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Estradiol protects against ovariectomy-induced susceptibility to the anabolic effects of glucocorticoids in rats

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ABSTRACT

Glucocorticoids increase appetite and body weight gain in rats and ovariectomy (OVX) induces obesity, while estrogen (E) replacement attenuates OVX-induced changes. It is known that animals with obesity are more responsive to glucocorticoids anabolic effects than lean ones. This study aimed to evaluate the effects of ovariectomy and the protective role of estradiol on the responses induced by prolonged treatment with corticosterone or dexamethasone on energy homeostasis. For this, female Wistar rats subjected to SHAM or OVX surgery, composing the SHAM, OVX, and OVX + E groups, received water/ETOH or corticosterone (15 mg/l) and water or dexamethasone (0.5 μg/l) as drinking fluid for 28 days. The OVX + E group, since the first day, was daily treated with estradiol (10 μg/0.2 ml/rat SC). OVX induced enhancement of body weight gain, food intake, area of the adipocytes and weight of retroperitoneal adipose tissue, plasma cholesterol and glucose intolerance, with reduction on uterus weight. In OVX animals, treatment with glucocorticoids induced increases on body weight gain, food intake, weight of retroperitoneal adipose tissue, area of adipocytes of retroperitoneal and perigonadal + perirenal fat depots, plasma triglycerides (corticosterone), and glycemic response after GTT (dexamethasone), with minor effects on SHAM group. Estradiol treatment to OVX rats prevented these effects induced by glucocorticoids, in addition to decrease body weight gain, fat accumulation and glucose intolerance, and to increase weight of uterus, triglycerides and free fatty acids plasma levels. These data demonstrate that protection against glucocorticoids-induced anabolic responses in females is eliminated by ovariectomy and estradiol can prevent these responses.

1. Introduction

Cortisol in humans and corticosterone in rodents are examples of endogenous glucocorticoids and important regulators of energy intake and expenditure through actions on hypothalamic peptides [1]. It is known that there are differential affinities to corticosteroids receptors to endogenous or exogenous glucocorticoids. Dexamethasone is an example of exogenous glucocorticoid and it can bind to glucocorticoid receptor, while corticosterone binds to mineralocorticoid (MR) and glucocorticoid receptors (GR) [2]. Glucocorticoids are known to increase appetite and body weight in humans and rodents [3,4], and their excess may increase central adiposity, as seen in Cushing's syndrome, besides contributing to insulin and leptin resistance [5].

In addition, it is known that glucocorticoid plasma concentrations are increased in different models of obesity and bilateral removal of adrenal glands, named adrenalectomy, is able to reduce hyperphagia and obesity in different experimental models [6–9]. Conversely, mice and rats with obesity present higher sensitivity to the anabolic effects of glucocorticoids than lean animals [10–12].

Other important factors that regulate energy homeostasis are

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ovarian steroids, particularly estrogens. It is known that estrogens may lead to decreased food intake and body weight gain, and its absence, as observed experimentally after ovariectomy or physiologically at menopause, induces increase on food intake and body weight gain, leading to obesity and a positive status in the energy balance [13]. Accordingly, body changes, such as augment of adiposity in the body composition mainly in the abdominal area can be observed in humans during menopause [14]. Estrogen replacement therapy is able to attenuate and even to reverse several metabolic parameters altered due to loss of ovarian function, observed at menopause or induced by ovariectomy [15–18]. In addition, estradiol prevents visceral fat accumulations, favoring deposition of lipids in subcutaneous over visceral depots [19].

Thus, it is well established in the literature that glucocorticoids trigger important anabolic responses and animals with obesity are more responsive to these effects of glucocorticoid than lean animals, and finally that loss of ovarian function can lead to obesity and its comorbidities, and estrogens play a protective role in the development of obesity. The hypothesis of the present study was that ovariectomy-induced obesity becomes the animals more susceptible to side effects of glucocorticoids than sham animals due to the lack of circulating estrogen in animals without the ovaries. Thus, estrogen replacement would be able to reverse these effects of ovariectomy. Therefore, the present study aimed to evaluate the effects of ovariectomy and the role of estrogens on the anabolic effects of glucocorticoids, using for this the treatment with dexamethasone and corticosterone for 28 days.

2. Material and methods

2.1. Animals

Adult female Wistar rats (230–250 g body weight), from the Central Animal Care Facility of the State University of Londrina (UEL) were used. Animals were kept in cages at controlled temperature (22 ± 2 °C), with a fixed light–dark cycle (light from 6:00 AM to 6:00 PM) and with ad libitum access to pelleted rat chow and fluid, unless otherwise specified. All experimental procedures were conducted after 2:00 PM and were approved by the Ethics Commission on the Use of Animals of UEL (Protocol Number 14638.2016.42). Animals were subjected to SHAM or OVX surgery, as described below, composing the groups SHAM, OVX and OVX + E. During the 28 days period of experiment, SHAM and OVX groups, since of the first day, were daily treated with corn oil (0.2 ml/rat, SC), while OVX + E group daily received estradiol cypionate (Pfizer, New York-USA - 10 μg/0.2 ml/rat, SC). Glucocorticoids treatment was performed in the drinking fluid for 28 days, and animals received tap water containing 0.5% ethanol (water/ETOH) or corticosterone diluted in 0.5% ethanol (Sigma, Saint Louis-USA - CORT: 15 mg/l), as well as tap water (water) or dexamethasone (Ache, Sao Paulo-Brazil - DEXA: 0.5 μg/l). The dose of estradiol cypionate was selected based on doses previously used in the literature [20], and the concentrations of corticosterone and dexamethasone were chosen based on pilot studies of our laboratory (data not shown). After surgery, animals were daily monitored for 28 days, when body weight, food and fluid intakes, as well as estrus cycle phase were evaluated.

The efficiency of the OVX and E treatment was supported by reductions ($P < 0.05$) in the weights of the uterus of OVX groups and by the increases ($P < 0.05$) in this parameter in OVX + E groups, respectively, with no effects of glucocorticoids (Table 1). The statistical analysis demonstrated an effect of group (SHAM or OVX: $F_{3;63} = 94.580$; $P < 0.001$; $F_{3;29} = 116.288$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;63} = 0.133$; $P = 0.717$; Water or DEXA: $F_{3;29} = 1.317$; $P = 0.259$) on the weight of the uterus, with no interactions between group and treatment ($F_{3;63} = 0.821$; $P = 0.369$; $F_{3;29} = 2.285$; $P = 0.140$). We observed an effect of group (OVX or OVX + E: $F_{3;58} = 548.552$; $P < 0.001$; $F_{3;38} = 131.175$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;58} = 0.343$; $P = 0{,}560$; Water or DEXA: $F_{3;38} = 0.239$; $P = 0.627$) on the weight of the uterus, with no interactions between group and treatment ($F_{3;58} = 0.107$; $P = 0.745$; $F_{3;38} = 0.372$; $P = 0.545$).

As the glucocorticoids were given in the drinking water in a fixed concentration along 28 days and considering the changes in the body weight gain and in the volume of fluid intake among the groups, the doses of corticosterone and dexamethasone received by each group were calculated. Thus, based on the drugs concentrations and on daily body weight and fluid intake, the doses of corticosterone and dexamethasone ingested by SHAM, OVX and OVX-E groups from the day 1 up to the day 28 were calculated and this whole response was integrated in the area under curve (AUC). It could be observed that all OVX + E groups showed higher ($P < 0.05$) AUC of doses of corticosterone and dexamethasone compared to their respective OVX groups (Fig. 1). In addition, the AUC of the dose of dexamethasone dose was reduced in OVX animals compared with SHAM animals (Fig. 1).

2.2. Experimental design and animal model

2.2.1. Effects of ovariectomy and estrogen replacement on glucocorticoids treatment-induced changes on food intake, body weight, plasma metabolic parameters, glucose tolerance test, and white adipose tissue

On the 27th day, SHAM, OVX and OVX + E animals, receiving tap water, Water/ETOH, CORT or DEXA, as described above, had their food removed at 8:00 AM, and the animals were subjected to glucose tolerance test (GTT) at 2:00 PM, when a drop of blood from the tail was collected for the determination of basal glycemia by the test tape of the Accu-Check Advantage II device (Roche, Taquara, RJ, Brazil). Then, intraperitoneal administration of 5% glucose at the dose of 1.0 g/kg of body weight was carried out. Thereafter, blood glucose measurements were performed by the test strip 15, 30, 60 and 120 min after glucose overload. Food was returned to the cages in the end of the GTT. On day 28th, these animals were food restricted at 8:00 AM, and they were decapitated after 6 h. Trunk blood was collected, and plasma was stored at −20 °C for plasma measurements. Perigonadal + perirenal and retroperitoneal white adipose tissues were collected at autopsy, when ovariectomy was also confirmed.

2.3. Surgical procedures

Animals were intraperitoneally anesthetized with a cocktail of ketamine (100 mg/kg, 10%, Agener União, Apucarana, PR, Brazil) and xylazine hydrochloride (20 mg/kg, 2%, Anasedan®, Vetbrands, Jacareí, SP, Brazil) and they were submitted to bilateral ovariectomy (OVX) or SHAM surgery of OVX. Both OVX and SHAM surgeries were performed via bilateral incisions in the skin and small bilateral sections through the muscle layer at the angle between the last rib and vertebral column. SHAM-operated animals underwent similar surgical procedures but without removal of the ovaries, according to the method described by Lasota and Danowska-Klonowskza [21]. After suturing, animals received prophylactic antibiotic (penicillin G, Zoetis, Chapecó, SC, Brazil: 50,000 units of 0.1 ml per 100 g of body weight, intramuscularly) and paracetamol (CIMED, Pouso Alegre, MG, Brazil, 200 mg/kg, orally), and they were placed in cages containing fluid and food, where they remained until the day of the experiment.

2.4. Determination of the estrous cycle

Vaginal smears of the rats were daily collected at 8 AM to follow the phase of the estrous cycle throughout the experiment. For this purpose, vaginal smear was performed, and the collected material was placed on histological glass slide, which was analyzed in optical microscope at 10× magnification for the analysis of proportions and types of the cells (cornified epithelial cells, nucleated epithelial cells and leukocytes). The phases of the estrous cycle were then determined by the following criteria: in proestrus there was predominance of nucleated epithelial

Table 1
Weight of the uterus (g) of SHAM, OVX and OVX + E groups, treated with Water/ETOH or corticosterone (CORT) and Water or dexamethasone (DEXA).

	SHAM		OVX		OVX + E	
	Water/ETOH	CORT	Water/ETOH	CORT	Water/ETOH	CORT
Weight of uterus (g)	0.5 ± 0.03 (21)	0.4 ± 0.02 (10)	0.2 ± 0.01$^{\#}$ (17)	0.2 ± 0.02$^{\#}$ (15)	1.3 ± 0.12Z$^{\#\#}$ (12)	1.3 ± 0.11$^{\#\#}$ (14)

	SHAM		OVX		OVX + E	
	Water	DEXA	Water	DEXA	Water	DEXA
Weight of uterus (g)	0.5 ± 0.06 (4)	0.4 ± 0.04 (7)	0.2 ± 0.01$^{\#}$ (9)	0.2 ± 0.01$^{\#}$ (9)	0.9 ± 0.12$^{\#\#}$ (10)	0.9 ± 0.12$^{\#\#}$ (10)

Data are expressed as mean ± SEM.
$^{\#}$ P < 0.05 SHAM vs. OVX.
$^{\#\#}$ P < 0.05 OVX vs. OVX + E.

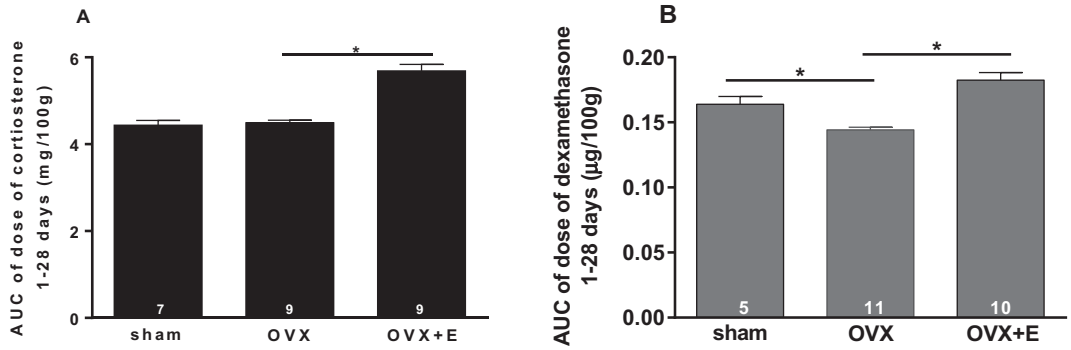

Fig. 1. Area under curve of the doses of corticosterone (mg/100 g) (A) and dexamethasone (µg/100 g) (B) received by SHAM, OVX and OVX-E groups, from day 1 to day 28 of treatment with glucocorticoids. Data are expressed as mean ± SEM. *P < 0.05.

cells, the estrous phase was identified by the presence of greater proportion of cornified epithelial cells, the metaestrus (diestrus I) was characterized by cornified and other nucleated cells, already accompanied by large number of leukocytes, and the diestrus (diestrus II) was identified by the presence of leukocytes cells. The phase of the estrous cycle chosen for SHAM animals was diestrus I, since only SHAM animals in this phase were considered in the study.

2.5. Body weight, food and fluid intake

The body weight of all rats was daily measured throughout the experimental period and expressed in grams. In each day, all the animals received pelleted rat chow (100 g/day, Nuvilab CR1, Nuvital®), and the scraps were removed after 24 h. The difference between the supply and the leftover food was calculated, and food intake was expressed as the AUC of daily amount of ingested food (g/100 g of body weight) from day 1 to day 28. For the analysis of the dose of glucocorticoids ingested by each group throughout the experiment, fluid intake was also daily evaluated by calculating the volume ingested by each animal (ml/100 g of body weight).

2.6. Lee index

The Lee index, expressed as $g^{1/3}$/cm, was evaluated at the end of each experiment and calculated by considering body weight and naso-anal distance, whereas the cubic root of body weight in grams was divided by naso-anal length in centimeters [22].

2.7. Euthanasia

All animals used in the experiments were euthanized by decapitation. Trunk blood was immediately collected in heparinized tubes and centrifuged at 14,000 ×*g* for 20 min at 4 °C to obtain the plasma, which was stored at −20 °C for biochemical analysis.

2.8. Removal and histology of white adipose tissue

Immediately after decapitation, visceral white adipose tissues, comprising the perigonadal, perirenal and retroperitoneal subdivisions were carefully removed, weighted, and the values were expressed in grams. Samples of adipose tissues were fixed in 4% formaldehyde in phosphate buffered saline (PBS), pH 7.4, for 48 h at 4 °C. After fixation, tissues were dehydrated in graded ethanol, cleared in xylol, and embedded in paraffin at 62 °C. The sections of 12 mm obtained on microtome were stained by hematoxylin and eosin, and then captured with the aid of an image system attached to the microscope (Motic, Xiamen, China), where 3 fields of each animal were analyzed, using image J software, to determine the area of adipocytes (µm^2) [23].

2.9. Measurement of cholesterol, triglycerides, free fatty acids and corticosterone plasma levels

The spectrophotometric determination of cholesterol and triglycerides plasma concentrations was performed using the BioLiquid Cholesterol Commercial Kit (Laborclin, PR) and BioLiquid Tryglicérides GPO-Trinder Commercial Kit (Laborclin, PR), respectively, according to the manufacturer protocol and the values were expressed as mg/dl. By means of the spectrophotometric method of Falholt et al. [24], using a spectrophotometer at wavelength of 550 nm, the results obtained for the determination of free fatty acids were expressed in µmoles/dl. For this, 100 µl of the plasma samples were used to perform two extractions, followed by shaking, aspiration of the upper phase and centrifugation. For the standard, 50 µl of palmitic acid (2 mmolar) was used in 1.0 ml of phosphate buffer (pH 6.4) and 6.0 ml of extractive solution, and the blank had only the extractive solution. Finally, by the fluorometric method of Guillemin et al. [25], based on the fluorescence of

corticosterone in sulfuric acid, the concentration of corticosterone expressed in μg/dl was obtained. The analysis was performed on a fluorimeter (Victor3TM, PerkinElmer) with excitation at 477 nm, emission 520 nm and sensitivity 11.

2.10. Statistical analysis

The normal distribution and homogeneity of the data were tested, and results were analyzed by two-way Anova, followed by the Newman–Keuls post hoc test. Data are expressed as mean ± standard error of the mean (SEM). Differences were considered significant at $P < 0.05$.

3. Results

3.1.1. Effects of ovariectomy and estradiol on glucocorticoids treatment-induced changes on food intake, body weight gain, Lee index, plasma metabolic parameters, visceral white adipose tissue and glucose tolerance test

All OVX groups showed higher ($P < 0.05$) body weight gain and food intake compared to their respective SHAM groups (Fig. 2). Corticosterone induced increases ($P < 0.05$) in body weight gain and food intake only in OVX animals, with no effects in SHAM groups (Fig. 2). Treatment with exogenous glucocorticoid, dexamethasone, did not alter body weight gain and food intake in SHAM or OVX groups (Fig. 2). We observed an effect of group (SHAM or OVX: $F_{3;62} = 133.50$; $P < 0.001$) and treatment (Water/ETOH or CORT: $F_{3;62} = 12.94$; $P < 0.001$) on body weight gain, with no interactions between group and treatment ($F_{3;62} = 1.49$; $P = 0.227$). There was also an effect of group (SHAM or OVX: $F_{3;62} = 27.80$; $P < 0.001$) and treatment (Water/ETOH or CORT: $F_{3;62} = 6.37$; $P = 0.014$) on food intake, with no interactions between group and treatment ($F_{3;62} = 0.560$; $P = 0.457$). We observed an effect of group (SHAM or OVX: $F_{3;40} = 84.517$; $P < 0.001$), but no effect of treatment (Water or DEXA: $F_{3;40} = 1.018$; $P = 0.320$) on body weight gain, with no interactions between group and treatment ($F_{3;40} = 1.092$; $P = 0.303$). There was an effect of group (SHAM or OVX: $F_{3;40} = 10.848$; $P = 0.002$) and no effect of treatment (Water or DEXA: $F_{3;40} = 1.476$; $P = 0.233$) on food intake, with no interactions between group and treatment ($F_{3;40} = 0.008$; $P = 0.930$).

Estradiol treatment reduced body weight gain ($P < 0.05$), and none of treatments, CORT and DEXA, changed body weight gain and food intake in OVX treated with estradiol (Fig. 2). We observed an effect of group (OVX or OVX + E: $F_{3;60} = 181.64$; $P < 0.001$) and treatment (Water/ETOH or CORT: $F_{3;60} = 10.44$; $P = 0.002$) on body weight gain, with no interactions between group and treatment ($F_{3;60} = 3.84$; $P = 0.055$). There was an effect of group (OVX or OVX + E: $F_{3;54} = 161.888$; $P < 0.001$) but no effect of treatment (Water or DEXA: $F_{3;54} = 0.0986$; $P = 0.755$) on body weight gain, with no interactions between group and treatment ($F_{3;54} = 0.130$; $P = 0.720$). For food intake, there was no effect of group (OVX or OVX + E: $F_{3;60} = 3.13$; $P = 0.082$) and effect of treatment (Water/ETOH or CORT: $F_{3;60} = 8.20$; $P = 0.006$), with no interactions between group and treatment ($F_{3;60} = 0.292$; $P = 0.591$). We observed no effect of group (OVX or OVX + E: $F_{3;54} = 0.212$; $P = 0.647$) and no effect of treatment (Water or DEXA: $F_{3;54} = 3.298$; $P = 0.075$) on food intake, with no interactions between group and treatment ($F_{3;54} = 0.030$; $P = 0.863$).

In corticosterone protocol, Lee index was not different among the groups, but in dexamethasone experiment, OVX animals treated with DEXA had higher ($P < 0.05$) Lee index compared to SHAM-DEXA group, with no differences between SHAM-Water and OVX-Water groups, as well as between Water and DEXA animals (Table 2). There was decrease ($P < 0.05$) in the Lee index of OVX + E animals of both protocols without effects of glucocorticoids in any group (Table 2). We observed no effect of group (SHAM or OVX: $F_{3;64} = 2.143$; $P = 0.149$) and treatment (Water/ETOH or CORT: $F_{3;64} = 0.079$; $P = 0.780$) on Lee index with no interactions between group and treatment ($F_{3;64} = 1.191$; $P = 0.280$). There was effect of group (SHAM or OVX: $F_{3;34} = 9.247$; $P = 0.005$) and no effect of treatment (Water or DEXA: $F_{3;34} = 0.00225$; $P = 0.962$) on Lee index, with no interactions between group and treatment ($F_{3;34} = 0.548$; $P = 0.465$). We observed an effect of group (OVX or OVX + E: $F_{3;63} = 20.973$; $P < 0.001$; $F_{3;42} = 51.764$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;63} = 2.201$; $P = 0.143$; Water or DEXA: $F_{3;42} = 0.406$; $P = 0.528$) on Lee index, with no interactions between group and treatment ($F_{3;63} = 1.664$; $P = 0.202$; $F_{3;42} = 0.349$; $P = 0.558$).

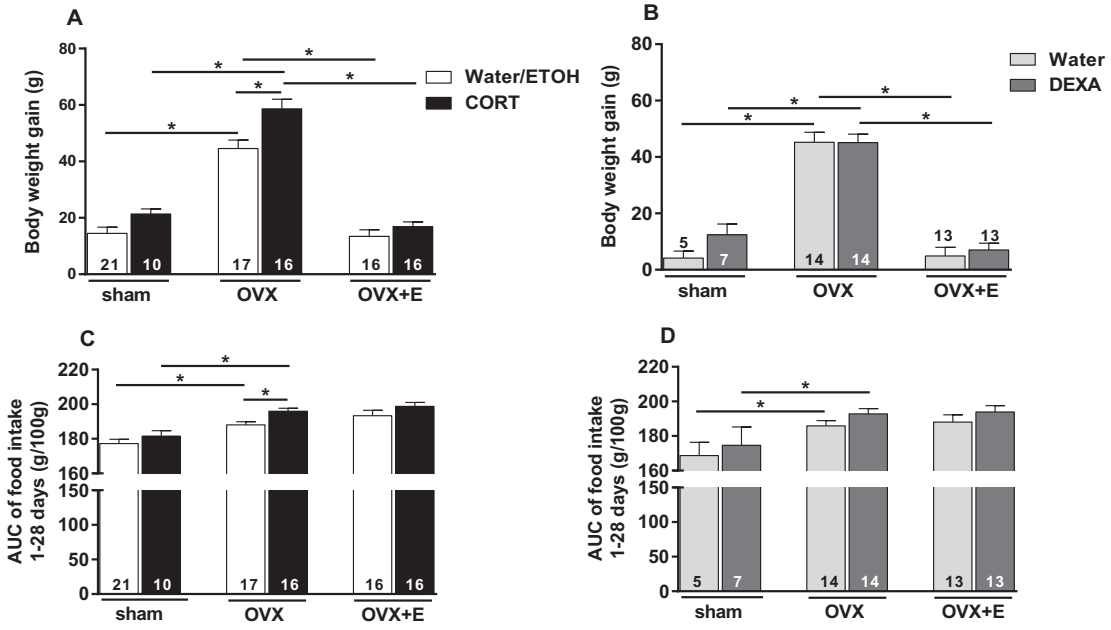

Fig. 2. Body weight gain (A, B) and food intake (C, D) of SHAM, OVX and OVX + E groups, which were treated with Water/ETOH or corticosterone (CORT: 15 mg/l) (A and C) and Water or dexamethasone (DEXA: 0.5 μg/l) (B and D). Data are expressed as mean ± SEM. *$P < 0.05$.

Table 2
Lee Index ($g^{1/3}$/cm) of SHAM, OVX and OVX + E groups, treated with Water/ETOH or CORT and Water or DEXA.

	SHAM		OVX		OVX + E	
	Water/ETOH	CORT	Water/ETOH	CORT	Water/ETOH	CORT
Lee Index ($g^{1/3}$/cm)	0.291 ± 0.003 (21)	0.287 ± 0.004 (10)	0.291 ± 0.002 (17)	0.291 ± 0.002 (16)	0.280 ± 0.002## (14)	0.285 ± 0.002## (16)

	SHAM		OVX		OVX + E	
	Water	DEXA	Water	DEXA	Water	DEXA
Lee Index ($g^{1/3}$/cm)	0.308 ± 0.003 (5)	0.306 ± 0.005 (7)	0.315 ± 0.002 (11)	0.318 ± 0.003# (11)	0.301 ± 0.002## (10)	0.301 ± 0.002## (10)

Data are expressed as mean ± SEM.
# P < 0.05 SHAM vs. OVX.
P < 0.05 OVX vs. OVX + E.

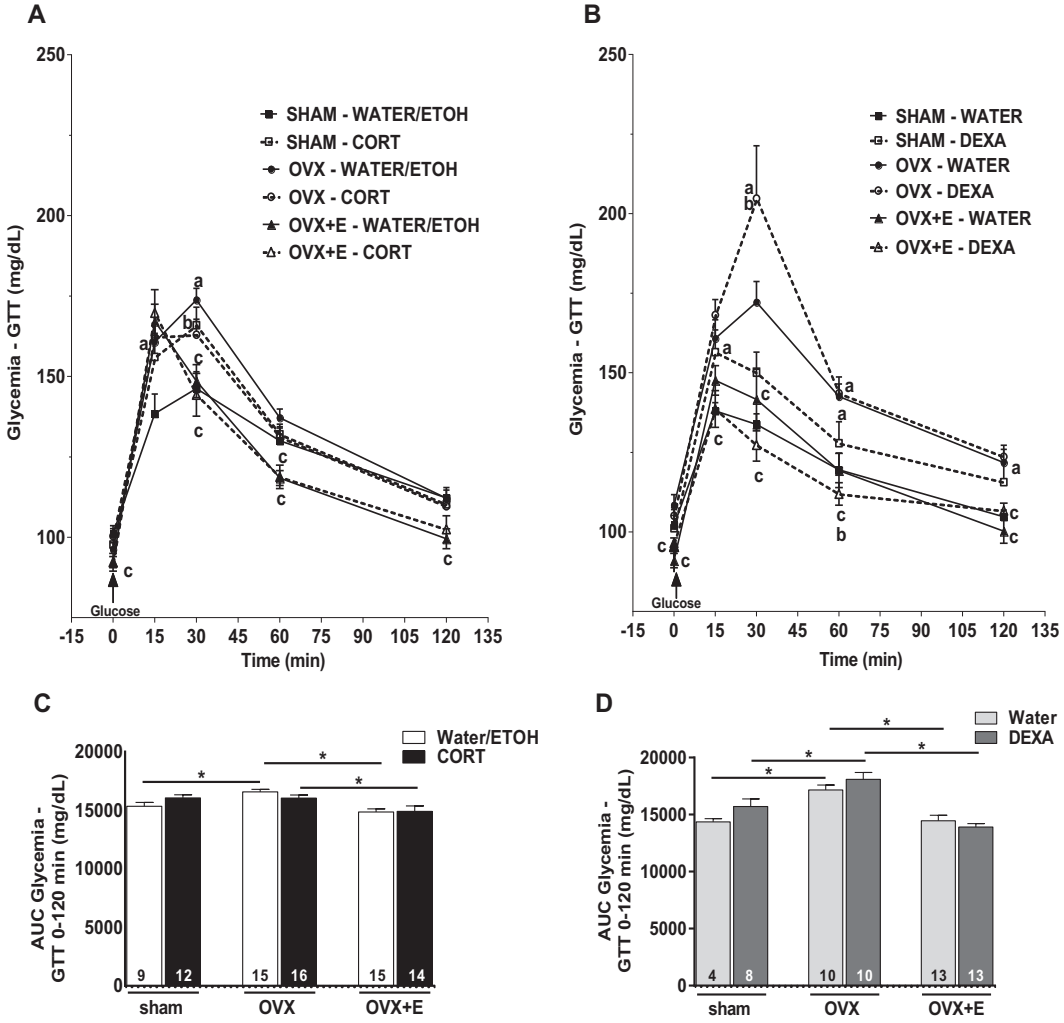

Fig. 3. Glucose tolerance test (GTT) (A, B) and area under the curve (AUC) of GTT (C, D) of SHAM, OVX and OVX + E groups, which were treated with Water/ETOH or corticosterone (CORT: 15 mg/l) (A, C) and Water or dexamethasone (DEXA: 0.5 μg/l) (B, D). Data are expressed as mean ± SEM. a: P < 0.05 vs. respective sham; b: P < 0.05 vs. respective OVX; c: P < 0.05 vs. Water or Water/ETOH. *P < 0.05.

According with the Fig. 3A, in the corticosterone treatment protocol, there was no differences among the groups in the basal glycemia, but after 15 and 30 min of glucose overload, OVX-Water/ETOH animals showed higher (P < 0.05) glycemia than SHAM-Water/ETOH group. In addition, glycemia of SHAM animals treated with CORT was higher at 30 min of GTT than SHAM-Water/ETOH group. For dexamethasone experiments (Fig. 3B), OVX-Water animals showed increased (P < 0.05) glycemia at 15, 60, and 120 min of GTT compared to SHAM-Water group. At points 30 and 60 min of GTT, OVX-DEXA group had higher (P < 0.05) glycemia in comparison to SHAM-DEXA animals, dexamethasone enhancing (P < 0.05) glycemia at 30 min of GTT in OVX animals, compared to Water treatment in this group.

The GTT of the OVX and OVX + E groups in corticosterone studies (Fig. 3A) showed that animals of OVX + E group showed smaller

($P < 0.05$) values of basal glycemia and after glucose overload in relation to OVX groups. These differences had statistical significance in the basal time for CORT treatment, at times 30 and 60 min for both Water/ETOH and CORT treatments, and at 120 min for Water/ETOH treatment. In dexamethasone experiments (Fig. 3B), OVX + E animals responded with lower ($P < 0.05$) values of glycemia than OVX groups. OVX + E animals treated with Water or DEXA showed reduced glycemia compared to the respective OVX groups at times 0, 30, and 120 min. DEXA decreased (60 min) glycemia in OVX + E group, and finally, at times 15 and 60 min, OVX + E-DEXA animals had lower values of glycemia than OVX-DEXA groups.

The whole response of GTT was integrated in the AUC of GTT, which showed that, in corticosterone experiments, OVX animals treated with Water/ETOH presented higher values ($P < 0.05$) of AUC of GTT than its respective SHAM group (Fig. 3C). In dexamethasone protocol, AUC of GTT of OVX groups treated with Water or DEXA was increased in comparison with their respective SHAM animals (Fig. 3D). There was an effect of group (SHAM or OVX: $F_{3;52} = 5.058$; $P = 0.029$) and no effect of treatment (Water or CORT: $F_{3;52} = 0.111$; $P = 0.740$) on AUC of GTT, with interactions between group and treatment ($F_{3;52} = 5.204$; $P = 0.027$). We observed an effect of group (SHAM or OVX: $F_{3;32} = 15.496$; $P < 0.001$) and no effect of treatment (Water or DEXA: $F_{3;32} = 2.947$; $P = 0.095$) on AUC of GTT, with no interactions between group and treatment ($F_{3;32} = 0.102$; $P = 0.752$). In addition, OVX + E animals presented reduced values of AUC of GTT than OVX groups in both protocols (Fig. 3C and D). There was an effect of group (OVX or OVX + E: $F_{3;60} = 20.96$; $P < 0.001$; $F_{3;46} = 53.332$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT) ($F_{3;60} = 0.594$; $P = 0.444$; Water or DEXA: $F_{3;46} = 0.152$; $P = 0.699$) on AUC of GTT, with no interactions between group and treatment ($F_{3;60} = 0.862$; $P = 0.357$; $F_{3;46} = 2.437$; $P = 0.126$).

For the results of visceral white adipose tissues (Figs. 4, 5, 6), the area of the adipocytes of both fat depots was enhanced ($P < 0.05$) by OVX (Water/ETOH or Water) compared to their respective SHAM group. Corticosterone and dexamethasone had no effects on the area of adipocytes and weight of white retroperitoneal adipose tissue in the SHAM groups compared to Water or Water/ETOH treatments. However, both treatments were effective in inducing increases ($P < 0.05$) in the area of adipocytes and weight of retroperitoneal adipose tissue in OVX animals, which presented significantly higher values ($P < 0.05$) compared to their respective SHAM animals (Figs. 4, 5A, B, D, E, and 6A, B, D, E). Dexamethasone increased the size of adipocytes of perigonadal + perirenal adipose tissues in OVX group, being this parameter higher in OVX animals treated with both glucocorticoids than SHAM groups treated with Water/ETOH or Water (Figs. 4, 5G, H, K, J, and 6G, H, K, J). Corticosterone and dexamethasone did not change the weight of perigonadal + perirenal adipose tissues in SHAM or OVX groups, but it was observed a decrease ($P < 0.05$) of this parameter in OVX-Water/ETOH animals in relation to SHAM-Water/ETOH of the corticosterone protocol, without differences in dexamethasone. We observed an effect of group (SHAM or OVX: $F_{3;26} = 26.243$; $P < 0.001$; $F_{3;24} = 14.976$; $P < 0.001$) and treatment (Water/ETOH or CORT: $F_{3;26} = 5.896$; $P = 0.024$; Water or DEXA: $F_{3;24} = 13{,}190$; $P = 0.001$) on the area of adipocyte in the retroperitoneal adipose tissue, with no interactions between group and treatment ($F_{3;26} = 2.127$; $P = 0.159$; $F_{3;24} = 0.00166$; $P = 0.968$). There was an effect of group (SHAM or OVX: $F_{3;26} = 13.101$; $P = 0.002$; $F_{3;22} = 27.036$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;26} = 0.278$; $P = 0.603$; Water or DEXA: $F_{3;22} = 2.023$; $P = 0.172$) on the area of adipocyte in the perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;26} = 1.015$; $P = 0.325$; $F_{3;22} = 3.127$; $P = 0.094$). We observed an effect of group (SHAM or OVX: $F_{3;62} = 9.244$; $P = 0.004$; $F_{3;38} = 4.569$; $P = 0.040$) and no effect of treatment (Water/ETOH or CORT: $F_{3;62} = 0.344$; $P = 0.560$; Water or DEXA: $F_{3;38} = 0.713$; $P = 0.404$) on the weight of retroperitoneal adipose tissue, with interactions between group and treatment in corticosterone protocol ($F_{3;62} = 6.654$; $P = 0.012$), but without interactions between group and treatment in dexamethasone protocol ($F_{3;38} = 2.723$; $P = 0.108$). There was no effect of group (SHAM or OVX: $F_{3;62} = 2.143$; $P = 0.149$; $F_{3;38} = 2.515$; $P = 0.122$) and treatment (Water/ETOH or CORT: $F_{3;62} = 0.079$; $P = 0.780$; Water or DEXA: $F_{3;38} = 0.00736$; $P = 0.932$) on the weight of perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;62} = 1.191$; $P = 0.280$; $F_{3;38} = 0.489$; $P = 0.489$).

Estradiol significantly decreased ($P < 0.05$) the size of the adipocytes and weight of retroperitoneal and perigonadal + perirenal adipose tissues of the animals treated with corticosterone, as well as the size of the adipocytes of both fat depots of dexamethasone treated animals, compared to the respective OVX group. Figs. 4 and 5 show that corticosterone and dexamethasone did not change the area of the adipocytes and weight of retroperitoneal adipose tissue in OVX + E groups. We observed an effect of group (OVX or OVX + E: $F_{3;60} = 6.796$; $P = 0.012$) and no effect of treatment (Water/ETOH or CORT: $F_{3;60} = 0.817$; $P = 0.370$) on the weight of retroperitoneal adipose tissue, with interactions between group and treatment ($F_{3;60} = 4.636$; $P = 0.036$). There was no effect of group (OVX or OVX + E: $F_{3;53} = 1.249$; $P = 0.269$) and treatment (Water or DEXA: $F_{3;53} = 3.288$; $P = 0.076$) on the weight of retroperitoneal adipose tissue, with no interactions between group and treatment ($F_{3;53} = 0.903$; $P = 0.347$). We observed an effect of group (OVX or OVX + E: $F_{3;60} = 4.364$; $P = 0.041$) and no effect of treatment (Water/ETOH or CORT: $F_{3;60} = 0.062$; $P = 0.804$) on the weight of the perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;60} = 1.338$; $P = 0.252$). There was no effect of group (OVX or OVX + E: $F_{3;53} = 1.365$; $P = 0.248$) and treatment (Water or DEXA: $F_{3;53} = 1.547$; $P = 0.219$) on the weight of perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;53} = 0.0686$; $P = 0.794$). We observed an effect of group (OVX or OVX + E: $F_{3;26} = 23.922$; $P < 0.001$; $F_{3;26} = 8.835$; $P < 0.007$) and no effect of treatment (Water/ETOH or CORT: $F_{3;26} = 3.011$; $P = 0.097$; Water or DEXA: $F_{3;26} = 1.348$; $P = 0.258$) on the area of adipocyte in the retroperitoneal adipose tissue, with interactions between group and treatment ($F_{3;26} = 6.760$; $P = 0.016$; $F_{3;26} = 4.948$; $P = 0.037$). There was an effect of group (OVX or OVX + E: $F_{3;23} = 13.712$; $P = 0.002$) and no effect of treatment (Water/ETOH or CORT: $F_{3;23} = 1.343$; $P = 0.261$) on the area of adipocyte in the perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;23} = 0.324$; $P = 0.576$). We observed an effect of group (OVX or OVX + E: $F_{3;22} = 7.772$; $P = 0.012$) and treatment (Water or DEXA: $F_{3;22} = 4.958$; $P = 0.039$) on the area of adipocyte in the perirenal and perigonadal adipose tissues, with no interactions between group and treatment ($F_{3;22} = 0.194$; $P = 0.665$) (Figs. 4, 5 B, C, E, F, H, I, K, L and 6 B, C, E, F, H, I, K, L).

It can be observed in Table 3 that corticosterone treatment increased ($P < 0.05$) plasma levels of total cholesterol in SHAM group and triglycerides in OVX animals. In addition, in corticosterone experiments, OVX induced increase ($P < 0.05$) in cholesterol plasma levels both in Water/ETOH and CORT treatments, with no differences among the groups in cholesterol and triglycerides plasma levels in dexamethasone protocol (Table 4). There were no differences among the experimental groups in the plasma concentrations of free fatty acids (Tables 3 and 4). Corticosterone treatment promoted decrease ($P < 0.05$) in plasma levels of corticosterone in SHAM animals, with no differences in OVX group, as well as without effects of DEXA in any group. In both treatments, OVX-Water or OVX-Water/ETOH animals showed decreased ($P < 0.05$) plasma concentrations of corticosterone, when compared to their respective SHAM groups (Tables 3 and 4). We observed an effect of group (SHAM or OVX: $F_{3;58} = 28.709$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;58} = 2.498$; $P = 0.120$) on plasma levels of total cholesterol, with no interactions between group and treatment ($F_{3;58} = 1.917$; $P = 0.172$). There was no effect of group (SHAM or OVX: $F_{3;40} = 0.441$; $P = 0.511$)

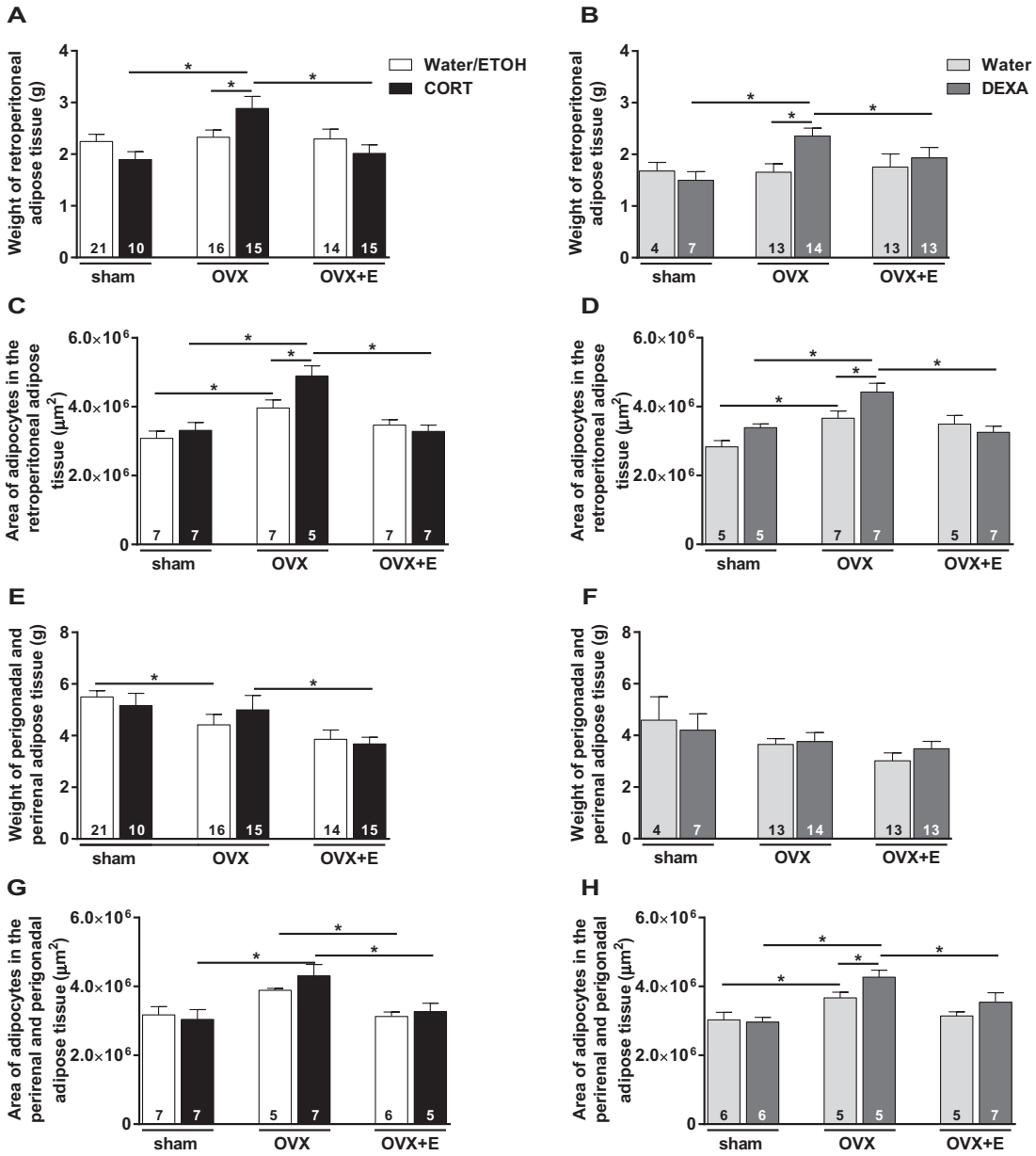

Fig. 4. Weights (g) and area of adipocytes (μm^2) of retroperitoneal (A–D) and perirenal + perigonadal adipose tissues (E–H) of SHAM, OVX and OVX + E groups, which were treated with Water/ETOH or corticosterone (CORT: 15 mg/l) (A, C, E, G) and Water or dexamethasone (DEXA: 0.5 µg/l) (B, D, F, H). Data are expressed as mean ± SEM. *P < 0.05.

and treatment (Water or DEXA: $F_{3;40} = 0.444$; P = 0.509) on plasma levels of total cholesterol, with no interactions between group and treatment ($F_{3;40} = 0.171$; P = 0.681). We observed no effect of group (SHAM or OVX: $F_{3;60} = 0.100$; P = 0.753) and effect of treatment (Water/ETOH or CORT: $F_{3;60} = 5.032$; P = 0.029) on plasma levels of triglycerides, with no interactions between group and treatment ($F_{3;60} = 0.761$; P = 0.387). There was no effect of group (SHAM or OVX: $F_{3;40} = 0.139$; P = 0.712) and treatment (Water or DEXA: $F_{3;40} = 0.219$; P = 0.642) on plasma levels of triglycerides, with no interactions between group and treatment ($F_{3;40} = 0.258$; P = 0.615). We observed no effect of group (SHAM or OVX: $F_{3;59} = 1.351$; P = 0.250; $F_{3;39} = 1.967$; P = 0.170) and treatment (Water/ETOH or CORT: $F_{3;59} = 0.664$; P = 0.419; Water or DEXA: $F_{3;39} = 0.375$; P = 0.545) on plasma levels of free fatty acids, with no interactions between group and treatment ($F_{3;59} = 0.173$; P = 0.679; $F_{3;39} = 0.120$; P = 0.731). We observed no effect of group (SHAM or OVX: $F_{3;60} = 0.004$; P = 0.948) and no effect of treatment (Water/ETOH or CORT: $F_{3;60} = 1.617$; P = 0.209) on plasma levels of corticosterone, with no interactions between group and treatment ($F_{3;60} = 1.950$; P = 0.168). There was an effect of group (SHAM or OVX: $F_{3;39} = 4.904$; P = 0.033) and no effect of treatment (Water or DEXA: $F_{3;39} = 0.348$; P = 0.559) on plasma levels of corticosterone, with no interactions between group and treatment ($F_{3;39} = 0.586$; P = 0.449).

The results of the OVX and OVX + E groups (Tables 3 and 4) showed that there was no significant difference in plasma total cholesterol concentration among the groups in both protocols. Additionally, corticosterone promoted no changes on plasma concentrations of triglycerides in OVX + E animals, and estradiol induced increase (P < 0.05) in plasma concentrations of triglycerides in animals treated with Water/ETOH and DEXA, in corticosterone and dexamethasone protocols, respectively. There was increase in plasma concentrations of free fatty acids in OVX + E/Water-ETOH group compared to the OVX/Water-ETOH animals in the corticosterone protocol. In dexamethasone experiments, DEXA increased free fatty acids

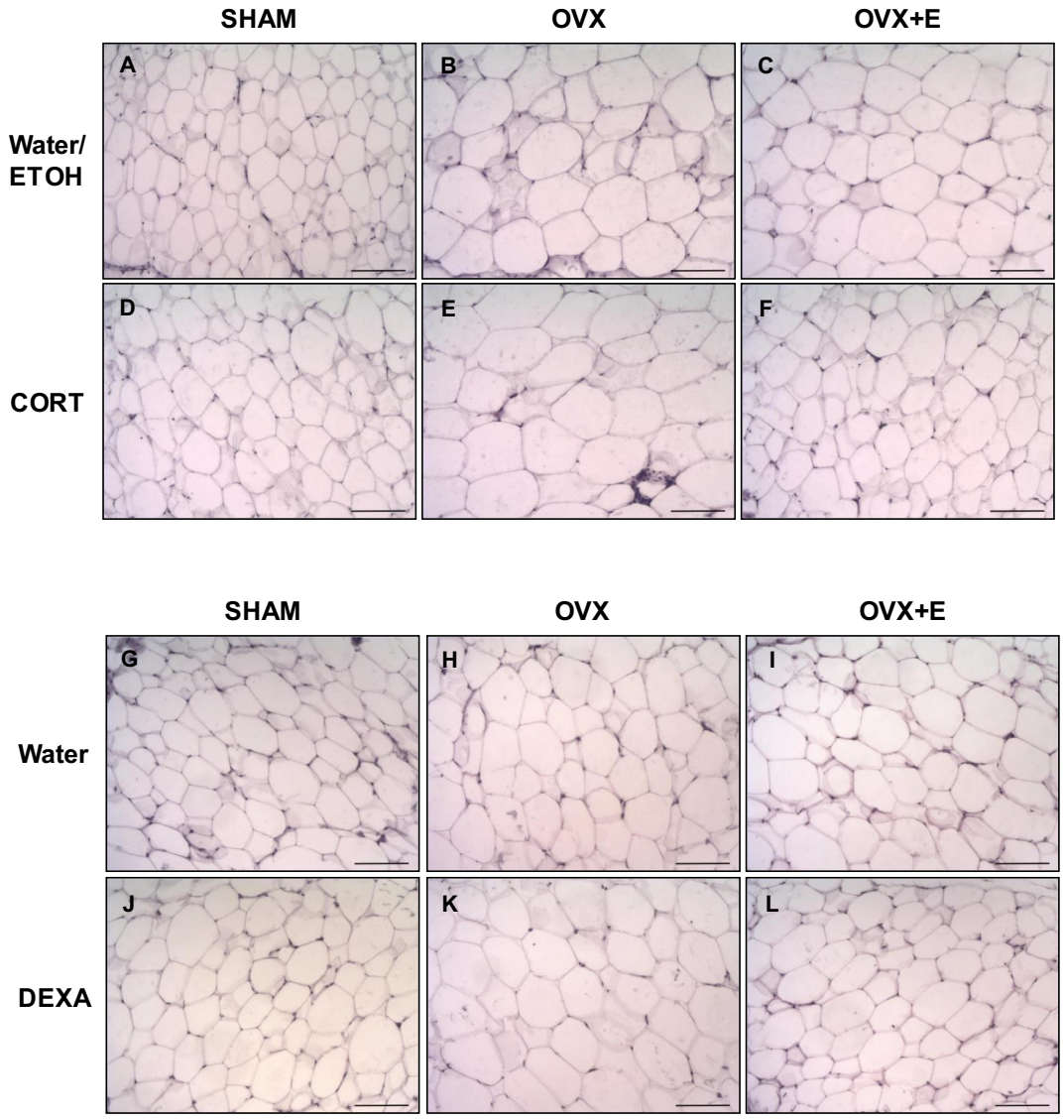

Fig. 5. Representative photomicrographs (100× magnification) of retroperitoneal adipose tissue, stained with hematoxylin-eosin, of SHAM, OVX and OVX + E groups, treated with Water/ETOH or corticosterone (CORT: 15 mg/l) (A–F) and Water or dexamethasone (DEXA: 0.5 μg/l) (G–L).

plasma concentrations in OVX + E animals, which showed higher values of this parameter compared to the respective OVX and OVX + E/Water groups. Both treatments increased ($P < 0.05$) plasma levels of corticosterone in OVX + E animals (CORT and DEXA) compared with their respective OVX groups, and in dexamethasone protocol there was enhancement ($P < 0.05$) of corticosterone plasma concentrations in animals OVX + E-DEXA compared to OVX + E-Water (Tables 3 and 4). We observed no effect of group (OVX or OVX + E: $F_{3;58} = 2.539$; $P = 0.117$; $F_{3;54} = 1.051$; $P = 0.310$) and treatment (Water/ETOH or CORT: $F_{3;58} = 1.160$; $P = 0.286$; Water or DEXA: $F_{3;54} = 0.0964$; $P = 0.757$) on plasma levels of total cholesterol, with no interactions between group and treatment ($F_{3;58} = 0.754$; $P = 0.389$; $F_{3;54} = 0.00204$; $P = 0.964$). There was an effect of group (OVX or OVX + E: $F_{3;55} = 5.599$; $P = 0.022$; $F_{3;54} = 7.165$; $P = 0.010$) and no effect of treatment (Water/ETOH or CORT: $F_{3;55} = 3.709$; $P = 0.060$; Water or DEXA: $F_{3;54} = 0.00741$; $P = 0.932$) on plasma levels of triglycerides, with no interactions between group and treatment ($F_{3;55} = 0.850$; $P = 0.361$; $F_{3;54} = 3.508$; $P = 0.067$). We observed an effect of group (OVX or OVX + E: $F_{3;60} = 18.552$; $P < 0.001$; $F_{3;52} = 16.003$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;60} = 3.984$; $P = 0.051$; Water or DEXA: $F_{3;52} = 2.616$; $P = 0.112$) on the plasma levels of free fatty acids, with no interactions between group and treatment in corticosterone protocol ($F_{3;60} = 0.260$; $P = 0.612$), but with interactions between group and treatment in dexamethasone protocol ($F_{3;52} = 8.275$; $P = 0.006$). There was an effect of group (OVX or OVX + E: $F_{3;59} = 7.902$; $P = 0.007$; $F_{3;54} = 16.300$; $P < 0.001$) and no effect of treatment (Water/ETOH or CORT: $F_{3;59} = 1.940$; $P = 0.169$; Water or DEXA: $F_{3;54} = 2.439$; $P = 0.125$) on plasma levels of corticosterone, with no interactions between group and treatment ($F_{3;59} = 1.052$; $P = 0.310$; $F_{3;54} = 1.685$; $P = 0.200$).

4. Discussion

The present study investigated whether ovariectomy becomes female rats more responsive to the anabolic effects of prolonged treatment with glucocorticoids, and if estradiol can be protective against these metabolic effects. Indeed, it was observed that the treatment for twenty-eight days with corticosterone or dexamethasone was able to induce, in OVX animals, anabolic effects including increases in body weight gain, food intake, size of adipocytes, weight of retroperitoneal adipose tissue and glycemia after GTT, and these responses were not observed in estradiol treated animals.

Classic data of the literature that demonstrate that ovariectomy can

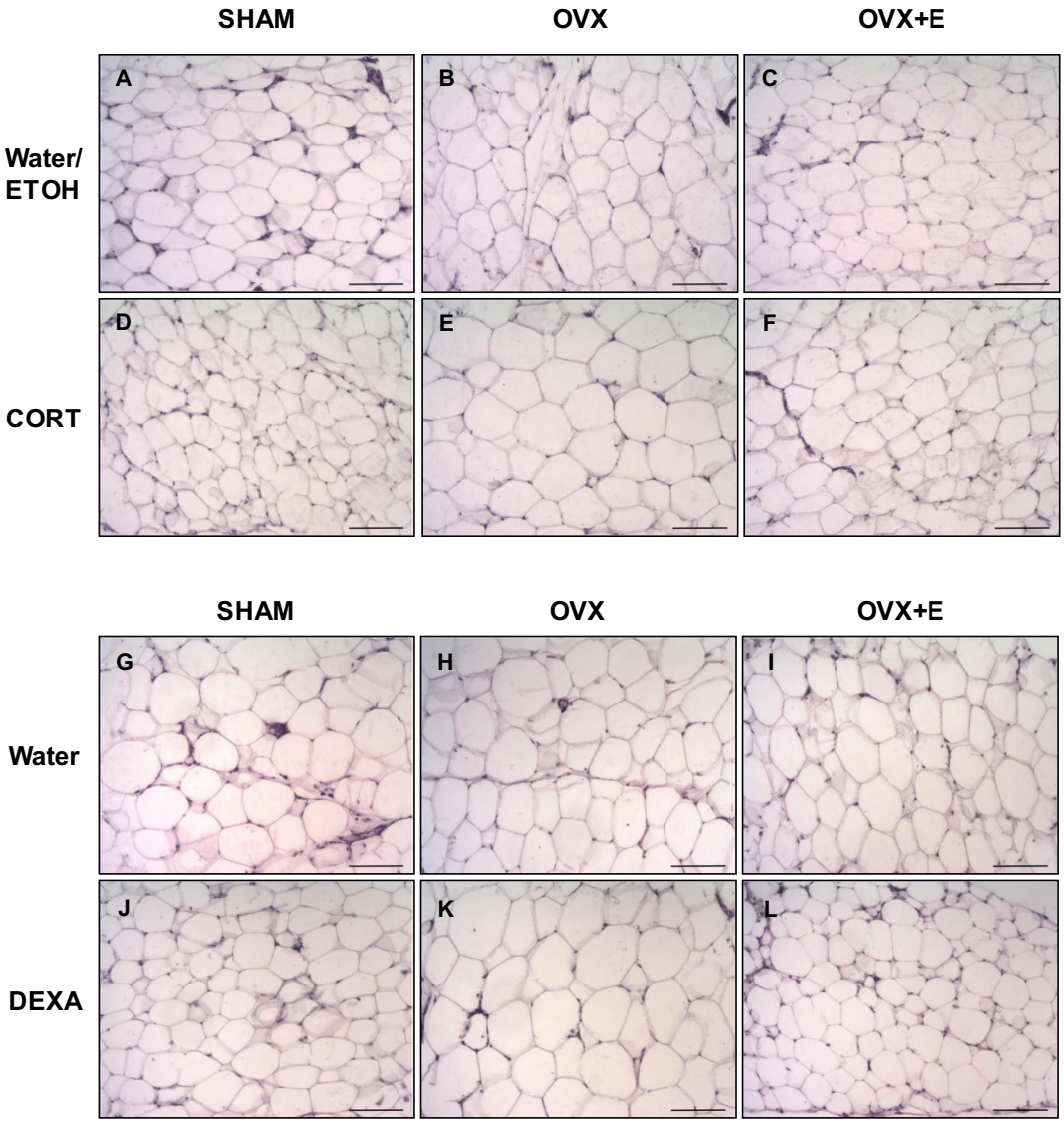

Fig. 6. Representative photomicrographs (100× magnification) of perirenal + perigonadal adipose tissues, stained with hematoxylin-eosin, of SHAM, OVX and OVX + E groups, treated with Water/ETOH or corticosterone (CORT: 15 mg/l) (A–F) and Water or dexamethasone (DEXA: 0.5 μg/l) (G–L).

lead to increase of food intake, body weight gain, visceral adiposity and plasma metabolic parameters [13,26] corroborate with the present findings, which show that OVX induces enhancement of body weight gain, food intake, weight of retroperitoneal adipose tissue, size of adipocytes in both visceral adipose tissues, glycemia after GTT, and plasma cholesterol. In this context, OVX-induced enhancement of food intake is related with increase and decrease in the expression of orexigenic and anorexigenic hypothalamic neuropeptides, respectively [18]. Accordingly, the classical effect of OVX increasing body weight gain and visceral fat pad may be due to the higher activity of the lipogenic enzyme lipoprotein lipase [27,28], while higher values of total cholesterol plasma levels may be ascribed to augmented plasma levels of low-

Table 3
Plasma levels of total cholesterol, triglycerides, free fatty acids and corticosterone of SHAM, OVX and OVX + E groups, treated with WATER/ETOH or corticosterone (CORT).

	SHAM		OVX		OVX + E	
	Water/ETOH	CORT	Water/ETOH	CORT	Water/ETOH	CORT
Cholesterol (mg/dl)	67.6 ± 4.2 (18)	79.6 ± 2.9* (9)	94.9 ± 4.8# (16)	95.7 ± 1.7# (15)	97.6 ± 3.3 (13)	105.0 ± 4.2 (16)
Triglycerides (mg/dl)	35.7 ± 2.4 (20)	37.7 ± 1.5 (8)	32.2 ± 1.8 (16)	39.8 ± 2.3* (16)	40.9 ± 3.5## (12)	43.6 ± 3.3 (11)
Free fatty acids (μmoles/dl)	65.2 ± 3.5 (20)	66.5 ± 2.5 (8)	67.6 ± 2.6 (16)	71.6 ± 2.2 (15)	78.4 ± 3.9## (13)	80.4 ± 4.1 (16)
Corticosterone (μg/dl)	5.0 ± 0.7 (21)	2.9 ± 0.7* (10)	3.0 ± 0.4# (15)	3.2 ± 0.4 (14)	3.9 ± 0.7 (15)	5.2 ± 0.6## (15)

Data are expressed as mean ± SEM.
* $P < 0.05$ Water/ETOH vs. CORT.
# $P < 0.05$ SHAM vs. OVX.
$P < 0.05$ OVX vs OVX + E.

Table 4
Plasma levels of total cholesterol, triglycerides, free fatty acids and corticosterone of SHAM, OVX and OVX + E groups, treated with WATER or dexamethasone (DEXA).

	SHAM		OVX		OVX + E	
	Water	DEXA	Water	DEXA	Water	DEXA
Cholesterol (mg/dl)	77.7 ± 2.2 (5)	84.6 ± 3.6 (7)	90.5 ± 3.2$^{\#}$ (14)	89.4 ± 3.6 (14)	95.1 ± 6.2 (13)	93.6 ± 3.6 (13)
Triglycerides (mg/dl)	38.9 ± 7.7 (5)	43.2 ± 4.3 (7)	39.5 ± 2.9 (14)	39.3 ± 3.5 (14)	43.3 ± 4.4 (13)	52.7 ± 5.9$^{\#\#}$ (13)
Free fatty acids (μmoles/dl)	86.2 ± 14.7 (4)	84.4 ± 6.3 (7)	79.1 ± 4.6 (14)	72.7 ± 4.5 (14)	84.9 ± 5.2 (12)	107.8 ± 6.3$^{\#\#,*}$ (12)
Corticosterone (μg/dl)	6.1 ± 1.6 (21)	4.9 ± 1.3 (10)	3.4 ± 0.5$^{\#}$ (15)	3.6 ± 0.7 (14)	4.2 ± 0.7 (15)	6.8 ± 0.6$^{\#\#,*}$ (15)

Data are expressed as mean ± SEM.
* $P < 0.05$ Water vs. DEXA.
$^{\#}$ $P < 0.05$ OVX vs. OVX + E.
$^{\#\#}$ $P < 0.05$ OVX vs OVX + E.

density lipoprotein cholesterol [29], and impaired glucose tolerance observed in OVX animals has been associated with defect in insulin-mediated glucose uptake in skeletal muscle [29]. In addition, OVX successfully suppressed endogenous production of ovarian steroids, which are known to have trophic roles in the uterus [30] and estradiol treatment in OVX animals reversed this effect [31]. Treatment with estradiol was able to attenuate several metabolic parameters that are altered by the loss of ovarian function, observed at menopause or induced by ovariectomy, decreasing body weight gain, fat accumulation and glycemic response after GTT, in accordance with previous data of literature [15–18,29,32]. Salpeter and colleagues [15] demonstrated that, in postmenopausal women who were treated for at least 8 weeks with oral estrogenic replacement, estradiol increased plasma triglycerides. Accordingly, Nigro et al. [33] showed that estradiol replacement-induced higher levels of serum triglycerides were due to increased production or secretion of very low-density lipoprotein cholesterol (VLDL). The lack of effects of estradiol on total cholesterol in the plasma may be a result of the increase of high-density lipoprotein cholesterol [29]. Though some studies have reported that estradiol classically reduces circulating free fatty acids following OVX, both in humans and rodents, [34,35], the current work demonstrated that estradiol increased circulating free fatty acids in OVX rats. However, other studies indicate that free fatty acids plasma levels are not changed by estradiol [36] and that estradiol induces a dose dependent increase in the activity of lipogenic enzymes in the liver, acetyl CoA carboxylase and fatty acid synthetase, which are key enzymes in the production of fatty acids [37], giving support to the present results. Thus, the discrepancies of estradiol effects on circulating free fatty acids in the literature could be explained by the differences in the time and dose of the treatment, animal strains and diet. Additionally, studies demonstrated that the increase of corticosterone levels in animals OVX with estradiol replacement occurs because the activity of the HPA axis is markedly influenced by sex steroids [38]. Estrogen stimulates the synthesis and release of ACTH, as reported by Kitay and colleagues [39], and estrogen acts centrally, in neurons that express corticotrophin releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus (PVN), to modulate hypothalamic-pituitary-adrenal axis and thus the neuroendocrine responses to stress [40]. Thus, the increase in corticosterone plasma concentrations in OVX + E animal and decrease in OVX group in the present work are in accordance with the literature.

Studies have shown that there is an interaction between glucocorticoids and ovarian steroids, since adrenalectomy was able to reverse obesity and some metabolic changes induced by chemical and physical ovariectomy [41,42], suggesting that glucocorticoids are required for the development of obesity induced by failure of ovarian function. Glucocorticoids are important regulators of energy and energy expenditure [1], increasing appetite and body weight gain in humans and rodents, and consequently leading to the development of obesity and its comorbidities [3,4]. In this context, glucocorticoids-induced enhancement of food intake and body weight gain is related with increase of orexigenic hypothalamic neuropeptides, neuropeptide Y and agouti-related protein, and decrease in the expression of anorexigenic hypothalamic neuropeptides, CRH and oxytocin [43–45], while estrogen have opposite effects [18]. Indeed, Karatsoreos et al. [46] demonstrated that chronic exposure to corticosterone (25 μg/ml) in the drinking water increased body weight gain, food intake and accumulation of adipose tissue in the mesenteric region. Asensio et al. [47] infused normal rats with dexamethasone intracerebroventricularly for 3 days and observed marked increase on food intake and body weight. However, these studies were conducted in male animals and studies evaluating the roles of glucocorticoids on energy homeostasis in female animals are rare. Thus, the current study is pioneer in evaluating glucocorticoids effects on this field in female rats, showing that both glucocorticoids basically had no effects on the parameters evaluated in SHAM rats.

Additionally, mice and rats with obesity are more sensitive than lean animals to effects of glucocorticoids on increased food intake, body weight gain, deposition of adipose tissue, and reduced brown adipose tissue activity [10,48]. However, this is the first report to demonstrate that, as in other experimental models of obesity, OVX-induced obesity also increases the susceptibility to the metabolic effects of glucocorticoids, and these responses are prevented by estradiol. In this context, previous data of the literature showed that OVX animals are more responsive to the increases in food intake and body weight gain induced by stress, which is known to induce marked release of glucocorticoids, and estradiol prevented these responses [49], supporting the data of the present study. Indeed, though the lack of direct evidences, these findings suggest that post-menopausal women may be more susceptible to stress-induced obesity. In addition, OVX animals also showed higher susceptibility to high-fat diet to increase body weight and adipose tissue than intact females [50], and estradiol abolished these effects [51]. Accordingly, the protective role of estrogens in the development of obesity induced by hyperlipidic diet, as well as their comorbidities, such as insulin resistance and dyslipidemias, were demonstrated not only in OVX animals [52–54], but also in males [55] and intact females [56].

The increase on the responsiveness to the anabolic effects of glucocorticoids in OVX animals and the protective effect of estradiol on these responses may be due to higher expression of GR and MR in the hypothalamus after OVX, especially the PVN, and a reversion of this effect by estradiol replacement [57,58]. However, local tissue concentrations of glucocorticoids depend not only on circulating levels of the hormone but also on pre-receptor conversion of 11-dehydrocorticosterone to corticosterone in rodents, via the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) [59]. In view of this, the stimulatory effect of OVX and inhibitory actions of estradiol on glucocorticoids-induced hypertrophy of visceral fat pads is reinforced by data that demonstrated that OVX upregulated 11βHSD1 expression in visceral adipose tissue, while estradiol replacement to OVX rats reduced the expression and activity of 11βHSD1 [60,61].

The effects of corticosterone and dexamethasone in OVX rats are in accordance with data of the literature in male animals. In this context, the enhancement of food intake induced by corticosterone in OVX rats may be ascribed changes in the expression of hypothalamic neuropeptides involved in the regulation of food intake, as already described. Accordingly, glucocorticoids-induced increase in body weight gain and visceral adiposity after OVX is likely to result of the greater susceptibility of visceral adipose tissues to the anabolic actions of glucocorticoids, due to higher amount and affinity of their receptors in these adipose depots [62,63], in association to the increased expression of LPL and other triglycerides-synthesis related genes induced by glucocorticoids, predominantly in the visceral adipose tissue [64–67]. Additionally, dexamethasone-induced higher glycemia after GTT in OVX animals may be explained by the known effect of glucocorticoids to induce impairment in glucose tolerance by reducing peripheral uptake of glucose uptake, especially in the skeletal muscle, because of a decrease of GLUT4 translocation to the cell surface [68–72]. Finally, corticosterone-induced increase in triglyceridemia after OVX could be consequence of the higher hepatic production of VLDL and impaired VLDL removal due to low LPL activity, possibly in the other fat depots than retroperitoneal, perigonadal + perirenal adipose tissues [73]. The difference in dexamethasone and corticosterone responses may be ascribed to the differential affinity of each glucocorticoid to corticosteroids receptor, GR and MR, since dexamethasone has more affinity to GR and corticosterone can bind to and activate both receptors [2]. Accordingly, corticosterone has more affinity to MR than to GR, and this higher affinity of glucocorticoids to MR is related to the ingestion and deposition of lipids throughout the circadian cycle, while GR have lower affinity for glucocorticoids, controlling carbohydrate intake in the first hours of the feeding period [2,74]. Altogether, these data suggest that both GR and MR need to be activated to mediate the effects of glucocorticoids after OVX, not only GR, and that MR are likely involved in the more prominent effects of corticosterone, since this glucocorticoid was more effective than DEXA to induce anabolic responses in OVX rats.

5. Conclusion

In summary, the present study is innovative in demonstrating that OVX animals are more susceptible to glucocorticoids-induced metabolic changes, and estradiol has a protective effect in these responses. Thus, it can be suggested that that the protection against the anabolic effects of glucocorticoids are like to be reduced in postmenopausal women, as in OVX rats, and estradiol replacement may be important to reestablish this protection.

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References

[1] F. Cavagnini, M. Croci, P. Putignano, M.L. Petroni, C. Invitti, Glucocorticoids and neuroendocrine function, Int. J. Obes. Relat. Metab. Disord. 24 (2000) S77–S79, https://doi.org/10.1038/sj.ijo.0801284.

[2] D.L. Tempel, S.F. Leibowitz, Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism, J. Neuroendocrinol. 6 (1994) 479–501.

[3] P.A. Tataranni, D.E. Larson, S. Snitker, J.B. Young, J.P. Flatt, E. Ravussin, Effects of glucocorticoids on energy metabolism and food intake in humans, Am. J. Phys. 271 (1996) E317–E325, https://doi.org/10.1152/ajpendo.1996.271.2.E317.

[4] K.E. Zakrzewska, I. Cusin, A. Stricker-Krongrad, D. Boss, D. Ricquer, B. Jeanrenaud, F. Rohner-Jeanrenaud, Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat, Diabetes 48 (1999) 365–370, https://doi.org/10.2337/diabetes.48.2.365.

[5] M. Rebuffé-Scrive, M. Krotkiewski, J. Elfverson, P. Bjorntorp, Muscle and adipose tissue morphology and metabolism in Cushing's syndrome, J. Clin. Endocrinol. Metab. 67 (1988) 1122–1128, https://doi.org/10.1210/jcem-67-6-1122.

[6] B.K. Bruce, B.M. King, G.R. Phelps, M.C. Veitia, Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats, Am. J. Phys. 243 (1982) E152–E157, https://doi.org/10.1152/ajpendo.1982.243.2.E152.

[7] Y. Yukimura, G.A. Bray, A.R. Wolfse, Some effects of adrenalectomy in the fatty rat, Endocrinology 103 (1978) 1924–1928, https://doi.org/10.1210/endo-103-5-1924.

[8] P.U. Dubuc, N.J. Wilden, Adrenalectomy reduces but does not reverse obesity in ob/ob mice, Int. J. Obes. 10 (1986) 91–98.

[9] H. Makimura, T.M. Mizuno, J. Roberts, J. Siverstein, J. Beasley, C.V. Mobbs, Adrenalectomy reverses obese phenotype and restores hypothalamic melanocortin tone in leptin-deficient *ob/ob* mice, Diabetes 49 (2000) 1917–1923, https://doi.org/10.2337/diabetes.49.11.1917.

[10] K. Tokuyama, J. Himms-Hagen, Increased sensitivity of the genetically obese mouse to corticosterone, Am. J. Phys. 252 (1987) E202–E208, https://doi.org/10.1152/ajpendo.1987.252.2.E202.

[11] K. Tokuyama, J. Himms-Hagen, Enhanced acute response to corticosterone in genetically obese (ob/ob) mice, Am. J. Phys. 257 (1989) E133–E138, https://doi.org/10.1152/ajpendo.1989.257.2.E133.

[12] M.R. Freedman, B.A. Horwitz, J.S. Stern, Effect of adrenalectomy and glucocorticoid replacement on development of obesity, Am. J. Phys. 250 (1986) R595–R607, https://doi.org/10.1152/ajpregu.1986.250.4.R595.

[13] L. Asarian, N. Geary, Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats, Horm. Behav. 42 (2002) 461–471, https://doi.org/10.1006/hbeh.2002.1835.

[14] F.A. Trémollieres, J.M. Pouilles, C.A. Ribot, Relative influence of age and menopause on total and regional body composition changes in postmenopausal women, Am. J. Obstet. Gynecol. 175 (1996) 1594–1600, https://doi.org/10.1016/S0002-9378(96)70111-4.

[15] S.R. Salpeter, J.M.E. Walsh, T.M. Ormiston, E. Greyber, N.S. Buckley, E.E. Salpeter, Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women, Diabetes Obes. Metab. 8 (2006) 538–554, https://doi.org/10.1111/j.1463-1326.2005.00545.x.

[16] D. Richard, P. Dekoninck, A. Lemay, S. Rivest, Reversible increase in energy deposition following castration induced by a gonadotropin releasing hormone agonist, Int. J. Obes. 15 (1991) 155–161.

[17] P.C. Butera, Estradiol and the control of food intake, Physiol. Behav. 99 (2010) 175–180, https://doi.org/10.1016/j.physbeh.2009.06.010.

[18] L.E.C.M. Silva, M. Castro, F.C. Amaral, J. Antunes-Rodrigues, L.L.K. Elias, Estradiol-induced hypophagia is associated with the differential mRNA expression of hypothalamic neuropeptides, Braz. J. Med. Biol. Res. 43 (2010) 759–766, https://doi.org/10.1590/S0100-879X2010007500059.

[19] D.J. Clegg, L.M. Brown, S.C. Woods, S.C. Benoit, Gonadal hormones determine sensitivity to central leptin and insulin, Diabetes 55 (2006) 978–987, https://doi.org/10.2337/diabetes.55.04.06.db05-1339.

[20] C.M. Leite, B. Kalil, E.T. Uchoa, J. Antunes-Rodrigues, L.K. Elias, J.E. Levine, J.A. Anselmo-Franci, Progesterone-induced amplification and advancement of GnRH/LH surges are associated with changes in kisspeptin system in preoptic area of estradiol-primed female rats, Brain Res. 1650 (2016) 30574–30581, https://doi.org/10.1016/j.brainres.2016.08.021.

[21] A. Lasota, D. Danowska-Klonowska, Experimental osteoporosis—different methods of ovariectomy in female white rats, Rocz. Akad. Med. Bialymst. 49 (2004) 129–131.

[22] L.L. Bernardis, B.D. Patterson, Correlation between "Lee index" and carcass fat content in weanling and adult female rats with hypothalamic lesions, J. Endocrinol. 40 (1968) 527–528.

[23] S.D. Parlee, S.I. Lentz, H. Mori, O.A. MacDougald, Quantifying size and number of adipocytes in adipose tissue, Methods Enzymol. 537 (2014) 93–122.

[24] K. Falholt, B. Lund, W. Falholt, An easy colorimetric method for routine determination of free fatty acids in plasma, Clin. Chim. Acta 46 (1973) 105–111, https://doi.org/10.1016/0009-8981(73)90016-8.

[25] R. Guillemin, G.W. Clayton, J.D. Smith, H.S. Lipscomb, Measurement of free corticosteroids in rat plasma: physiological validation of a method, Endocrinology 63 (1958) 349–358.

[26] G.N. Wade, J.M. Gray, Gonadal effects on food intake and adiposity: a metabolic hypothesis, Physiol. Behav. 22 (1979) 583–593, https://doi.org/10.1016/0031-9384(79)90028-3.

[27] F. Picard, Y. Deshaies, J. Lalonde, P. Samson, C. Labrie, A. Bélanger, F. Labrie, D. Richard, Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats, Int. J. Obes. Relat. Metab. Disord. 24 (2000) 830–840.

[28] P. Mauriège, D. Langin, V. Montminy, C. Martel, J.P. Després, A. Bélanger, F. Labrie, Y. Deshaies, Effect of a long-term percutaneous adrenal steroid treatment on rat adipose tissue metabolism, Int. J. Obes. 24 (2000) S148–S150, https://doi.org/10.1038/sj.ijo.0801308.

[29] V. Saengsirisuwan, S. Pongseeda, M. Prasannarong, K. Vichaiwong, C. Toskulkao, Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement, Metabolism 58 (2009) 38–47, https://doi.org/10.1016/j.metabol.2008.08.004.

[30] N.H. Rogers, J.W. Perfield 2nd, K.J. Strissel, M.S. Obin, A.S. Greenberg, Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity, Endocrinology 150 (2009) 2161–2168, https://doi.org/10.1210/en.2008-1405.

[31] U.I. Modder, B.L. Riggs, T.C. Spelsberg, D.G. Fraser, E.J. Atkinson, R. Arnold, S. Khosla, Dose-response of estrogen on bone versus the uterus in ovariectomized mice, Eur. J. Endocrinol. 151 (2004) 503–510, https://doi.org/10.1530/eje.0.1510503.

[32] T.L. MacDonald, K.L. Ritchie, S. Davies, M.J. Hamilton, D.T. Cervone, D.J. Dyck, Exercise training is an effective alternative to estrogen supplementation for improving glucose homeostasis in ovariectomized rats, Phys. Rep. 3 (2015) e12617, , https://doi.org/10.14814/phy2.12617.

[33] M. Nigro, A.T. Santos, C.S. Barthem, R.A.N. Louzada, R.S. Fortunato, L.A. Ketzer, D.P. Carvalho, L. Meis, A change in liver metabolism but not in brown adipose tissue thermogenesis is an early event in ovariectomy-induced obesity in rats, Endocrinology 155 (2014) 2881–2891, https://doi.org/10.1210/en.2013-1385.

[34] F. Pansini, G. Bonaccorsi, F. Genovesi, M.R. Folegatti, B. Bagni, C.M. Bergamini, G. Mollica, Influence of estrogens on serum free fatty acid levels in women, J. Clin. Endocrinol. Metab. 71 (1990) 1387–1389, https://doi.org/10.1210/jcem-71-5-1387.

[35] G. Koricanac, M. Stojiljkovic, S. Radivojsa, Z. Zakula, N. Ribarac-Stepic, E.R. Isenovic, Effects of dexamethasone on insulin receptor in aging, Acta Biol. Hung. 59 (2008) 17–29, https://doi.org/10.1556/ABiol.59.2008.1.2.

[36] T.L. MacDonald, R. MacPherson, L. Castellani, D. Cervone, E. Anderson, D.C. Wright, D.J. Dyck, Estradiol does not directly regulate adipose lipolysis, Adipocytes 6 (2017) 76–86, https://doi.org/10.1080/21623945.2017.1287638.

[37] T. Mandour, A.H. Kissebah, V. Wynn, Mechanism of oestrogen and progesterone effects on lipid and carbohydrate metabolism: alteration in the insulin: glucagon molar ratio and hepatic enzyme activity, Eur. J. Clin. Investig. 7 (1977) 181–187, https://doi.org/10.1111/j.1365-2362.1977.tb01595.x.

[38] I. Pollard, B.M. White, J.R. Bassett, K.D. Cairncross, Plasma glucocorticoid elevation and desynchronization of the estrous cycle following unpredictable stress in the rat, Behav. Biol. 14 (1975) 103–108.

[39] J.I. Kitay, M.D. Coyne, N.H. Swygert, K.E. Gaines, Effects of gonadal hormones and ACTH on the nature and rates of secretion of adrenalcortical steroids by the rat, Endocrinology 89 (1971) 565–570.

[40] M.P. Carey, C.H. Deterd, J. de Koning, F. Helmerhorst, E.R. de Kloet, The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat, J. Endocrinol. 144 (1995) 311–321.

[41] D. Ouerghi, S. Rivest, D. Richard, Adrenalectomy attenuates the effect of chemical castration on energy balance in rats, J. Nutr. 122 (1992) 369–373.

[42] Y. Deshaies, A. Dagnault, J. Lalonde, D. Richard, Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat, Am. J. Phys. 273 (1997) E355–E362.

[43] E.T. Uchôa, L.E.C.M. Silva, M. Castro, J. Antunes-Rodrigues, L.L.K. Elias, Hypothalamic oxytocin neurons modulate hypophagic effect induced by adrenalectomy, Horm. Behav. 56 (2009) 532–538, https://doi.org/10.1016/j.yhbeh.2009.09.007.

[44] E.T. Uchôa, L.E.C.M. Silva, M. Castro, J. Antunes-Rodrigues, L.L.K. Elias, Corticotrophin-releasing factor mediates the hypophagia after adrenalectomy, increasing meal-related satiety responses, Horm. Behav. 58 (2010) 714–719, https://doi.org/10.1016/j.yhbeh.2010.07.003.

[45] E.T. Uchôa, L.E.C.M. Silva, M. Castro, J. Antunes-Rodrigues, L.L.K. Elias, Glucocorticoids are required for meal-induced changes in the expression of hypothalamic neuropeptides, Neuropeptides 46 (2012) 119–124, https://doi.org/10.1016/j.npep.2012.02.002.

[46] I.N. Karatsoreos, S.M. Bhagat, N.P. Bowles, Z.M. Weil, D.W. Pfaff, B.S. McEwen, Endocrine and physiological changes in response to chronic corticosterone: a potential model of the metabolic syndrome in mouse, Endocrinology 151 (2010) 2117–2127, https://doi.org/10.1210/en.2009-1436.

[47] C. Asensio, P. Muzzin, F. Rohner-Jeanrenaud, Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance, Int. J. Obes. 28 (2004) S45–S52, https://doi.org/10.1038/sj.ijo.0802856.

[48] R. Poggioli, C.B. Ueta, R.A. Drigo, M. Castillo, T.L. Fonseca, A.C. Bianco, Dexamethasone reduces energy expenditure and increases susceptibility to diet-induced obesity in mice, Obesity (Silver Spring) 21 (9) (2013) E415–E420, https://doi.org/10.1002/oby.20338.

[49] E.A. Elbassuoni, Gender differences in ghrelin response to chronic immobilization stress in rats: possible role of estrogen, Gen. Physiol. Biophys. 33 (2014) 111–120, https://doi.org/10.4149/gpb_2013061.

[50] J. Hong, R.E. Stubbins, R.R. Smith, A.E. Harvey, N.P. Núñez, Differential susceptibility to obesity between male, female and ovariectomized female mice, Nutr. J. 8 (11) (2009), https://doi.org/10.1186/1475-2891-8-11.

[51] R.E. Stubbins, V.B. Holcomb, J. Hong, N.P. Núñez, Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance, Eur. J. Nutr. 51 (2012) 861–870, https://doi.org/10.1007/s00394-011-0266-4.

[52] 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.

[53] R. Matysková, B. Zelezná, J. Maixnerová, D. Koutová, M. Haluzík, L. Maletínská, Estradiol supplementation helps overcome central leptin resistance of ovariectomized mice on a high fat diet, Horm. Metab. Res. 42 (2010) 182–186, https://doi.org/10.1055/s-0029-1243250.

[54] L. Zhu, W.C. Brown, Q. Cai, A. Krust, P. Chambon, O.P. McGuinness, J.M. Stafford, Estrogen treatment after ovariectomy protects against fatty liver and may improve pathway-selective insulin resistance, Diabetes 62 (2013) 424–434, https://doi.org/10.2337/db11-1718.

[55] L. Zhu, M.N. Martinez, C.H. Emfinger, B.T. Palmisano, J.M. Stafford, Estrogen signaling prevents diet-induced hepatic insulin resistance in male mice with obesity, Am. J. Physiol. Endocrinol. Metab. 306 (2014) E1188–E1197, https://doi.org/10.1152/ajpendo.00579.2013.

[56] S.A. Litwak, J.L. Wilson, W. Chen, C. Garcia-Rudaz, M. Khaksari, M.A. Cowley, P.J. Enriori, Estradiol prevents fat accumulation and overcomes leptin resistance in female high-fat diet mice, Endocrinology 155 (2014) 4447–4460, https://doi.org/10.1210/en.2014-1342.

[57] L.H. Burgess, R.J. Handa, Estrogen-induced alterations in the regulation of mineralocorticoid and glucocorticoid receptor messenger RNA expression in the female rat anterior pituitary gland and brain, Mol. Cell. Neurosci. 4 (1993) 191–198, https://doi.org/10.1006/mcne.1993.1023.

[58] J.V. Seale, S.A. Wood, H.C. Atkinson, M.S. Harbuz, S.L. Lightman, Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats, J. Neuroendocrinol. 16 (2004) 989–998, https://doi.org/10.1111/j.1365-2826.2004.01258.x.

[59] B.R. Walker, R. Andrew, Tissue production of cortisol by 11betahydroxysteroid dehydrogenase type 1 and metabolic disease, Ann. N. Y. Acad. Sci. 1083 (2006) 165–184.

[60] S.K. Paulsen, M.P. Nielsen, B. Richelsen, J.M. Bruun, A. Flyvbjerg, S.B. Pedersen, Upregulation of adipose 11-beta-hydroxysteroid dehydrogenase type 1 expression in ovariectomized rats is due to obesity rather than lack of estrogen, Obesity (Silver Spring) 16 (4) (2008) 731–735, https://doi.org/10.1038/oby.2008.2.

[61] T. Andersson, I. Söderström, K. Simonyté, T. Olsson, Estrogen reduces 11beta-hydroxysteroid dehydrogenase type 1 in liver and visceral, but not subcutaneous, adipose tissue in rats, Obesity (Silver Spring) 18 (3) (2010) 470–475, https://doi.org/10.1038/oby.2009.294.

[62] M. Rebuffé-Scrive, M. Brönnegard, A. Nilsson, J. Eldh, J.A. Gustafsson, P. Björntorp, Steroid hormone receptors in human adipose tissues, J. Clin. Endocrinol. Metab. 71 (1990) 1215–1219, https://doi.org/10.1210/jcem-71-5-1215.

[63] B.L. Wajchenberg, D. Giannella-Neto, M.E.R. Da Silva, R.F. Santos, Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome, Horm. Metab. Res. 34 (2002) 616–621, https://doi.org/10.1055/s-2002-38256.

[64] A.J. Peckett, D.C. Wright, M.C. Riddell, The effects of glucocorticoids on adipose tissue lipid metabolism, Metabolism 60 (2011) 1500–1510, https://doi.org/10.1016/j.metabol.2011.06.012.

[65] S.J. Spencer, A. Tilbrook, The glucocorticoid contribution to obesity, Stress 14 (2011) 233–246, https://doi.org/10.3109/10253890.2010.534831.

[66] M.J. Lee, P. Pramyothin, K. Karastergiou, S.K. Fried, Deconstructing the roles of glucocorticoids in adipose tissue biology and the development of central obesity, Biochim. Biophys. Acta 1842 (2014) 473–481, https://doi.org/10.1016/j.bbadis.2013.05.029.

[67] T. Wu, L. Yang, J. Jiang, Y. Ni, J. Zhu, X. Zheng, Q. Wang, X. Lu, Z. Fu, Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats, Life Sci. 192 (2018) 173–182, https://doi.org/10.1016/j.lfs.2017.11.049.

[68] N.E. Block, M.G. Buse, Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo, Am. J. Phys. 256 (1989) E39–E48, https://doi.org/10.1152/ajpendo.1989.256.1.E39.

[69] G. Dimitriadis, B. Leighton, M. Parry-Billings, S. Sasson, M. Young, U. Krause, S. Bevan, T. Piva, G. Wegener, E.A. Newsholme, Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle, Biochem. J. 321 (1997) 707–712, https://doi.org/10.1042/bj3210707.

[70] H.C. Horner, A. Munck, G.E. Lienhard, Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts, J. Biol. Chem. 262 (1987) 17696–17702.

[71] R. Nosadini, S. Del Prato, A. Tiengo, A. Valerio, M. Muggeo, G. Opocher, F. Mantero, E. Duner, C. Marescotti, F. Mollo, F. Belloni, Insulin resistance in Cushing's syndrome, J. Clin. Endocrinol. Metab. 57 (1983) 529–536, https://doi.org/10.1210/jcem-57-3-529.

[72] R.A. Rizza, L.J. Mandarino, J.E. Gerich, Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action, J. Clin. Endocrinol. Metab. 54 (1982) 131–138, https://doi.org/10.1210/jcem-54-1-131.

[73] J.D. Bagdade, E.L. Bierman, D. Porte Jr., The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects, J. Clin. Invest. 46 (1967) 1549–1557, https://doi.org/10.1172/JCI105646.

[74] J.L. Arriza, R.B. Simerly, L.W. Swanson, R.M. Evans, The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response, Neuron 1 (1988) 887–900, https://doi.org/10.1016/0896-6273(88)90136-5.