from the Dex group exhibited an increase in the pancreatic beta-cell mass at PNM 6 ($p = 0.02$; F1, 14 = 6.15; r2 = 20.34; Fig. 5A) and PNM 12 ($p = 0.04$; F1, 15 = 4.59; r2 = 14.65; Fig. 5B). However, there was an interaction between the factors at PNM 6 ($p = 0.04$; F1, 14 = 4.61; r2 = 15.26) and PNM 12 ($p = 0.01$; F1, 15 = 8.03; r2 = 25.61), indicating that gestational administration with vitamin D blunted this phenotype. Vitamin D administration *per se* did not change this parameter in the VD group at PNM 6 and PNM 12. The groups of female offspring showed similar beta-cell mass either at PNM 6 (Fig. 5C) or PNM 12 (Fig. 5D).

Regarding the percentage of beta-cells, there was an effect of prenatal administration of vitamin D ($p = 0.02$; F1, 14 = 6.62; r2 = 27.21; Fig. 5E), only in the groups that received dexamethasone, leading to a reduction in this parameter in males at PNM 6. At PNM 12, there was an interaction between the factors ($p = 0.03$; F1, 16 = 5.37; r2 = 22.89; Fig. 5F), showing a reduction on the percentage of beta-cells in the DexVD group compared to Dex group. The female groups presented a similar beta-cell percentage either at PNM 6 (Fig. 5G) or PNM 12 (Fig. 5H).

4. Discussion

Our study was aimed to investigate the effects of maternal vitamin D administration in different aspects of the glucose homeostasis of the young and adult offspring prenatally exposed to GC. Our data confirmed the hypothesis that prenatal administration of vitamin D can attenuate, and even prevent, some glucose homeostasis parameters that were affected by in utero exposure to dexamethasone. This protection occurred only in the male offspring.

Regarding the birth weight, we corroborated previous findings showing that prenatal dexamethasone exposure causes IUGR in the male and female offspring, leading to smaller birth weight [24,29]. Low birth weight also correlates with decreased expression of 11-beta-hydroxysteroid dehydrogenase type 2 (Hsd11b2), an enzyme that, in rodents, catalyzes the conversion of corticosterone to inert 11-dehydrocorticosterone. This conversion protects the fetus from excessive maternal concentrations of GC and high fetal corticosterone concentration [30]. Although our data had not shown that maternal vitamin D administration affects birth weight, the relation between maternal vitamin D status and birth weight is controversial. An observational study suggests a positive correlation between maternal vitamin D status and neonatal birth weight [31] although another study have not found an effect of maternal vitamin D on this parameter [32]. These differences could be attributed to the time of gestation when vitamin D was introduced or to the method used to measure maternal vitamin D.

A reduction in the adult body weight of rodents exposed to high concentrations of GC during gestation, such as food restriction stress or prenatal administration of dexamethasone, has been shown previously [24,30]. In the present study, the prenatal use of dexamethasone promoted a different pattern of body weight changes during adulthood between male and female offspring. Although the literature has shown a catch-up growth in both male and female offspring with the same dose of dexamethasone [33,34], we only found it in the 6-month-old females. In the male offspring, the combination of prenatal dexamethasone and vitamin D resulted in a late weight reduction. Also, this combination led to a reduction of total adipose tissue, which might have contributed to the reduced body weight in the male offspring. There is no evidence in the literature showing a programming effect of prenatal administration of vitamin D on the offspring's food intake or body weight during adulthood. Although we did not assess food intake, Farhangi and colleagues [35] showed a decreased food intake in rats supplemented with vitamin D, which was related to reduced expression of *brain-derived neurotrophic factor* (BDNF), a factor that plays an essential role in energy balance regulation. Moreover, it seems to exist a relation between

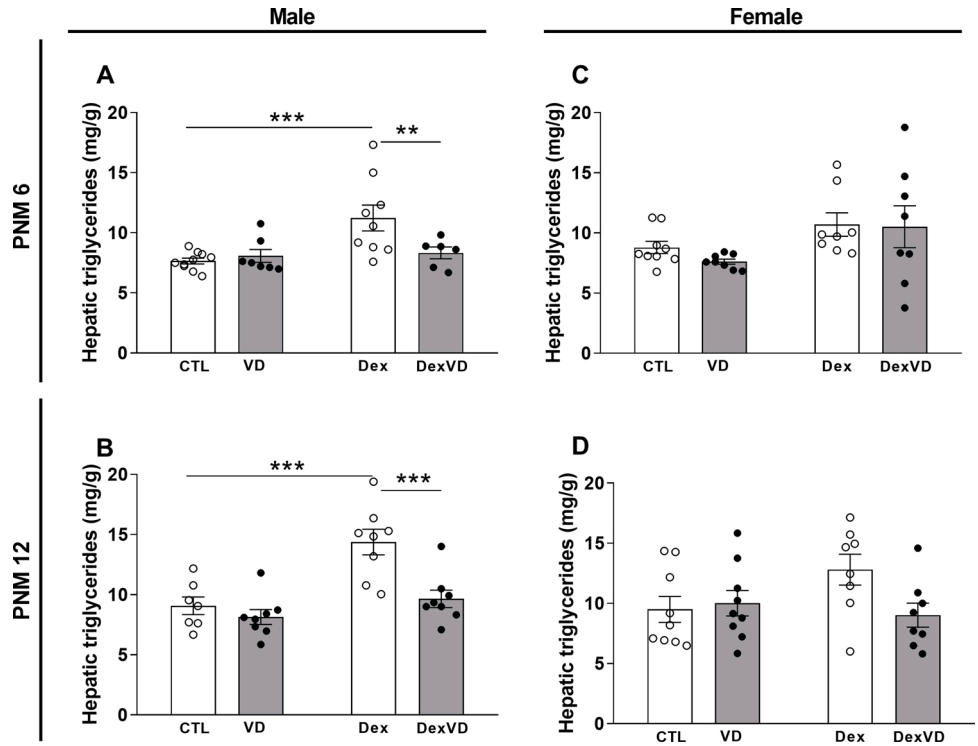

Fig. 4. Hepatic triglycerides concentrations at 6 (A) and 12 (B) month old male offspring and at 6 (C) and 12 (D) month old female offspring. Two-way ANOVA followed by Fisher's LSD *posthoc* test. Data are means ± SEM. ** $p \leq 0.009$, ***≤ 0.0009.

vitamin D and leptin concentrations, since 1α-hydroxylase-knockout mice present lower leptin concentrations associated with higher food intake than their wild-type counterparts [36]. Also, the gestational vitamin D administration may have programmed the adipose tissue expression of VDR, and VDR-*knockout* mice present an increased expression of UCP-1 and UCP-2, two important proteins involved in thermogenesis [37].

Consistent with prior observations, prenatal exposure to dexamethasone during the final third of pregnancy did not lead the offspring to become glucose-intolerant until the age of 6 months, irrespective of sex [29,38]. However, both male and female offspring became glucose intolerant at the age of 12 months. These data corroborate other findings showing that the priming effect of GC in the endocrine pancreas during fetal development occurred, but this effect can only be seen at later periods of life [29], when the beta-cell function might be reduced, like during the natural process of aging [39]. Indeed, prenatal administration of dexamethasone induces glucose intolerance in aged male rats (24 months old) [40]. The glucose intolerance in the male Dex group occurred in parallel to reduced insulin sensitivity and increased beta-cell mass. Yet, the glucose intolerance persisted in the DexVD groups of male and female rats, regardless of whether insulin sensitivity was normal or not. The latter can be explained probably by insufficient beta-cell mass or function instead of reduced hepatic function, since both male and female DexVD groups did not develop fatty liver. As well, GC administration to young rats induces glucose intolerance in 3 and 12-month-old males, but in females, this impairment occurs only at 12 months of age [26]. This is supported by a more robust endocrine pancreas function in young females as observed in the female offspring in our study. The absence of a protective effect of vitamin D administration during the gestational period on the offspring glucose tolerance is corroborated by a recent study showing that thirty days of vitamin D administration does not affect glucose tolerance in normal or obese rats [41].

Reduction in insulin sensitivity was previously shown in male offspring at 40 days of age, born from dams exposed to prenatal administration of dexamethasone [42]. Part of the mechanism by which this occurs is the increased activity of the hypothalamus-pituitary-adrenal axis and hepatic insulin resistance after fetal exposure to dexamethasone. These events can be associated either with a decreased insulin-suppression of mRNA expression of PEPCK [41], which leads to an increased glucose synthesis by gluconeogenesis; or to a decreased insulin-stimulated glucose uptake in the muscle [43]. In the current study, prenatal GC exposure decreased insulin sensitivity and increased hepatic triglycerides content in the male offspring in an age-dependent manner. This ectopic lipid accumulation occurs probably as a function of attenuated hepatic insulin signaling through the involvement of diacylglycerol and activation of protein kinase Cε [44]. This mechanism could also be implied in the glucose intolerance through increased hepatic gluconeogenesis. Pro-inflammatory adipocytokines could also act synergically favoring the insulin-resistant state. Indeed, prenatal exposure to dexamethasone increases adipose gene expression of inflammatory markers and increases the plasma concentration of interleukin 6 (Il-6), Il-1β, tumor necrosis factor-alpha (Tnfα) mRNAs and plasma free fatty acids concentrations in 6-month-old rats, independently of sex [45,46]. Elevated pro-inflammatory adipocytokines are associated with increased lipolysis and increased concentrations of serum fatty acids [45]. The present data suggest that the increase in the serum fatty acid found in male rats prenatally exposed to dexamethasone could be, at least in part, contributing as a substrate for the increased content of hepatic triglycerides and to the insulin resistance found in this group.

Our data showed that maternal vitamin D administration prevented the reduction in insulin sensitivity as well as the accumulation of hepatic triglycerides in the male offspring prenatally exposed to GC. It has been shown that the administration of calcitriol can suppress the expression of genes related to hepatic lipogenesis and pro-inflammatory markers, as well as reduce the hepatic accumulation of lipids in mice [47,48]. Furthermore, vitamin D reduces adipose tissue inflammation both *in vitro* and in *vivo* [49,50] and promotes down-regulation of the expression and production of IL-1β, TNF- α and IL-6 [51]. Other pathways by which vitamin D might have improved insulin sensitivity in the present work is

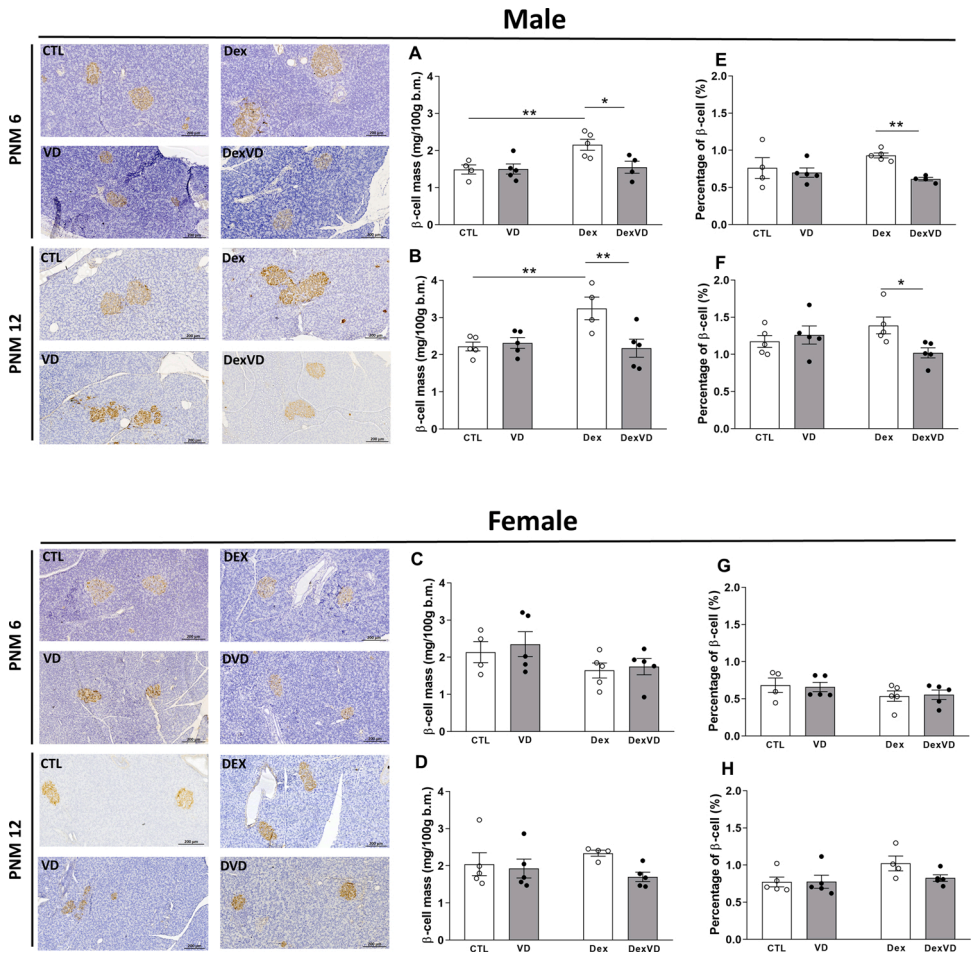

Fig. 5. Percentage and β-cell mass expressed in mg/kg b.m. and representative images of pancreas sections immunostained for insulin and counter-stained with hematoxylin in 6 (A, E) and 12 (B, F) month old male offspring and 6 (C, G) and 12 (D, H) female offspring. Data are means ± SEM. * $p < 0.05$, **≤ 0.009.

by a direct improvement of the insulin signaling pathways, or even by its antioxidative properties, since vitamin D can lower oxidative stress in diabetics [52].

Vitamin D administration during the gestational period leads to an increased concentration of plasma vitamin D and calcium concentrations during lactation in the offspring [53]. Although we have not measured the plasma concentration of vitamin D, calcium concentrations were increased in those male and female rats born from dams given vitamin D during pregnancy (data not shown), which could be indirectly related to higher concentrations of endogenous vitamin D and its protective effects shown in our study.

We showed that the male rats prenatally exposed to GC presented an expansion of the beta-cell mass. An increase in beta-cell mass is commonly coupled with high insulin demand, under reduced insulin sensitivity, and may prevent an increase of fasting glycemia at basal condition, as observed in our previous study [54]. Yet, since increasing beta-cell mass is not always enough to avoid glucose intolerance, a beta-cell dysfunction must also be considered during glucose intolerance [54]. However, since insulinemia has not been measured in the present study, our data do not allow us to attest the causes of glucose intolerance. Our DexVD offspring kept up glucose-intolerant at 12-months of age, independently of sex or the presence of insulin resistance. Thus, it is probable that the maternal benefits of vitamin D administration had not counteracted the impact of GC on the endocrine pancreas development, especially on transcription factors related to the beta-cell function. Although the DexVD offspring had not manifest neither insulin resistance nor compensatory upregulation of beta-cell mass, some impairment of beta-cell function (i.e., the machinery of insulin biosynthesis or exocytosis) was still present, preventing old rats to couple with the glucose overload. Another possible explanation for the prenatal GC-induced glucose intolerance involves changes in the hepatic insulin clearance. On the one hand, dexamethasone is known to reduce insulin-degrading enzyme (IDE) activity [55] which is correlated with increased insulin resistance and, in the long-term, with glucose-intolerant phenotype and diabetes in animal studies [56,57]. On the other hand, an increase of IDE activity decreases insulin availability and can lead to glucose intolerance. Because our data showed that females became glucose-intolerant but did not present insulin resistance, the latter alternative might be more likely.

From a metabolic perspective, we showed that males are more susceptible than females to fetal programming caused by prenatal GC exposure. We suggest that sex steroids may have a role in these outcomes. In fact, females have better peripheral insulin sensitivity in a model of high-fat diet [58]. Animal experiments demonstrated that estradiol regulates depolarization and Ca^{2+} influx [59] and inhibits K_{ATP} channels [60] in beta-cells, which favor optimal insulin secretion. Moreover, the administration of estradiol promotes protection against triglyceride accumulation and liver steatosis in female mice exposed to a high-fat diet [61]. Although males can also exhibit some circulating estradiol due to peripheral conversion of testosterone, it seems like the protective effects of estradiol depend on the progesterone concentrations as well, as shown previously [62]. Accordingly, they showed an improvement of insulin resistance in ovariectomized rats when estradiol and progesterone are replaced, but not when only estradiol or progesterone are replaced.

In conclusion, the effects of exposure to excessive concentrations of

GC in the late period of gestation on the metabolic outcomes of the adult offspring occured in an age and sex-dependent manner. The metabolic dysfunctions were more pronounced in older male rats. Although maternal vitamin D administration during the entire pregnancy period prevented late insulin insensitivity and hepatic lipid accumulation caused by prenatal GC exposure in the offspring, it was not able to prevent glucose intolerance. Thus, improving vitamin D concentrations during gestation may be an important and valuable tool for the mitigation of late children outcomes associated with prenatal exposure to GC.

Ethics approval

All protocols were performed under the Guidelines for Ethical Care of Experimental Animals and approved by the Animal Research Ethics Committee of the Federal University of Santa Catarina (Florianópolis, SC, Brazil- protocol number-7174170417).

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Authors statement

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Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] T. Roseboom, S. de Rooij, R. Painter, The Dutch famine and its long-term consequences for adult health, Early Hum. Dev. 82 (2006) 485–491, https://doi.org/10.1016/j.earlhumdev.2006.07.001.
[2] J.R. Seckl, M.C. Holmes, Mechanisms of disease: glucocorticoids, their placental metabolism and fetal' programming' of adult pathophysiology, Nat. Clin. Pract. Endocrinol. Metab. 3 (2007) 479–488, https://doi.org/10.1038/ncpendmet0515.
[3] A.J. Drake, P.J. Raubenheimer, D. Kerrigan, K.J. McInnes, J.R. Seckl, B.R. Walker, Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet, Endocrinology 151 (2010) 1581–1587, https://doi.org/10.1210/en.2009-1088.
[4] M. Bruschettini, D.L.A. Van den Hove, D. Gazzolo, H.W.M. Steinbusch, C.E. Blanco, Lowering the dose of antenatal steroids: the effects of a single course of bethamethasone on somatic growth and brain cell proliferation in the rat, Am. J. Obstet. Gynecol. 194 (2006) 1341–1346, https://doi.org/10.1016/j.ajog.2005.11.044.
[5] R.M. Reynolds, Programming effects of glucocorticoids, Clin. Obstet. Gynecol. 56 (2013) 602–609, https://doi.org/10.1097/GRF.0b013e31829939f7.
[6] D.J. Barker, K.L. Thornburg, Placental programming of chronic diseases, cancer and lifespan: a review, Placenta. 34 (2013) 841–845, https://doi.org/10.1016/j.placenta.2013.07.063.
[7] S.R. Dalziel, N.K. Walker, V. Parag, C. Mantell, H.H. Rea, A. Rodgers, J.E. Harding, Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial, Lancet. 365 (2005) 1856–1862, https://doi.org/10.1016/S0140-6736(05)66617-2.
[8] M.H. Hsu, J.M. Sheen, Y.C. Chen, H.R. Yu, Y.L. Tain, L.T. Huang, Rats with prenatal dexamethasone exposure and postnatal high-fat diet exhibited insulin resistance, and spatial learning and memory impairment: effects of enriched environment, Neuroreport. 3 (2020) 265–273, https://doi.org/10.1097/WNR.0000000000001406.
[9] M.J. Nyirenda, R.S. Lindsay, C.J. Kenyon, A. Burchell, J.R. Seckl, Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring, J. Clin. Invest. 101 (1998) 2174–2181, https://doi.org/10.1172/JCI1567.
[10] A. Garofano, P. Czernichow, B. Bréant, Beta-cell mass and proliferation following late fetal and early postnatal malnutrition in the rat, Diabetologia. 41 (1998) 1114–1120, https://doi.org/10.1007/s001250051038.
[11] K.L. Franko, A.J. Forhead, A.L. Fowden, Differential effects of prenatal stress and glucocorticoid administration on postnatal growth and glucose metabolism in rats, J. Endocrinol. 204 (2010) 319–329, https://doi.org/10.1677/JOE-09-0390.
[12] L. Zhou, A. Zhang, K. Wang, Q. Zhou, T. Duan, Folate ameliorates dexamethasone-induced fetal and placental growth restriction potentially via improvement of trophoblast migration, Int. J. Clin. Exp. Pathol. 8 (2015) 3008–3014.
[13] E. Hornsby, P.E. Pfeffer, N. Laranjo, W. Cruikshank, M. Tuzova, A.A. Litonjua, S. T. Weiss, V.J. Carey, G. O'Connor, C. Hawrylowicz, Vitamin D supplementation during pregnancy: effect on the neonatal immune system in a randomized controlled trial, J. Allergy Clin. Immunol. 141 (2018) 269–278, https://doi.org/10.1016/j.jaci.2017.02.039.
[14] A.A. Hidalgo, K.K. Deeb, J.W. Pike, C.S. Johnson, D.L. Trump, Dexamethasone enhances 1alpha,25-dihydroxyvitamin D3 effects by increasing vitamin D receptor transcription, J. Biol. Chem. 286 (2011) 36228–36237, https://doi.org/10.1074/jbc.M111.244061.
[15] P. Murthi, H.E. Yong, T.P. Ngyuen, S. Ellery, H. Singh, R. Rahman, H. Dickinson, D. W. Walker, M. Davies-Tuck, E.M. Wallace, P.R. Ebeling, Role of the placental vitamin d receptor in modulating feto-placental growth in fetal growth restriction and preeclampsia-affected pregnancies, Front. Physiol. 7 (2016) 43, https://doi.org/10.3389/fphys.2016.00043.
[16] N. Yates, R.C. Crew, C.S. Wyrwoll, Vitamin D deficiency and impaired placental function: potential regulation by glucocorticoids? Reproduction. 153 (2017) R163–R171, https://doi.org/10.1530/REP-16-0647.
[17] H. Zhang, X. Chu, Y. Huang, G. Li, Y. Wang, Y. Li, C. Sun, Maternal vitamin D deficiency during pregnancy results in insulin resistance in rat offspring, which is associated with inflammation and Iκbα methylation, Diabetologia. 57 (2014) 2165–2172, https://doi.org/10.1007/s00125-014-3316-7.
[18] J. Wen, Q. Hong, X. Wang, L. Zhu, T. Wu, P. Xu, Z. Fu, L. You, X. Wang, C. Ji, X. Guo, The effect of maternal vitamin D deficiency during pregnancy on body fat and adipogenesis in rat offspring, Sci. Rep. 8 (2018) 365, https://doi.org/10.1038/s41598-017-18770-4.
[19] A.W. Norman, J.B. Frankel, A.M. Heldt, G.M. Grodsky, Vitamin D deficiency inhibits pancreatic secretion of insulin, Science. 209 (1980) 823–825, https://doi.org/10.1126/science.6250216.
[20] P. Autier, Vitamin D status as a synthetic biomarker of health status, Endocrine. 51 (2016) 201–202, https://doi.org/10.1007/s12020-015-0837-x.
[21] C. Palacios, L.M. De-Regil, L.K. Lombardo, J.P. Peña-Rosas, Vitamin D supplementation during pregnancy: updated meta-analysis on maternal outcomes, J. Steroid Biochem. Mol. Biol. 164 (2016) 148–155, https://doi.org/10.1016/j.jsbmb.2016.02.008.
[22] C.R. Villa, J. Chen, B. Wen, S.M. Sacco, A. Taibi, W.E. Ward, E.M. Comelli, Maternal vitamin D beneficially programs metabolic, gut and bone health of mouse male offspring in an obesogenic environment, Int. J. Obes. (Lond). 40 (2016) 1875–1883, https://doi.org/10.1038/ijo.2016.177.
[23] D. O'Regan, C.J. Kenyon, J.R. Seckl, M.C. Holmes, Glucocorticoid exposure in late gestation in the rat permanently programs gender-specific differences in adult cardiovascular and metabolic physiology, Am. J. Physiol. Endocrinol. Metab. 287 (5) (2021) E863–E870, https://doi.org/10.1152/ajpendo.00137.2004.
[24] K. Motta, P.R.L. Gomes, P.M. Sulis, S. Bordin, A. Rafacho, Dexamethasone administration during late gestation has No major impact on lipid metabolism, but reduces newborn survival rate in wistar rats, Front. Physiol. 9 (2018) 783, https://doi.org/10.3389/fphys.2018.00783.
[25] V.A. Giozzet, A. Rafacho, A.C. Boschero, E.M. Carneiro, J.R. Bosqueiro, Dexamethasone treatment in vivo counteracts the functional pancreatic islet alterations caused by malnourishment in rats, Metabolism. 57 (2008) 617–624, https://doi.org/10.1016/j.metabol.2007.12.004.
[26] C. dos Santos, F.B. Ferreira, L.M. Gonçalves-Neto, S.R. Taboga, A.C. Boschero, A. Rafacho, Age- and gender-related changes in glucose homeostasis in glucocorticoid-treated rats, Can. J. Physiol. Pharmacol. 92 (2014) 867–878, https://doi.org/10.1139/cjpp-2014-0259.
[27] F.G. Battiston, C. Dos Santos, A.M. Barbosa, S. Sehnem, E.C.R. Leonel, S.R. Taboga, J.A. Anselmo-Franci, F.B. Lima, A. Rafacho, Glucose homeostasis in rats treated with 4-vinylcyclohexene diepoxide is not worsened by dexamethasone treatment, J. Steroid Biochem. Mol. Biol. 165 (2017) 170–181, https://doi.org/10.1016/j.jsbmb.2016.06.001.

[28] F. Natividade da Silva, H.S. Brunetta, M.A. Bruxel, F.A. Gomes, A. Rafacho, Impact of glucocorticoid treatment before pregnancy on glucose homeostasis of offspring exposed to glucocorticoid in adult life, Life Sci. 237 (2019), 116913, https://doi.org/10.1016/j.lfs.2019.116913.
[29] Y.C. Chen, Y.H. Huang, J.M. Sheen, Y.L. Tain, H.R. Yu, Y.C. Chen, M.M. Tiao, H. C. Kuo, L.T. Huang, Prenatal dexamethasone exposure programs the development of the pancreas and the secretion of insulin in rats, Pediatr. Neonatol. 58 (2017) 135–144, https://doi.org/10.1016/j.pedneo.2016.02.008.
[30] B. Valtat, C. Dupuis, D. Zenaty, A. Singh-Estivalet, F. Tronche, B. Bréant, B. Blondeau, Genetic evidence of the programming of beta cell mass and function by glucocorticoids in mice, Diabetologia. 54 (2011) 350–359, https://doi.org/10.1007/s00125-010-1898-2.
[31] C. Morgan, L. Dodds, D.B. Langille, H.A. Weiler, B.A. Armson, J.C. Forest, Y. Giguère, C.G. Woolcott, Cord blood vitamin D status and neonatal outcomes in a birth cohort in Quebec, Canada, Arch. Gynecol. Obstet. 293 (2016) 731–738, https://doi.org/10.1007/s00404-015-3899-3.
[32] R. Morley, J.B. Carlin, J.A. Pasco, J.D. Wark, Maternal 25-hydroxyvitamin D and parathyroid hormone concentrations and offspring birth size, J. Clin. Endocrinol. Metab. 91 (2006) 906–912, https://doi.org/10.1210/jc.2005-1479.
[33] C.C. Tsai, M.M. Tiao, J.M. Sheen, L.T. Huang, Y.L. Tain, I.C. Lin, Y.J. Lin, Y.J. Lai, C.C. Chen, K.A. Chang, H.R. Yu, Obesity programmed by prenatal dexamethasone and postnatal high-fat diet leads to distinct alterations in nutrition sensory signals and circadian-clock genes in visceral adipose tissue, Lipids Health Dis. 18 (2019) 19, https://doi.org/10.1186/s12944-019-0963-1.
[34] G. Chen, H. Xiao, J. Zhang, H. Zhang, B. Li, T. Jiang, Y. Wen, Y. Jiang, K. Fu, D. Xu, Y. Guo, Y. Ao, H. Bi, H. Wang, Prenatal dexamethasone exposure-induced a gender-difference and sustainable multi-organ damage in offspring rats via serum metabolic profile analysis, Toxicol. Lett. 316 (2019) 136–146, https://doi.org/10.1016/j.toxlet.2019.09.007.
[35] M.A. Farhangi, M. Mesgari-Abbasi, G. Nameni, G. Hajiluian, P. Shahabi, The effects of vitamin D administration on brain inflammatory markers in high fat diet induced obese rats, BMC Neurosci. 18 (2017) 81, https://doi.org/10.1186/s12868-017-0400-1.
[36] C.J. Narvaez, D. Matthews, E. Broun, M. Chan, J. Welsh, Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue, Endocrinology. 150 (2009) 651–661, https://doi.org/10.1210/en.2008-1118.
[37] Y. Xu, Y. Lou, J. Kong, VDR regulates energy metabolism by modulating remodeling in adipose tissue, Eur. J. Pharmacol. 865 (2019), 172761, https://doi.org/10.1016/j.ejphar.2019.172761.
[38] T.B. Payolla, C.J. Teixeira, F.T. Sato, G.M. Murata, G.A. Zonta, F.S. Sodré, C. V. Campos, F.N. Mesquita, G.F. Anhê, S. Bordin, In utero dexamethasone exposure exacerbates hepatic steatosis in rats that consume fructose during adulthood, Nutrients. 11 (2019) 2114, https://doi.org/10.3390/nu11092114.
[39] A.M. Chang, J.B. Halter, Aging and insulin secretion, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E7–12, https://doi.org/10.1152/ajpendo.00366.2002.
[40] J. Lesage, F. Del-Favero, M. Leonhardt, H. Louvart, S. Maccari, D. Vieau, M. Darnaudery, Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat, J. Endocrinol. 181 (2004) 291–296, https://doi.org/10.1677/joe.0.1810291.
[41] Z.M. Guareschi, A.C. Valcanaia, V.M. Ceglarek, P. Hotz, B.K. Amaral, D.W. de Souza, T.A. de Souza, T. Nardelli, T.R. Ferreira, N.C. Leite, C. Lubackzeuski, H. R. de O Emilio, S. Grassiolli, The effect of chronic oral vitamin D supplementation on adiposity and insulin secretion in hypothalamic obese rats, Br. J. Nutr. 121 (2019) 1334–1344, https://doi.org/10.1017/S0007114519000667.
[42] E.S. Buhl, S. Neschen, S. Yonemitsu, J. Rossbacher, D. Zhang, K. Morino, A. Flyvbjerg, P. Perret, V. Samuel, J. Kim, G.W. Cline, K.F. Petersen, Increased hypothalamic-pituitary-adrenal axis activity and hepatic insulin resistance in low-birth-weight rats, Am. J. Physiol. Endocrinol. Metab. 293 (2007) E1451–E1458, https://doi.org/10.1152/ajpendo.00356.2007.
[43] E.S. Buhl, T.K. Jensen, N. Jessen, B. Elfving, C.S. Buhl, S.B. Kristiansen, R. Pold, L. Solskov, O. Schmitz, G. Wegener, S. Lund, K.F. Petersen, Treatment with an SSRI antidepressant restores hippocampo-hypothalamic corticosteroid feedback and reverses insulin resistance in low-birth-weight rats, Am. J. Physiol. Endocrinol. Metab. 298 (2010) E920–9, https://doi.org/10.1152/ajpendo.00606.2009.
[44] R.J. Perry, V.T. Samuel, K.F. Petersen, G.I. Shulman, The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes, Nature. 510 (2014) 84–91, https://doi.org/10.1038/nature13478.
[45] C.S. Wyrwoll, P.J. Mark, T.A. Mori, B.J. Waddell, Developmental programming of adult hyperinsulinemia, increased proinflammatory cytokine production, and altered skeletal muscle expression of SLC2A4 (GLUT4) and uncoupling protein 3, J. Endocrinol. 198 (2008) 571–579, https://doi.org/10.1677/JOE-08-0210.
[46] P.J. Mark, C.S. Wyrwoll, I.S. Zulkafli, T.A. Mori, B.J. Waddell, Rescue of glucocorticoid-programmed adipocyte inflammation by omega-3 fatty acid supplementation in the rat, Reprod. Biol. Endocrinol. 12 (2014) 39, https://doi.org/10.1186/1477-7827-12-39.
[47] M. Kong, L. Zhu, L. Bai, X. Zhang, Y. Chen, S. Liu, S. Zheng, S.J. Pandol, Y.P. Han, Z. Duan, Vitamin D deficiency promotes nonalcoholic steatohepatitis through impaired enterohepatic circulation in animal model, Am. J. Physiol. Gastrointest. Liver Physiol. 307 (2014) G883–93, https://doi.org/10.1152/ajpgi.00427.2013.
[48] R. Li, E. Guo, J. Yang, A. Li, Y. Yang, S. Liu, A. Liu, X. Jiang, 1,25$(OH)_2$ D_3 attenuates hepatic steatosis by inducing autophagy in mice, Obesity Silver Spring (Silver Spring) 25 (2017) 561–571, https://doi.org/10.1002/oby.21757.
[49] I. González-Molero, G. Rojo-Martínez, S. Morcillo, C. Gutierrez, E. Rubio, V. Pérez-Valero, I. Esteva, M.S. Ruiz de Adana, M.C. Almaraz, N. Colomo, G. Olveira, F. Soriguer, Hypovitaminosis D and incidence of obesity: a prospective study, Eur. J. Clin. Nutr. 67 (2013) 680–682, https://doi.org/10.1038/ejcn.2013.48.
[50] J.F. Landrier, E. Karkeni, J. Marcotorchino, L. Bonnet, F. Tourniaire, Vitamin D modulates adipose tissue biology: possible consequences for obesity? Proc. Nutr. Soc. 75 (2016) 38–46, https://doi.org/10.1017/S0029665115004164.
[51] A. Giulietti, E. van Etten, L. Overbergh, K. Stoffels, R. Bouillon, C. Mathieu, Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory, Diabetes Res. Clin. Pract. 77 (2007) 47–57, https://doi.org/10.1016/j.diabres.2006.10.007.
[52] H. Yaribeygi, M. Maleki, T. Sathyapalan, H. Iranpanah, H.M. Orafai, T. Jamialahmadi, A. Sahebkar, The molecular mechanisms by which vitamin D improve glucose homeostasis: a mechanistic review, Life Sci. 244 (2020), 117305, https://doi.org/10.1016/j.lfs.2020.117305.
[53] P. Pan, D.H. Jin, M. Chatterjee-Chakraborty, K. Halievski, D. Lawson, D. Remedios, C. Smetka, V. Pinto, E. Parra, A.S. Fleming, The effects of vitamin D_3 during pregnancy and lactation on offspring physiology and behavior in sprague-dawley rats, Dev. Psychobiol. 56 (2014) 12–22, https://doi.org/10.1002/dev.21086.
[54] A. Rafacho, T.M. Cestari, S.R. Taboga, A.C. Boschero, J.R. Bosqueiro, High doses of dexamethasone induce increased beta-cell proliferation in pancreatic rat islets, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E681–E689, https://doi.org/10.1152/ajpendo.90931.2008.
[55] A.O. Protzek, L.F. Rezende, J.M. Costa-Júnior, S.M. Ferreira, A.P. Cappelli, F.M. de Paula, J.C. de Souza, M.A. Kurauti, E.M. Carneiro, A. Rafacho, A.C. Boschero, Hyperinsulinemia caused by dexamethasone treatment is associated with reduced insulin clearance and lower hepatic activity of insulin-degrading enzyme, J. Steroid Biochem. Mol. Biol. 155 (2016) 1–8, https://doi.org/10.1016/j.jsbmb.2015.09.020.
[56] H. Fakhrai-Rad, A. Nikoshkov, A. Kamel, M. Fernström, J.R. Zierath, S. Norgren, H. Luthman, J. Galli, Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats, Hum. Mol. Genet. 9 (2000) 2149–2158, https://doi.org/10.1093/hmg/9.14.2149.
[57] R. Deprez-Poulain, N. Hennuyer, D. Bosc, W.G. Liang, E. Enée, X. Marechal, J. Charton, J. Totobenazara, G. Berte, J. Jahklal, T. Verdelet, J. Dumont, S. Dassonneville, E. Woitrain, M. Gauriot, C. Paquet, I. Duplan, P. Hermant, F. X. Cantrelle, E. Sevin, M. Culot, V. Landry, A. Herledan, C. Piveteau, G. Lippens, F. Leroux, W.J. Tang, P. van Endert, B. Staels, B. Deprez, Catalytic site inhibition of insulin-degrading enzyme by a small molecule induces glucose intolerance in mice, Nat. Communi. 8250 (2015), https://doi.org/10.1038/ncomms9250.
[58] E. Riant, A. Waget, H. Cogo, J.F. Arnal, R. Burcelin, P. Gourdy, Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice, Endocrinology. 150 (2009) 2109–2117, https://doi.org/10.1210/en.2008-0971.
[59] P. Alonso-Magdalena, S. Morimoto, C. Ripoll, E. Fuentes, A. Nadal, The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance, Environ. Health Perspect. 114 (2006) 106–112, https://doi.org/10.1289/ehp.8451.
[60] A. Nadal, P. Alonso-Magdalena, S. Soriano, I. Quesada, A.B. Ropero, The pancreatic beta-cell as a target of estrogens and xenoestrogens: implications for blood glucose homeostasis and diabetes, Mol. Cell. Endocrinol. 304 (2009) 63–68, https://doi.org/10.1016/j.mce.2009.02.016.
[61] G. Bryzgalova, L. Lundholm, N. Portwood, J.A. Gustafsson, A. Khan, S. Efendic, K. Dahlman-Wright, Mechanisms of antidiabetogenic and body weight-lowering effects of estrogen in high-fat diet-fed mice, Am. J. Physiol. Endocrinol. Metab. 295 (2008) E904–12, https://doi.org/10.1152/ajpendo.90248.2008.
[62] C. González, A. Alonso, N. Alvarez, F. Díaz, M. Martínez, S. Fernández, A. M. Patterson, Role of 17beta-estradiol and/or progesterone on insulin sensitivity in the rat: implications during pregnancy, J. Endocrinol. 166 (2000) 283–291, https://doi.org/10.1677/joe.0.1660283, 2017.