Steroids 188 (2022) 109132

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Effect of chronic administration of 17β-estradiol on the vasopressor responses induced by the sympathetic nervous system in insulin resistance rats

Erika J. Gutiérrez-Lara [a], Araceli Sánchez-López [a], Janet Murbartián [a], Selene J. Acosta-Cota [b], David Centurión [a,*]

[a] Departamento de Farmacobiología, Cinvestav Unidad Coapa, Czda. de los Tenorios 235, Col. Granjas-Coapa, Deleg. Tlalpan, C.P. 14330 México City, México
[b] Departamento de Ciencias de la Salud, Universidad Autónoma de Occidente, Blv. Lola Beltrán y Blv. Rolando Arjona. S/N, Col. 4 de marzo, C.P. 80020 Culiacán, Sinaloa, México

ARTICLE INFO

Keywords:
17β-estradiol
Insulin resistance
Hyperinsulinemia
Hypertension
Vasopressor response

ABSTRACT

Several studies have demonstrated that the underlying mechanism of insulin resistance (IR) is linked with developing diseases like diabetes mellitus, hypertension, metabolic syndrome, and polycystic ovary syndrome. In turn, the dysfunction of female gonadal hormones (especially 17β-estradiol) may be related to the development of IR complications since different studies have shown that 17β-estradiol has a cardioprotector and vasorelaxant effect. This study aimed was to determine the effect of the 17β-estradiol administration in insulin-resistant rats and its effects on cardiovascular responses in pithed rats. Thus, the vasopressor responses are induced by sympathetic stimulation or i.v. bolus injections of noradrenaline ($\alpha_{1/2}$), methoxamine (α_1), and UK 14,304 (α_2) adrenergic agonist were determined in female pithed rats with fructose-induced insulin resistance or control rats treated with: 1) 17β-estradiol or 2) its vehicle (oil) for 5 weeks. Thus, 17β-estradiol decreased heart rate, prevented the increase of blood pressure induced by ovariectomy, but with the opposite effect on sham-operated rats; and decreased vasopressor responses induced by i.v. bolus injections of noradrenaline on sham-operated (control and fructose group) and ovariectomized (control) rats, and those induced by i.v. bolus injections of methoxamine (α_1 adrenergic agonist). Overall, these results suggest 17β-estradiol has a cardioprotective effect, and its effect on vasopressor responses could be mediated mainly by the α1 adrenergic receptor. In contrast, IR with ovariectomy 17β-estradiol decreases or loses its cardioprotector effect, this could suggest a possible link between the adrenergic receptors and the insulin pathway.

1. Introduction

Insulin resistance (IR) is defined as the decrease in insulin action at the cellular level, characterized by the inability of insulin to increase glucose uptake and its utilization, leading to compensatory hyperinsulinemia [1]. In addition, several studies have shown that the underlying mechanism of IR provides the basis for the subsequent development of many metabolic and cardiovascular diseases, such as diabetes mellitus (DM) and hypertension [1–4]; and it is also linked with polycystic ovary syndrome (PCOS) [5,6]. On the other hand, IR has a progressive increase after menopause, which is thought to be due to an increase in central adipose tissue and the effects of hypoestrogenism [7,8]. The relationship between IR and PCOS, as well as with menopause, suggests that gonadal hormones may be involved in the development of IR, or well a disfunction of female gonadal hormones (mainly 17β-estradiol) could be an active driver of the development of insulin resistance and its complications, as those described above.

Several studies have demonstrated that 17β-estradiol not only improves insulin sensitivity [7,9–11] but also has a cardioprotector [12–14] and vasorelaxant [15–18] effect. Some mechanisms have been proposed to explain the action of 17β-estradiol in decreasing IR. 17β-Estradiol has been shown to decrease gluconeogenesis by the hepatic Foxo1 and phosphatidylinositol-3 kinase (PI3K)-Akt pathway in male mice [9]; also, it improves insulin signaling in the heart increasing the

* Corresponding author.
E-mail address: dcenturi@cinvestav.mx (D. Centurión).
URL: http://farmacobiologia.cinvestav.mx/PersonalAcad%C3%A9mico/DrDavidCenturi%C3%B3nPacheco (D. Centurión).

https://doi.org/10.1016/j.steroids.2022.109132
Received 23 July 2022; Received in revised form 14 September 2022; Accepted 14 October 2022
Available online 20 October 2022

activity of the insulin receptor substrate 1 (IRS1) and p-Akt [11] in rats. While concerning the vasorelaxant effects, several mechanisms have been proposed by which 17β-estradiol can generate its action; for example, by inhibition of calcium channels type L [16] or by an increase of nitric oxide as a consequence of increasing the expression of endothelial nitric oxide synthase (eNOS) and activating the PI3K/Akt pathway [17,18]. In addition, in rats with post-menopausal metabolic syndrome induced by high-fat diet and ovariectomy, 17β-estradiol decreased contractile responses induced by angiotensin II and noradrenaline, increased vasorelaxation responses, and improved endothelial dysfunction by the activation of eNOS, silent information regulation 2 homologue (SIRT1) and AMP-activated PK (AMPK) [19]. The vasorelaxant effects of 17β-estradiol have been observed in isolated rat aortas [19–21], while 17β-estradiol reduces the vasoconstrictor responses in isolated rat mesenteric arteries [22,23]. This effect has also been observed in a pithed rat model, where it was observed a decrease in vasopressor responses in ovariectomized diabetic rats [15]. Due to the relevance of these pathologies, their relationship with 17β-estradiol, and the fact that no studies demonstrate its effect on vasopressor responses in insulin-resistant rats, this study aimed to determine the effect of the 17β-estradiol administration in insulin-resistant rats and its effects on cardiovascular responses in pithed rats.

2. Experimental procedures

2.1. Animals

Female Wistar rats weighing 200–220 g (n = 48) were used. The animals were housed in plastic cages and maintained under standardized conditions (22 ± 2 °C, 50 % humidity and 12/12-h light–dark cycle), with food and water freely available. All animal procedures and protocols of the present study were approved by our Institutional Ethics Committee (Cicual-Cinvestav) following the regulations established by the Mexican Official Norm for the Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999), following the Guide for the Care and Use of Laboratory Animals in U.S.A.

2.2. Induction of insulin resistance

At the beginning of the study, animals were divided into two groups (control group and fructose group). The control group (n = 24) was fed with a regular diet and tap water freely available for 23 weeks, while the fructose group (n = 24) was fed with a regular diet and fructose solution (15 % wt./vol.) freely available for 23 weeks to induce IR.

2.3. Bilateral ovariectomy

Sixteen weeks after animals started with IR induction, they were ovariectomized or sham-operated. The animals were anaesthetized with ketamine (70 mg/kg, i.m.) and xylazine (5 mg/kg, i.m.). After anaesthesia, a small incision (1 cm) in the lower abdomen was performed, and both ovaries were removed (Ovx; n = 24). While sham-operated animals (n = 24) only make a small incision without remotion of ovaries. Both subgroups received postoperative treatment with antibiotics (penicillin G procaine: 80,000 IU/kg and dihydrostreptomycin: 2.5 mg/kg) and anti-inflammatory drugs (flumethasone; 12.5 μg/kg) (Fluvicina®, Pfizer of Mexico). Then, the animals were allowed to recover for two weeks.

2.4. Chronic administration of 17β-estradiol

Two weeks after the ovariectomy or sham operation, the animals received daily s.c. injections of 17β-estradiol (10 μg/kg; n = 24) or its vehicle (corn oil; 0.5 ml/kg; n = 24) for 35 days, respectively [15,24].

2.5. Biochemical measurements

Fasting blood glucose and triglyceride (TG) levels were determined before and after treatment with 17β-estradiol or its vehicle (Fig. 1A) using a glucometer (AccuCheck® and Accutrend® Plus; Roche of Mexico). Plasma insulin levels were measured during the oral glucose tolerance test; basal levels were quantified at time cero (before administration of glucose (1 g/kg, p.o.)) with a rat insulin ELISA (Enzyme-linked immunosorbent Assay) kit from ALPCO. Absorbance of enzymatic reaction was quantified at 450 nm. All determinations were performed by duplicate and a standard curve was included in each experiment as suggested by the provider.

The insulin resistance was determined according to the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) using the following formula: HOMA-IR = [(glucose (mmol/L) × insulin (μIU/mL))/ 22.5] [25].

2.6. Oral glucose tolerance tests (OGTT)

Before and after treatment with 17β-estradiol or its vehicle, rats fasted for 12 h. Subsequently, for insulin plasma levels, tail blood samples were collected at 0 min (before) and 5, 10, 15, 30, 60 min after oral administration of glucose (1 g/kg, p.o.). For glucose levels, samples were collected at the same time as those for insulin levels, including 90 and 120 min (see Fig. 1A).

2.7. Measurement of arterial blood pressure and heart rate in conscious animals

Heart rate (HR), systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure were measured by a tail-cuff method using a LE 5001 automatic blood pressure recorder (Letica1, PanLab, Barcelona, Spain). Determinations were obtained after treatment with 17β-estradiol or its vehicle (Fig. 1A).

2.8. Evaluation of the vasopressor responses in pithed rats

Here, we used a pithed rat model because this model allows *in vivo* observation of cardiovascular reactions induced by several vasoactive substances without the participation of circulatory reflexes; it also allows to induce cardiovascular responses through electrical stimuli by an electrode through the spinal nerve roots [26].

Therefore, at the end of the treatment period with 17β-estradiol or its vehicle, the animals were anesthetized with isoflurane (3 %). Next, the trachea was cannulated and the central nervous system was destroyed by inserting a stainless-stell rod through the orbit and *foramen magnum* into the *vertebral foramen* (pithed) [27]. Then, the animals were artificially ventilated with a positive pressure pump (7025 rodent ventilator, Ugo Basile, Comerio, VA, Italy) at 56 S/min and a stroke volume of 20 ml/kg [28]. After that, a bilateral cervical vagosympathectomy was made, and catheters were placed in: 1) the left and right femoral veins for drug administration and 2) the left carotid artery to measure blood pressure and heart rate. This catheter was connected to a pressure transducer (*P*23 XL, Grass Technologies, Warwick, RI, U.S.A.). Blood pressure and heart rate were recorded simultaneously using a data acquisition unit (MP150A- CE, Biopac Systems Inc., Goleta, CA) and Acqknowledge software v4.2 (Biopac Systems Inc., Goleta, CA). Diastolic blood pressure was determined, as this is the blood pressure when the left ventricle is relaxed and thus could indirectly represent the systemic vascular resistance that regulates arterial blood pressure and blood flow within organs [29].

2.8.1. Stimulation of the vasopressor sympathetic outflow

After surgical intervention, the stainless-steel rod was replaced by an enameled electrode except for 1 cm long section 9 cm from the tip. The uncovered segment was situated at the T_7-T_9 region of the spinal cord to

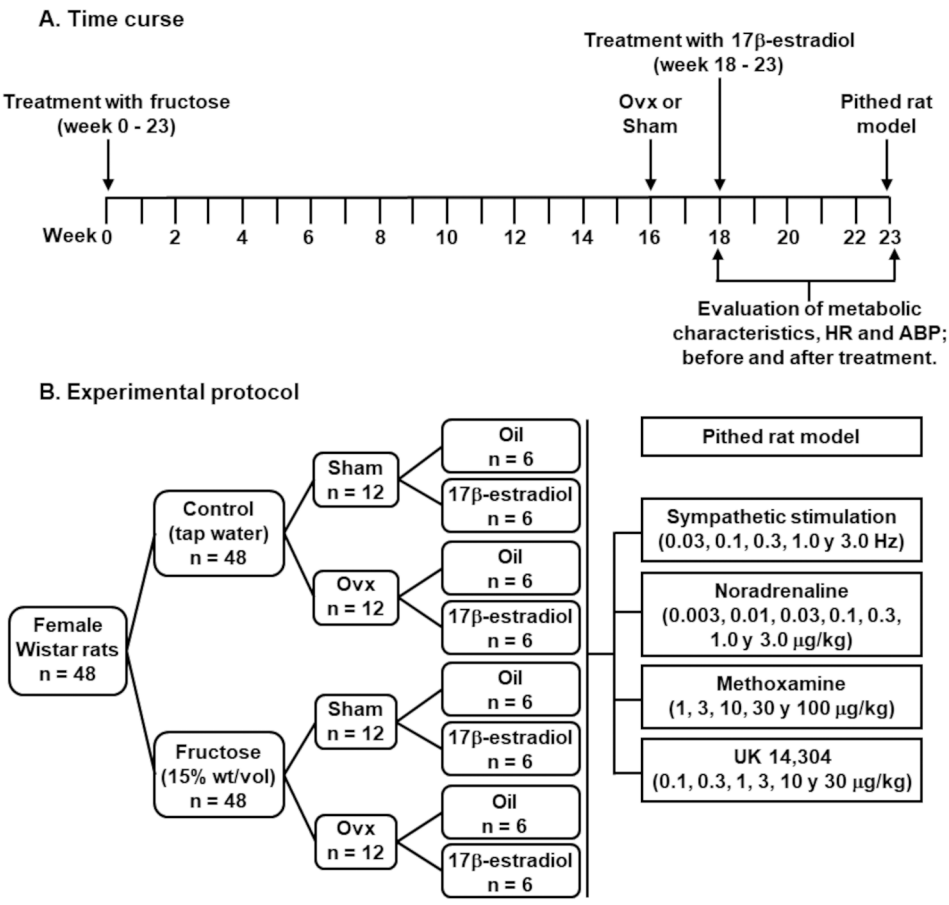

Fig. 1. Experimental design. (A) Time curse. (B) Experimental protocol in pithed rats. All animals received a regular diet freely available plus tap water (control) or fructose 15 % wt./vol., respectively, for 23 weeks. On week 16, animals underwent sham surgery (Sham) or ovariectomy (Ovx); then, after two weeks of recovery, the animals received treatment with 17β-estradiol (10 μg/kg, s.c.) or its vehicle (corn oil; 0.5 ml/kg) for 5 weeks. Before and after 17β-estradiol treatment, metabolic characteristics (oral glucose tolerance test, plasma insulin levels, HOMA-index, glucose and triglyceride blood vessels), heart rate (HR) and arterial blood pressure (ABP) were evaluated. Finally, at week 23, the pithed rat model was developed to evaluate the vasopressor responses.

allow for selective stimulation of the sympathetic vasopressor outflow [26]. Before electrical stimulation, the animals received gallamine (25 mg/Kg, i.v.) to avoid electrically induced muscular twitching. 30 min after surgery the hemodynamic conditions stabilized and baseline values of diastolic pressure and heart rate were determined. Next, the preganglionic vasopressor sympathetic outflow was stimulated with an S88X square pulse stimulator (Grass Technologies, Warwick, RI, U.S.A.) by applying 10 s trains of monophasic, rectangular pulses (2 ms, 60 V) at increasing frequencies of stimulation frequencies (0.03, 0.1, 0.3, 1 and 3 Hz). An SIU-V isolation unit (Grass Technologies, Warwick, RI, U.S.A.) was used to minimize artefacts resulting from the stimuli. When diastolic blood pressure returned to baseline levels, the subsequent frequency was applied; this procedure was performed systematically until the stimulus–response curve was completed (approximately 30 min).

2.8.2. Vasopressor responses

The vasopressor responses induced by sympathetic stimulation (as described above) or i.v. bolus injections of the adrenoceptor agonists: (1) exogenous noradrenaline (endogenous ligand; 0.003, 0.01, 0.03, 0.1, 0.3 1 and 3 μg/kg), (2) methoxamine (α_1 adrenoceptor; 1, 3, 10, 30 and 100 μg/kg) and (3) UK 14,304 (α_2 adrenoceptor; 0.03, 0.1, 0.3, 1, 3, 10 and 30 μg/kg) were determined in all groups (Fig. 1B).

2.9. Experimental design

Forty-eight animals were divided into two groups. The first group (n = 24) received tap water (control group), and the second group (n = 24) that developed IR received fructose solution (15 % wt/vol) for 23 weeks. At week sixteen, each group were divided into two groups that underwent sham surgery (Sham; n = 12 each) or ovariectomy (ovx; n = 12 each). Two weeks later, each subgroup was divided accord of treatment with 17β-estradiol (10 μg/kg, s.c.; n = 6 each) or its vehicle (corn oil; 0.5 ml/kg, s.c.; n = 6 each) for 35 days (5 weeks). It is important to mention that before and after the treatment with 17β-estradiol or its vehicle biochemical parameters (glucose and TG levels, plasma insulin levels, HOMA-IR), OGTT, as well as heart rate and arterial blood pressure (SBP and DBP) were evaluated after 12 h of fasting. Finally, after 17β-estradiol treatment, the pithed rat model was developed to evaluate the vasopressor responses (Fig. 1).

2.10. Drugs

In addition to the anaesthetic isoflurane (Fluriso ™, Vet One®, Boise, ID, U.S.A.), the following compounds were used in this study: gallamine triethiodide (PubChem CID: 6172), (±)-noradrenaline bitartrate (PubChem CID: 168929), methoxamine hydrochloride (PubChem CID: 6081), 5-Bromo-*N*-(2-imidazolin-2-yl)-6-quinoxalinamine (UK 14,304) (PubChem CID: 2435), 1,1 Dimethylbiguanide hydrochloride (metformin) (PubChem CID: 14219), 17β-estradiol (PubChem CID: 5757) (Sigma Chemical Co., St. Louis, MO, U.S.A.) and Crystalline fructose (KRYSTAR®, Tate and Lyle). All the compounds were dissolved in saline solution except for UK 14,304, which was dissolved in dimethyl sulfoxide (DMSO) (PubChem CID: 679) 10 % and 17β-estradiol (dissolved in corn oil).

2.11. Statistical analysis

All results are presented as mean ± standard error of the mean (s.e.m.), and the number of animals is represented by n. Under the normality of the data, an analysis of variance (ANOVA) was performed, which was one-way or two-way repeated measures depending on the case. Then the differences among the changes in all the analyzed parameters were

evaluated using the Tukey post hoc test. Moreover, the area under the curve (AUC) was calculated using the trapezoid rule, and the comparison was evaluated with a student's *t*-test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Metabolic and hemodynamic parameters

As shown in Table 1, fructose consumption did not cause changes in glucose blood levels or body weight in any of the groups. In the sham group, fructose consumption increased insulin levels, HOMA-IR index, and TG compared with the sham + control group. In contrast, fructose consumption did not change these parameters in the ovariectomy group compared with its respective control group; however, these changes in the ovariectomy + fructose group were statistically significant only compared with the sham + control group. Interestingly, insulin levels were increased after ovariectomy and were significantly higher than those shown in the sham + control group.

Table 2 shows that in the control group, 17β-estradiol significantly increased fasting insulin levels and values of HOMA-IR; in contrast, this treatment diminished body weight in control animals with sham surgery (sham) when compared with vehicle (oil). Further, in animals with fructose consumption and sham surgery, the treatment with 17β-estradiol increased the triglycerides blood levels compared with sham-operated and control animals treated with its vehicle (oil). On the other hand, in both control and IR animals with ovariectomy, the treatment with 17β-estradiol did not significantly modify the determined parameters (glucose, TG, insulin levels, HOMA-IR index, and body weight).

Moreover, Table 3 shows the effect of fructose (IR animals) on hemodynamic variables (heart rate, systolic, diastolic, and mean blood pressure). After 23 weeks of fructose consumption, the values of heart rate, systolic, diastolic, and mean blood pressure were increased in both groups of sham-operated and ovariectomized rats compared with their respective control groups.

The effect of treatment with 17β-estradiol on hemodynamic variables is shown in Table 4. In sham-operated rats, both in control and IR (fructose consumption) groups, 17β-estradiol significantly increased systolic, diastolic, and mean blood pressure, and reduce heart rate but with no significant difference compared with their respective vehicle (oil) groups. In contrast, in control-ovariectomized rats, the treatment with 17β-estradiol reduced all the hemodynamic variables analyzed compared with the vehicle (oil) group. However, these changes were not statistically significant. Interestingly, in ovariectomized rats with IR, the treatment with 17β-estradiol significantly reduced systolic, diastolic, and mean blood pressure; it also diminished the heart rate but with no significant difference when compared with its vehicle group.

3.2. Blood glucose and plasma insulin during oral glucose tolerance test

To know the handling of glucose in animals, we performed an OGTT after 23 weeks of fructose consumption or its vehicle (tap water). Both blood glucose and plasma insulin levels were determined during the test. The results are shown in Fig. 2 as the area under the curve of the time course. In this respect, blood glucose values were similar in all groups (Fig. 2A). Nevertheless, plasma insulin levels (Fig. 2B) were significantly increased in animals treated with fructose in ovariectomized or sham-operated rats.

Next, Fig. 3A shows the effect of 17β-estradiol on glucose levels sham operated or ovariectomized rats. In control animals, these values did not significantly change. In contrast, 17β-estradiol significantly increased insulin plasma levels (Fig. 3B) during OGTT in both sham-operated and ovariectomized rats. On the other hand, in animals with IR (fructose group), the treatment with 17β-estradiol significantly diminished blood glucose values in sham-operated rats; the treatment with 17β-estradiol also diminished glucose values in ovariectomized rats but without statistically significant differences (Fig. 3C). Conversely, insulin levels were not modified in any of the groups with fructose consumption after treatment with 17β-estradiol (Fig. 3D).

3.3. Effect of insulin resistance induced by fructose consumption on the vasopressor responses induced by sympathetic stimulation or i.v. injections for several α-adrenoceptor agonists

As shown in Fig. 4, electrical stimulation of the vasopressor sympathetic outflow or i.v. bolus injection of noradrenaline, methoxamine and UK 14,304 produced frequency or dose-dependent increases in diastolic blood pressure both in the control and fructose group. Interestingly, in animals with sham surgery, the vasopressor responses to sympathetic stimulation were significantly smaller in the group treated with fructose than in the control group, specifically at the frequencies of 1 and 3 Hz (Fig. 4A; $F_{(1,4)} = 5.60$, $P < 0.05$). This decrease was also observed in the responses generated by noradrenaline at the doses of 0.1–3 μg/kg (Fig. 4B; $F_{(1,4)} = 6.00$, $P < 0.05$) and UK 14,304 at the doses of 0.3–30 μg/kg (Fig. 4D; $F_{(1,4)} = 29.264$, $P = 0.03$). While the fructose consumption did not modify methoxamine responses (Fig. 4C; $F_{(1,4)} = 1.66$, $P = 0.26$). Likewise, in ovariectomized rats, fructose consumption did not modify the vasopressor responses generated by sympathetic stimulation or the different adrenergic agonists used (Fig. 4E-H).

3.4. Effect of ovariectomy on the vasopressor responses induced by sympathetic stimulation or i.v. injections for several α-adrenoceptor agonists

As show in Fig. 5A and 5B the vasopressor responses generated by sympathetic stimulation (at 1 and 3 Hz; $F_{(1,4)} = 7.55$, $P = 0.040$) and noradrenaline (at the doses of 0.1 to 3 μg/kg; $F_{(1,4)} = 6.66$, $P = 0.05$) were decreased after ovariectomy. However, this surgery did not significantly affect the responses produced by α_1 (methoxamine) and α_2 (UK 14,304) adrenergic agonists. Meanwhile, in animals with IR, the surgery failed to modify the vasopressor responses, when compared with sham-operated rats.

3.5. Effect of the 17β-estradiol on the vasopressor responses induced by sympathetic stimulation or i.v. injections for several α-adrenoceptor agonists

As observed in Fig. 6, in sham-operated rats, the treatment with 17β-estradiol significantly decreased vasopressor responses induced by intravenous bolus injection of noradrenaline both in control ($F_{(1,4)} = 12.36$, $P = 0.02$; Fig. 6B) and in IR (with fructose consumption) ($F_{(1,4)} =$

Table 1
Effect of fructose 15 % consumption on biochemical parameters and body weight in sham-operated (sham) and ovariectomized (Ovx) rats.

Group		Glucose (mmol/l)	Insulin (μIU/ml)	HOMA-IR	Triglyceride (mg/dl)	Body weight (kg)
Sham	**Control**	5.65 ± 0.15	3.68 ± 0.46	0.95 ± 0.15	120 ± 21	0.38 ± 0.01
	Fructose	5.68 ± 0.25	11.06 ± 2.31*	2.81 ± 0.72*	212 ± 27*	0.43 ± 0.03
Ovx	**Control**	6.16 ± 0.25	8.26 ± 1.54*	2.26 ± 0.37*	138 ± 26	0.41 ± 0.03
	Fructose	5.29 ± 0.24	12.68 ± 3.54*	2.91 ± 0.82*	214 ± 24*	0.47 ± 0.03

*, $P < 0.05$ vs sham + control.

Table 2
Effect of 17β-estradiol on biochemical parameters and body weight in animals with water or fructose 15 % consumption.

Group		Glucose (mmol/l)	Insulin (μIU/ml)	HOMA-IR	Triglyceride (mg/dl)	Body weight (kg)
Control						
Sham	**Oil**	5.65 ± 0.15	3.68 ± 0.46	0.95 ± 0.15	120 ± 21	0.38 ± 0.01
———	**17β-Estradiol**	5.51 ± 0.22	15.27 ± 0.14*	3.50 ± 0.62*	160 ± 17	0.26 ± 0.02*
Ovx	**Oil**	6.16 ± 0.25	8.26 ± 1.54	2.26 ± 0.37	138 ± 26	0.41 ± 0.03
	17β-Estradiol	5.63 ± 0.10	13.01 ± 0.80	3.32 ± 0.23	179 ± 16	0.35 ± 0.01
Fructose						
Sham	**Oil**	5.68 ± 0.25	11.06 ± 2.31	2.81 ± 0.72	212 ± 27	0.43 ± 0.03
	17β-Estradiol	5.29 ± 0.14	12.62 ± 2.57	3.02 ± 0.56	339 ± 21*	0.43 ± 0.02
	Oil	5.29 ± 0.24	12.68 ± 3.54	2.91 ± 0.82	214 ± 24	0.47 ± 0.03
Ovx	**17β-Estradiol**	5.66 ± 0.23	14.70 ± 2.38	3.57 ± 0.56	287 ± 38	0.40 ± 0.01

Administration of 17β-estradiol (10 μg/kg·day, s.c.); or its vehicle (oil, 0.5 ml/kg·day; s.c.) for 5 weeks, in animals with water (control) or fructose 15 % consumption. *, $P < 0.05$ vs control + sham + oil.

Table 3
Effect of fructose on hemodynamic variables in sham or ovx rats.

Group		HR (BPM)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)
Sham	**Control**	368 ± 17	105 ± 4	84 ± 5	91 ± 5
———	**Fructose**	443 ± 6*	137 ± 4*	109 ± 4*	118 ± 4*
Ovx	**Control**	401 ± 22	131 ± 3*	101 ± 5*	110 ± 4*
	Fructose	439 ± 7*	153 ± 4*,[a]	130 ± 4*	138 ± 4*

Water consumption (control); heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP), and mean blood pressure (MBP). *, $P < 0.05$ vs Sham + control; [a], $P < 0,05$ vs Ovx + control.

Table 4
Effect of 17β-estradiol on hemodynamic variables, in animals with water or fructose 15 % consumption.

Group		HR (BPM)	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)
Control					
Sham	**Oil**	368 ± 17	105 ± 4	84 ± 5	91 ± 5
———	**17β-Estradiol**	356 ± 12	131 ± 3[a]	112 ± 3[a]	115 ± 5[a]
Ovx	**Oil**	401 ± 22	131 ± 3	101 ± 5	110 ± 4
	17β-Estradiol	366 ± 23	118 ± 2	91 ± 3	100 ± 2
Fructose					
Sham	**Oil**	443 ± 6	137 ± 4[a]	109 ± 4[a]	118 ± 4
	17β-Estradiol	415 ± 12	166 ± 6[b]	146 ± 6[b]	152 ± 6[b]
Ovx	**Oil**	439 ± 7	153 ± 4	130 ± 4	138 ± 4
	17β-Estradiol	431 ± 5	123 ± 4[c]	97 ± 5[c]	105 ± 4[c]

17β-Estradiol (10 μg/kg·day; s.c.; for 5 weeks), oil (0.5 ml/kg·day; s.c.; for 5 weeks); control (water consumption); heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP). [a], $P < 0.05$ vs control + sham + oil; [b], $P < 0.05$ vs fructose + sham + oil; [c], $P < 0.05$ vs fructose + ovx + oil.

3.70, $P < 0.05$; Fig. 6F) group compared with the corresponding group treated with oil. Also, 17β-estradiol: (1) decreased vasopressor responses at the highest dose of methoxamine (100 μg/kg, Fig. 6G) in the group with fructose consumption, and (2) did not change the vasopressor responses in the control group (Fig. 6C). However, the treatment with 17β-estradiol did not modify vasopressor responses induced by sympathetic stimulation (Fig. 6A and 6E), or UK 14,304 (Fig. 6D and 6H), in the control groups and in those that had fructose consumption.

On the other hand, in ovariectomized and control rats (Fig. 7A-D), the treatment with 17β-estradiol decreased vasopressor responses induced by noradrenaline (at 3 μg/kg; Fig. 7B) and methoxamine (at 100 μg/kg; Fig. 7C), without changes in vasopressor responses induced by sympathetic stimulation (Fig. 7A) and UK 14,304 (Fig. 7D), when compared with oil. Meanwhile, in animals with fructose consumption and ovariectomy, the treatment with 17β-estradiol increased vasopressor responses induced by sympathetic stimulation, specifically at the frequencies 1 and 3 Hz ($F_{(1,4)} = 3.42$, $P < 0.05$; Fig. 7E). Further, this treatment did not modify the vasopressor responses induced by intravenous bolus injection of noradrenaline (Fig. 7F), methoxamine (Fig. 7G) or UK 14,304 (Fig. 7H).

4. Discussion

In this study, we analyzed the effect of ovariectomy which leads to the loss of sex hormones, and insulin resistance, as well as the effect of chronic administration of 17β-estradiol on several cardiovascular responses.

4.1. Effect of fructose consumption on metabolic variables

In our model, we observed that chronic fructose (15 % wt./vol.; 23 weeks) consumption in female rats did not change body weight compared with control groups, both in sham and ovariectomized rats (Table 1). These results were similar to previous reports [30,31]; animals with fructose consumption had lower food and liquid intake than the control groups.

On the other hand, chronic fructose (15 % wt./vol.; 23 weeks) consumption significant increased: i) fasting insulin levels (Table 1), ii) levels of insulin during OGTT (Fig. 2B), iii) HOMA-IR index (Table 1), and iv) fasting triglycerides levels (Table 1) in the sham group, but in ovariectomy group, these changes were not statistically significant when compared with its control group. In contrast, fructose consumption did not modify fasting glucose (Table 1), glucose values of OGTT (Fig. 2A), or body weight (Table 1) both in sham and ovariectomy groups. These results are similar to previous reports [32–34]. Since hyperinsulinemia is a compensatory mechanism to maintain normoglycemia [35,36], and IR may reduce VLDL (very low-density lipid) breakdown and, as a consequence, increase hypertriglyceridemia [37], our data suggest that fructose consumption (15 %) during 23 weeks induces insulin resistance, hypertriglyceridemia without dysregulation of glucose metabolism. Additionally, according to the results in ovariectomized rats, it is suggested that ovariectomy and its consequent loss of female sex hormones could contribute to the development of IR leading to a less difference between the control and fructose groups than that observed in these groups in sham-operated.

4.2. Evidence that ovariectomy contributes to the development of insulin resistance

Our results show that in the control group, ovariectomy significantly increased: i) fasting insulin levels (Table 1), ii) HOMA-IR index (Table 1), and iii) fasting triglycerides levels (without statistical significance, Table 1) compared with the sham-operated group. In contrast, ovariectomy did not modify fasting glucose (Table 1), glucose or insulin values of OGTT (Fig. 2A, 2B), or body weight (Table 1) compared with the sham-operated group. While in groups with fructose consumption, ovariectomy showed the same tendency but without becoming a

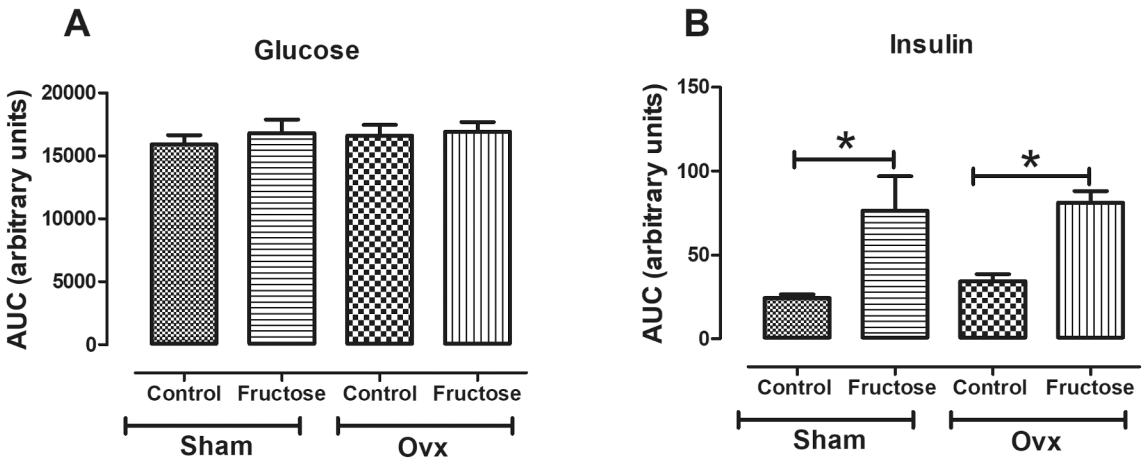

Fig. 2. Effect of fructose consumption on glucose and insulin levels in sham or ovariectomized rats. The area under the curve (AUC) of blood glucose (A) and plasma insulin values (B) obtained during oral glucose tolerance test after 23 weeks of consumption of fructose 15 % or water (control) in both groups sham surgery (sham) and ovariectomized (ovx) animals. Each column represents the mean ± s.e.m. *, $P < 0.05$ vs control.

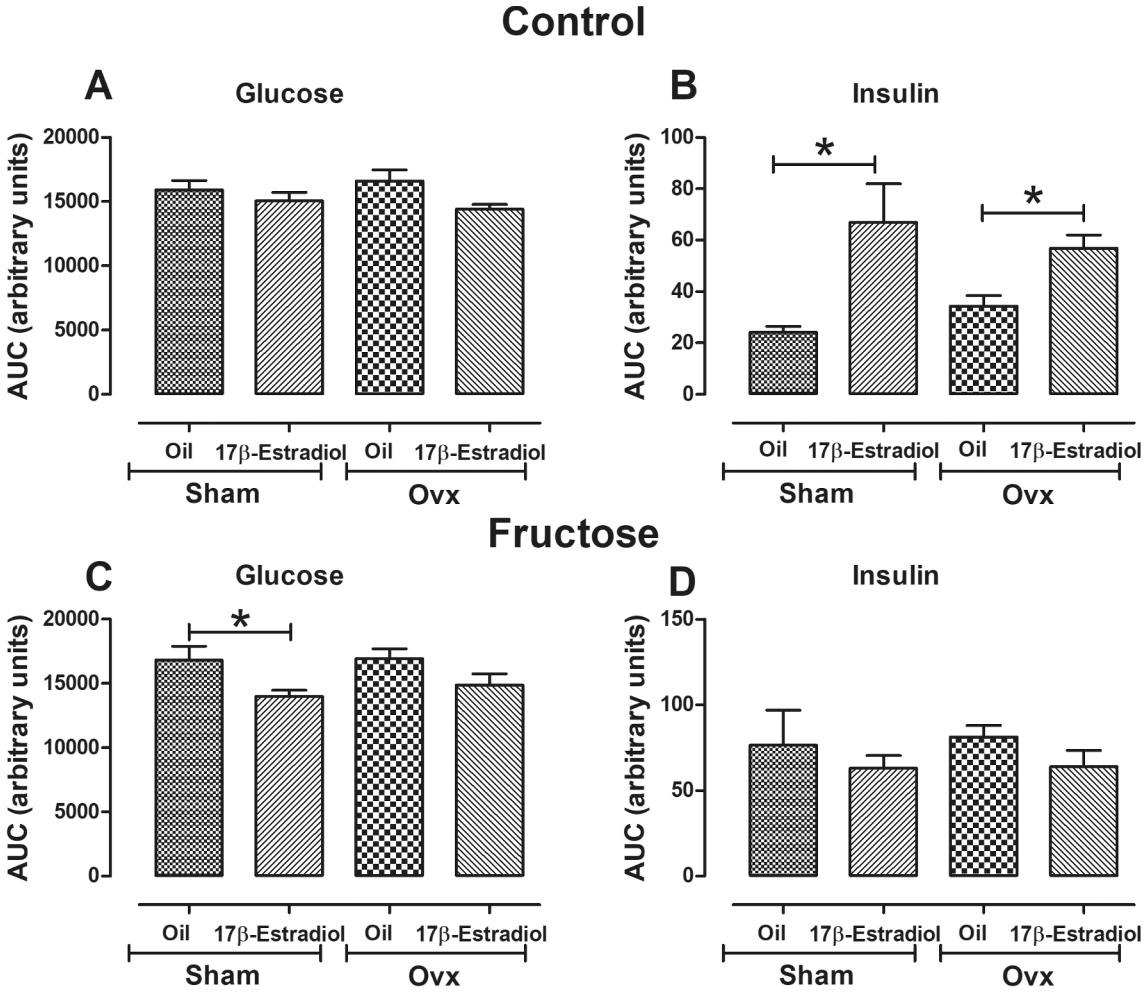

Fig. 3. Effect of 17β-estradiol administration on glucose and insulin levels in animals with fructose or water consumption with sham surgery or ovariectomized. The area under the curve (AUC) of blood glucose (A, C) and plasma insulin values (B, D) obtained during oral glucose tolerance test after (week 23) of s.c. administration of vehicle (oil, 0.5 ml/kg day) or 17β-estradiol (10 μg/kg day) in both groups sham (sham surgery) and ovx (ovariectomized) animals. The upper panel shows the differences in control groups (tap water consumption; A, B) and the bottom panel shows the differences in groups with fructose 15 % consumption (fructose; C, D). Each column represents the mean ± s.e.m. *, $P < 0.05$ vs control.

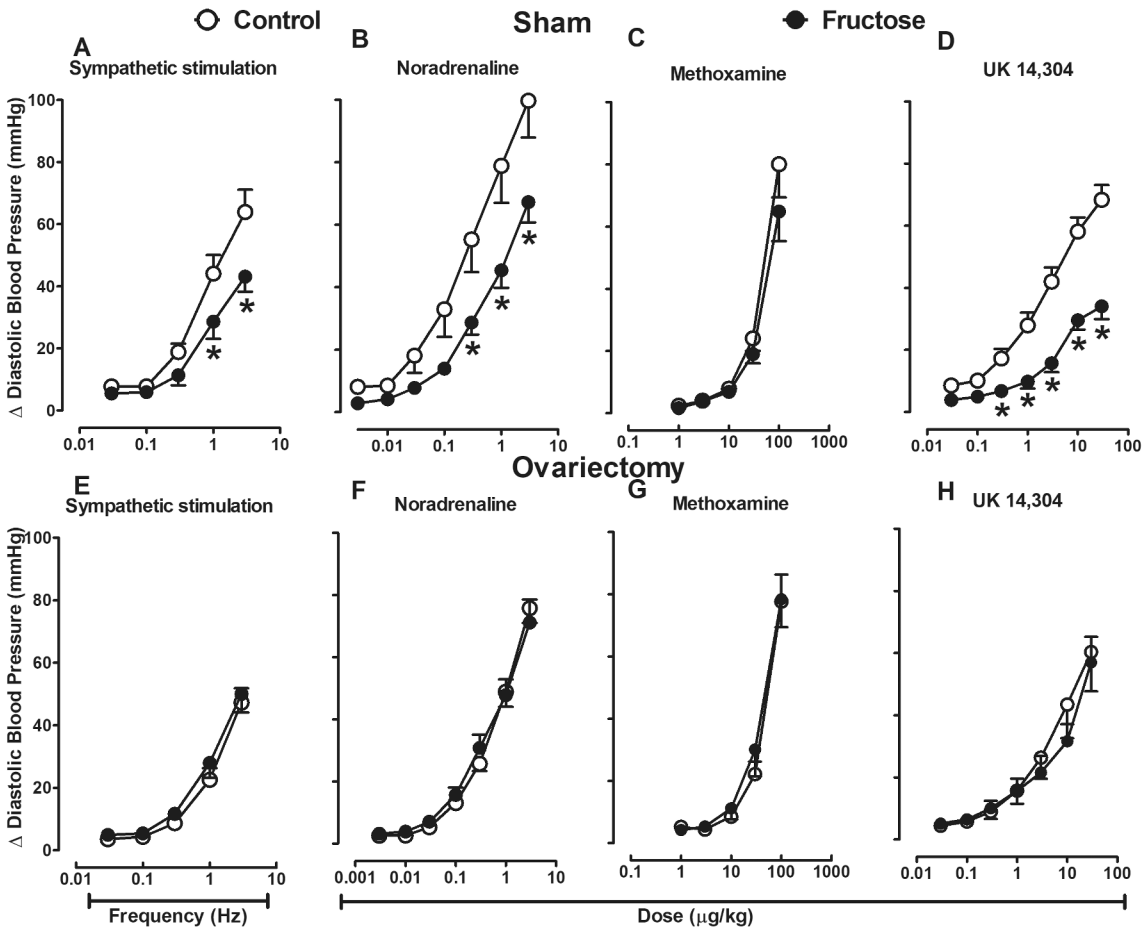

Fig. 4. Effect of fructose consumption on vasopressor responses. Effect of fructose 15 % (23 weeks) on vasopressor responses induced by sympathetic stimulation (0.03–3 Hz; A, E) or i.v. bolus injections of noradrenaline (0.003–3 μg/kg; B, F), methoxamine (1–100 μg/kg; C, G) or UK 14,304 (0.03–30 μg/kg; D, H) both in sham surgery (sham; A-D) and ovariectomized (Ovx; E-H) groups. Each point represents the mean ± s.e.m. *, $P < 0.05$ vs control.

statistically significant change (Table 1, and Fig. 2). Our results are similar to previous reports [38,39] and suggest that ovariectomy contributes to the development of IR; meanwhile, the combination of ovariectomy and fructose consumption exacerbates the development of IR.

4.3. *Effect of chronic administration of 17β-estradiol on metabolic variables*

17β-Estradiol has an essential role in glucose homeostasis and insulin secretion. However, dysregulation (increase or decrease) of physiological levels could cause an affectation on the metabolism. Indeed, we observed that chronic administration of 17β-estradiol in the sham-control group significantly increased fasting insulin levels, HOMA-IR index (Table 2), and insulin levels in OGTT (Fig. 3). Besides, this treatment: i) increased fasting triglyceride levels but without statistical significance (Table 2); ii) significantly decreased body weight, iii) had no significant change in fasting glucose levels (Table 2) or glucose levels in OGTT (Fig. 3A); although it shows a tendency to decrease these parameters. On the other hand, the administration of 17β-estradiol has the same tendency in the ovariectomy-control group as in the sham-control group, but only with a statistically significant change in the increase of insulin levels in OGTT (Table 2; and Fig. 3B). These results are similar with that presented by Marchand et al., 2018 [40] who observed that women with higher levels of 17β-estradiol had less insulin sensitivity and could be related with lipid alterations, because we also observed an impaired insulin sensitivity and increase in fasting triglycerides in groups with 17β-estradiol administration. Thus, we suggest that these insulin alterations could be related to concomitant lipid changes.

On the other hand, in the group with fructose consumption and sham surgery, the chronic administration of 17β-estradiol: i) significantly decreased glucose levels in OGTT (Fig. 3C), ii) significantly increased triglycerides (Table 2), and iii) increased fasting insulin levels and HOMA-IR index (Table 2) but without statistical significance compared with the vehicle of 17β-estradiol. Finally, in the group with fructose consumption and ovariectomy, the chronic administration of 17β-estradiol decreased glucose and insulin levels in OGTT (Fig. 3), as well as fasting insulin levels and HOMA-IR index (Table 2), but without statistical significance. Taken together, these results suggest that chronic treatment with 17β-estradiol improves insulin sensitivity in animals with fructose consumption. The increase in insulin levels observed in all groups treated with 17β-estradiol may be explained in terms that 17β-estradiol can improve the function of pancreatic beta cells by the promotion of the GLP-1 secretion or by regulation of the ATP-sensitive potassium (K_{ATP}) channels through cyclic GMP (cGMP) and cGMP-dependent protein kinase (PKG) activity which stimulates insulin secretion [41,42]. Furthermore, 17β-estradiol has been shown to improve insulin sensitivity by activating Era-Akt-Foxo1 signaling pathway, which can reduce gluconeogenesis, leading to a decrease in glucose levels [9].

4.4. *Effect of insulin resistance on hemodynamic parameters and vasopressor responses*

Chronic consumption of 15 % fructose solution significantly increased all hemodynamic parameters in the sham-operated group

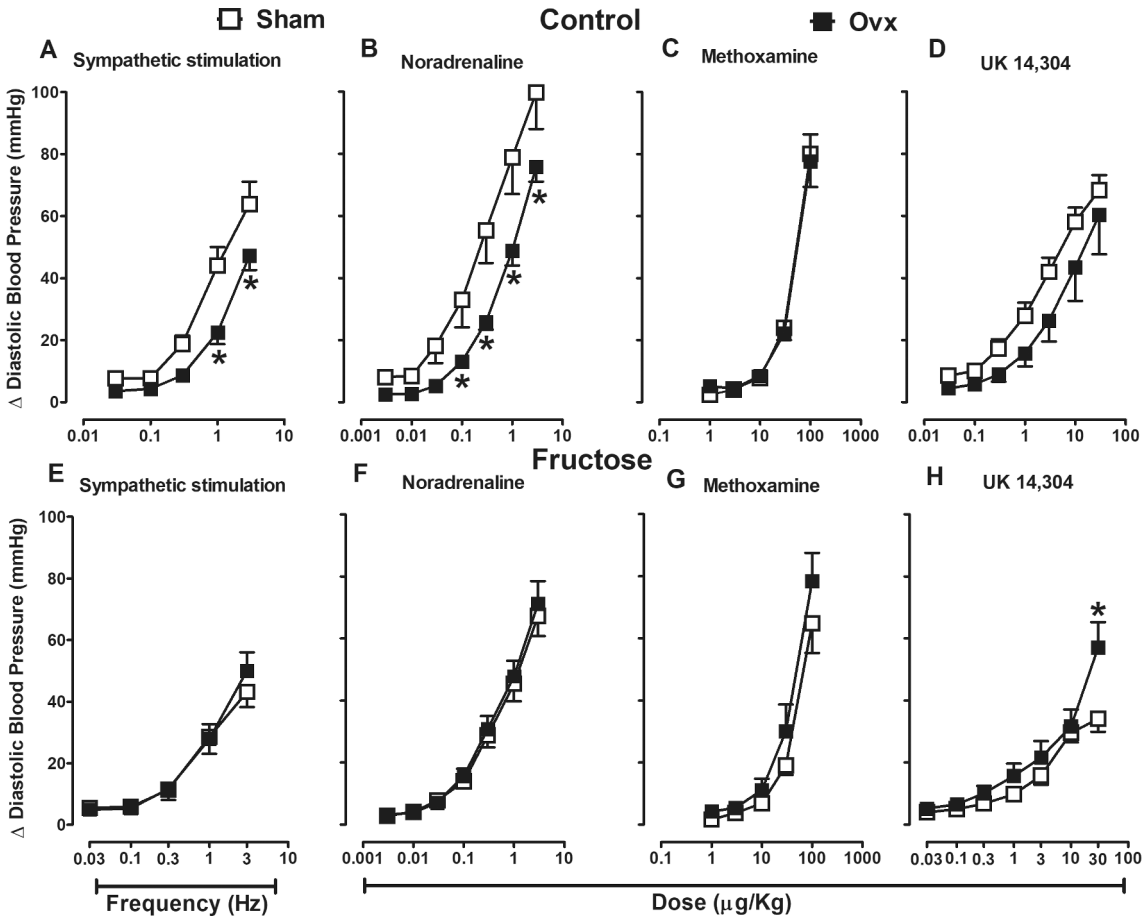

Fig. 5. Effect of ovariectomy on vasopressor responses. Effect of ovariectomy on vasopressor responses induced by sympathetic stimulation (0.03–3 Hz; A, E) or i. v. bolus injections of noradrenaline (0.003–3 μg/kg; B, F), methoxamine (1–100 μg/kg; C, G) or UK 14,304 (0.03–30 μg/kg; D, H) in control (tap water consumption, A-D) and fructose (fructose 15 % consumption) groups (E-H). Each point represents the mean ± s.e.m. *, $P < 0.05$ vs Sham.

compared with its control group. While in the ovariectomy group, fructose consumption increased all hemodynamic parameters compared with its control group; however, it was statistically significant only to SBP (Table 3). These results are consistent with previous reports which show that fructose consumption induces hypertension in rats [32,43] and humans [44], and in different studies. In this respect, it has been proposed that this effect may be because fructose consumption increases uric acid production and induces IR, and both of them are considered to be a possible cause of hypertension [44–46].

In contrast, although fructose consumption induced significant increases in blood pressure measurements in the awake animals, the vasopressor responses were measured by changes in DBP in a pithed rat model. The baseline values of DBP were similar in the control and fructose groups at time cero. After the stimulus or intravenous bolus injections, the vasopressor responses were significantly decreased, specifically those induced by sympathetic stimulation and by intravenous bolus injections of noradrenaline (α_1/α_2 adrenergic agonist) and UK 14,304 (α_2 adrenergic agonist) (Fig. 4A-D). The results could be because fructose consumption also induced both IR and hyperinsulinemia, leading to these contradictory effects probably due to the fact that insulin produces vasodilatation and vasoconstriction effects [47,48]. In this case, due to the obtained results, we suggested that the decreases on vasopressor responses are mainly mediated by an action on α_2 adrenergic receptors.

In ovariectomized animals, the fructose consumption did not change the vasopressor responses by sympathetic stimulation, or those generated by the intravenous bolus injections of noradrenaline, methoxamine, or UK 14,304 (Fig. 4). These results show that the loss of female sex hormones could contribute to the development of cardiovascular diseases and masks the effect of hyperinsulinemia induced by fructose consumption.

4.5. *Effect of ovariectomy on hemodynamic parameters and vasopressor responses*

Our results show that in the control group, ovariectomy significantly increased SBP, DBP and MBP; it also increased HR, although no statistical significance was observed (Table 3). This effect was similar in the fructose group, where the ovariectomy increased all hemodynamic parameters but was statistically significant only to SBP (Table 3). These results are consistent with those presented by Honigberg et al. [49], who observed that premature menopause increases the risk of cardiovascular disease with an increase in hypertension incidence.

On the other hand, on vasopressor responses, in the control group, ovariectomy only diminished those induced by sympathetic stimulation and intravenous bolus injections of noradrenaline (Fig. 5A and 5B), while in the fructose group ovariectomy did not change the above vasopressor responses (Fig. 5E- 5H). These results suggest that the ovariectomy decreased the sympathetic tone in the control group, similar to the reported by Shimojo et al. [50], and considering the results in the control group, probably ovariectomy also decreased the functionality of α_1/α_2 adrenergic receptors but with more action on α_2 adrenergic receptors, since the vasopressor responses induced by UK 14,304 were decreased but with no significant differences, and no changes were observed in those vasopressor responses induced by methoxamine. All these effects (hemodynamic and vasopressor

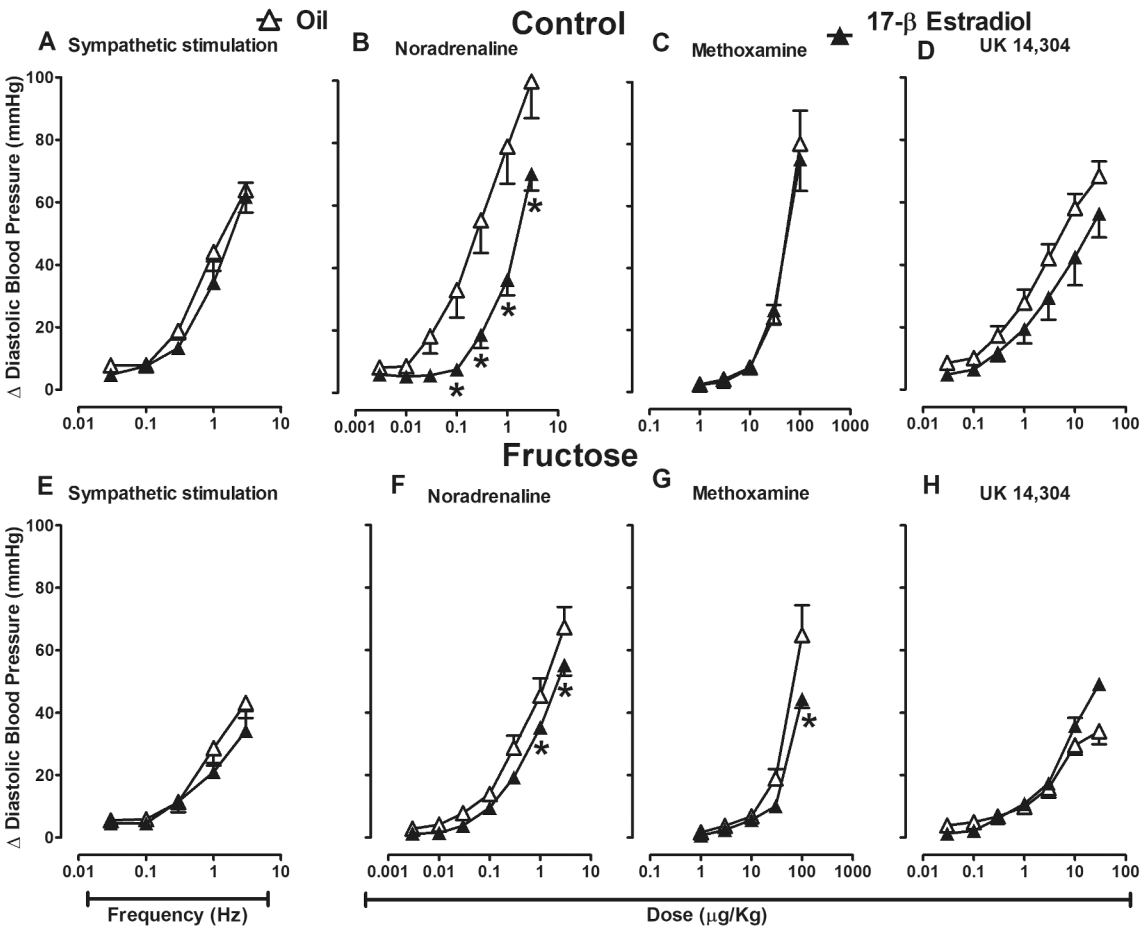

Fig. 6. Effect of 17β-estradiol on vasopressor responses in sham-operated rats. Effect of 17β-estradiol (10 μg/kg·day) or its vehicle (corn oil, 0.5 ml/kg·day) in sham-operated rats on vasopressor responses induced by sympathetic stimulation or i.v. bolus injections of noradrenaline, methoxamine, or UK 14,304 in control (tap water, A-D) and fructose (E-H) groups. Each point represents the mean ± s.e.m. *, $P < 0.05$ vs vehicle.

responses) maybe be less marked in the fructose group because the effect of IR could mask the effect of ovariectomy.

4.6. Effect of chronic administration of 17β-estradiol on hemodynamic parameter and vasopressor responses

Our results show that chronic administration of 17β-estradiol decreased HR but was not statistically significant, compared with its vehicle, in the control group with sham surgery or ovariectomy, and in the fructose group with ovariectomy. While in the fructose group with sham surgery, 17β-estradiol significantly decreased HR (Table 4). Therefore, 17β-estradiol shows a cardioprotective effect which could be due to its effect on estrogen receptors, leading to a reduction in Ca^{++} influx and an increase in Ca^{++} uptake on cardiomyocytes, as previously demonstrated [51,52]. However, future studies are required to test this hypothesis.

In contrast, 17β-estradiol significantly increased SBP, DBP and MBP in the control and fructose groups with sham surgery. Nevertheless, in the control and fructose group with ovariectomy, the treatment with 17β-estradiol decreased SBP, DBP and MBP. Notwithstanding, these changes were statistically significant only in the fructose group (Table 4). This paradox effect has also been reported previously on women with hormone replacement therapy [52]. Here, we suggest that these discrepancies may be due to the 17β-estradiol levels in the body because sham-operated rats have average production of natural 17β-estradiol plus administered 17β-estradiol, leading to higher 17β-estradiol levels than presented under physiologically normal conditions. In turn, this suggests that high levels of 17β-estradiol could induce an adverse effect on blood pressure, but its replacement in ovariectomized rats has a positive effect on blood pressure and heart rate; similar data were found by Li et al. [53]. In this sense, they found a correlation between hormonal steroid levels and cardiac function and showed that high 17β-estradiol levels increased the diastolic cardiac function due to a decrease in ejection fraction; however, the mechanisms are not fully understood. Besides, these effects were lower on IR rats (with fructose consumption) causing the effect of IR masks the effect of 17β-estradiol.

On the other hand, in sham-operated control rats, 17β-estradiol significantly decreased vasopressor responses induced by i.v. bolus injections of noradrenaline compared with the vehicle of 17β-estradiol (Fig. 6B). Moreover, in sham-operated IR rats, 17β-estradiol significantly decreased vasopressor responses induced by i.v. bolus injections of noradrenaline (Fig. 6F) and methoxamine (Fig. 6G) compared with its vehicle (oil). These last results were similar to those obtained on ovariectomized control rats (Fig. 7B and 7C). Overall, these results suggest that the effect of chronic administration of 17β-estradiol on vasopressor responses could be mediated at least in part by down-regulation or decrease in functionality of postganglionic alpha-adrenergic receptors, mainly by the α1 adrenergic receptor. However, future studies are required to test this hypothesis.

On ovariectomized fructose rats, 17β-estradiol increased vasopressor responses induced by sympathetic stimulation (Fig. 7E) but did not change vasopressor responses induced by i.v. bolus injections with the agonist of adrenergic receptors. These results suggest that both IR and ovariectomy could lead to loss of effect of the treatment with 17β-estradiol.

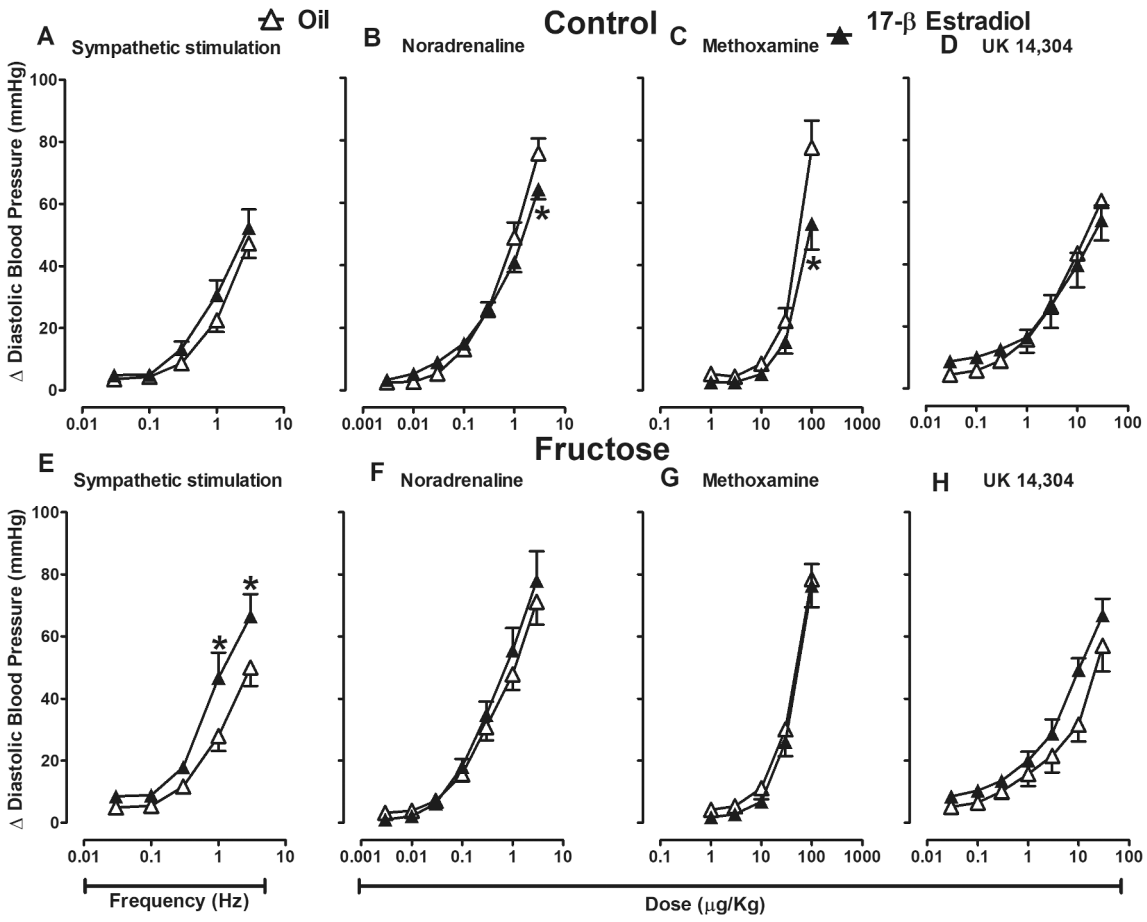

Fig. 7. Effect of 17β-estradiol on vasopressor responses in ovariectomized rats. Effect of 17β-estradiol (10 μg/kg·day) or its vehicle (corn oil, 0.5 ml/kg·day) in ovariectomized rats on vasopressor responses induced by sympathetic stimulation or i.v. bolus injections of noradrenaline, methoxamine, or UK 14,304 in control (tap water, A-D) and fructose (E-H) groups. Each point represents the mean ± s.e.m. *, $P < 0.05$ vs vehicle.

5. Conclusions

This study demonstrated that treatment with 17β-estradiol: i) increased the metabolic disbalance (hyperinsulinemia, IR, and TG) induced by ovariectomy both in control and fructose groups; ii) increased triglycerides induced by fructose consumption; iii) prevented the increase of blood pressure induced by ovariectomy both in control and fructose group, but with the opposite effect on sham-operated rats; and iv) decreased vasopressor responses induced by i.v. bolus injections of noradrenaline on sham-operated (control and fructose group) and ovariectomized (control) rats, and those induced by i.v. bolus injections of methoxamine (α_1 adrenergic agonist). Overall, these results suggest that 17β-estradiol has a cardioprotective effect, and the effect of 17β-estradiol on vasopressor responses could be mediated mainly by the α1 adrenergic receptor, while IR and ovariectomy lead to a loss or decrease of the effect of the treatment with estradiol.

CRediT authorship contribution statement

Erika J. Gutiérrez-Lara: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Araceli Sánchez-López:** Methodology, Validation, Investigation, Data curation, Writing – review & editing. **Janet Murbartián:** Resources, Methodology, Data curation, Writing – review & editing. **Selene J. Acosta-Cota:** Formal analysis, Writing – original draft. **David Centurión:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank CONACyT (Mexico) for their financial support (Grant No. 252702).

References

[1] F. Wang, L. Han, D. Hu, Fasting insulin, insulin resistance and risk of hypertension in the general population: A meta-analysis, Clin. Chim. Acta 464 (2017) 57–63, https://doi.org/10.1016/j.cca.2016.11.009.
[2] N. Sasaki, R. Ozono, Y. Higashi, R. Maeda, Y. Kihara, Association of insulin resistance, plasma glucose level, and serum insulin level with hypertension in a population with different stages of impaired glucose metabolism, J. Am. Heart Assoc. 9 (2020), https://doi.org/10.1161/JAHA.119.015546.
[3] F. Artunc, E. Schleicher, C. Weigert, A. Fritsche, N. Stefan, H.-U. Häring, The impact of insulin resistance on the kidney and vasculature, Nat. Rev. Nephrol. 12 (2016) 721–737, https://doi.org/10.1038/nrneph.2016.145.
[4] J.F. Ndisang, A. Vannacci, S. Rastogi, Insulin resistance, type 1 and type 2 diabetes, and related complications 2017, J. Diabetes Res. 2017 (2017) 1–3, https://doi.org/10.1155/2017/1478294.
[5] K. Polak, A. Czyzyk, T. Simoncini, B. Meczekalski, New markers of insulin resistance in polycystic ovary syndrome, J. Endocrinol. Invest. 40 (2017) 1–8, https://doi.org/10.1007/s40618-016-0523-8.
[6] D. Macut, J. Bjekić-Macut, D. Rahelić, M. Doknić, Insulin and the polycystic ovary syndrome, Diabetes Res. Clin. Pract. 130 (2017) 163–170, https://doi.org/10.1016/j.diabres.2017.06.011.

[7] A. Đogo, M. Stojanovic, M. Ivovic, M. Tancic Gajic, L.V. Marina, G. Citlucanin, M. Brkic, S. Popovic, S. Vujovic, Menopausal hyperinsulinism and hypertension – new approach, Gynecol. Endocrinol. 36 (2020) 709–713, https://doi.org/10.1080/09513590.2020.1768370.

[8] S. Whitcroft, A. Herriot, Insulin resistance and management of the menopause: a clinical hypothesis in practice, Menopause Int. 17 (2011) 24–28, https://doi.org/10.1258/mi.2011.011003.

[9] H. Yan, W. Yang, F. Zhou, X. Li, Q. Pan, Z. Shen, G. Han, A. Newell-Fugate, Y. Tian, R. Majeti, W. Liu, Y. Xu, C. Wu, K. Allred, C. Allred, Y. Sun, S. Guo, Estrogen improves insulin sensitivity and suppresses gluconeogenesis via the transcription factor Foxo1, Diabetes 68 (2019) 291–304, https://doi.org/10.2337/db18-0638.

[10] B.M. Galmés-Pascual, M.R. Martínez-Cignoni, A. Morán-Costoya, M. Bauza-Thorbrügge, M. Sbert-Roig, A. Valle, A.M. Proenza, I. Lladó, M. Gianotti, 17β-estradiol ameliorates lipotoxicity-induced hepatic mitochondrial oxidative stress and insulin resistance, Free Radic. Biol. Med. 150 (2020) 148–160, https://doi.org/10.1016/j.freeradbiomed.2020.02.016.

[11] M. Esmailidehaj, F. Kuchakzade, M.E. Rezvani, Z. Farhadi, H. Esmaeili, H. Azizian, 17β-Estradiol improves insulin signalling and insulin resistance in the aged female hearts: Role of inflammatory and anti-inflammatory cytokines, Life Sci. 253 (2020), 117673, https://doi.org/10.1016/j.lfs.2020.117673.

[12] C.J. Lagranha, T.L.A. Silva, S.C.A. Silva, G.R.F. Braz, A.I. da Silva, M.P. Fernandes, D.F. Sellitti, Protective effects of estrogen against cardiovascular disease mediated via oxidative stress in the brain, Life Sci. 192 (2018) 190–198, https://doi.org/10.1016/j.lfs.2017.11.043.

[13] B. Cong, Y. Xu, H. Sheng, X. Zhu, L. Wang, W. Zhao, Z. Tang, J. Lu, X. Ni, Cardioprotection of 17β-estradiol against hypoxia/reoxygenation in cardiomyocytes is partly through up-regulation of CRH receptor type 2, Mol. Cell. Endocrinol. 382 (2014) 17–25, https://doi.org/10.1016/j.mce.2013.09.002.

[14] A.A. Knowlton, D.H. Korzick, Estrogen and the female heart, Mol. Cell. Endocrinol. 389 (2014) 31–39, https://doi.org/10.1016/j.mce.2014.01.002.

[15] S.J. Acosta-Cota, A. Sánchez-López, T. Molina-Muñoz, N.L. Gómez-Viquez, D. Centurión, Evidence that chronic administration of 17β-oestradiol decreases the vasopressor responses to adrenergic system stimulation in streptozotocin-diabetic female rats, Steroids 83 (2014) 1–9, https://doi.org/10.1016/j.steroids.2014.01.011.

[16] E. Cairrão, E. Alvarez, J.M. Carvas, A.J. Santos-Silva, I. Verde, Non-genomic vasorelaxant effects of 17β-estradiol and progesterone in rat aorta are mediated by L-type Ca2+ current inhibition, Acta Pharmacol. Sin. 33 (2012) 615–624, https://doi.org/10.1038/aps.2012.4.

[17] C. Stirone, Y. Chu, L. Sunday, S.P. Duckles, D.N. Krause, 17β-Estradiol increases endothelial nitric oxide synthase mRNA copy number in cerebral blood vessels: quantification by real-time polymerase chain reaction, Eur. J. Pharmacol. 478 (2003) 35–38, https://doi.org/10.1016/j.ejphar.2003.08.037.

[18] A. Satake, M. Takaoka, M. Nishikawa, M. Yuba, Y. Shibata, K. Okumura, K. Kitano, H. Tsutsui, K. Fujii, S. Kobuchi, M. Ohkita, Y. Matsumura, Protective effect of 17β-estradiol on ischemic acute renal failure through the PI3K/Akt/eNOS pathway, Kidney Int. 73 (2008) 308–317, https://doi.org/10.1038/sj.ki.5002690.

[19] D.S. Bendale, P.A. Karpe, R. Chhabra, S.P. Shete, H. Shah, K. Tikoo, 17-β Oestradiol prevents cardiovascular dysfunction in post-menopausal metabolic syndrome by affecting SIRT1/AMPK/H3 acetylation, Br. J. Pharmacol. 170 (2013) 779–795, https://doi.org/10.1111/bph.12290.

[20] Y.M. Seok, E.J. Jang, O. Reiser, M. Hager, I.K. Kim, 17β-Estradiol induces vasorelaxation in a G-protein-coupled receptor 30-independent manner, Naunyn. Schmiedebergs, Arch. Pharmacol. 385 (2012) 945–948, https://doi.org/10.1007/s00210-012-0770-y.

[21] O.M. Reslan, Z. Yin, G.R.A. do Nascimento, R.A. Khalil,, Subtype-specific Estrogen Receptor-mediated Vasodilator Activity in the Cephalic, Thoracic, and Abdominal Vasculature of Female Rat, J. Cardiovasc. Pharmacol. 62 (2013) 26–40, https://doi.org/10.1097/FJC.0b013e31828bc88a.

[22] W. Keung, R.Y.K. Man, Circulating sex hormones modulate vascular contractions and acute response to 17β-estradiol in rat mesenteric arteries, Pharmacology 88 (2011) 55–64, https://doi.org/10.1159/000329426.

[23] D. Song, V.G. Yuen, L. Yao, J.H. McNeill, Chronic estrogen treatment reduces vasoconstrictor responses in insulin resistant rats, Can. J. Physiol. Pharmacol. 84 (2006) 1139–1143, https://doi.org/10.1139/y06-061.

[24] J.O. Strom, E. Theodorsson, L. Holm, A. Theodorsson, Different methods for administering 17β-estradiol to ovariectomized rats result in opposite effects on ischemic brain damage, BMC Neurosci. 11 (2010) 39, https://doi.org/10.1186/1471-2202-11-39.

[25] A. Schultz, S. Barbosa-da-Silva, M.B. Aguila, C.A. Mandarim-de-Lacerda, Differences and similarities in hepatic lipogenesis, gluconeogenesis and oxidative imbalance in mice fed diets rich in fructose or sucrose, Food Funct. 6 (2015) 1684–1691, https://doi.org/10.1039/C5FO00251F.

[26] J.S. Gillespie, A. Maclaren, D. Pollock, A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat, Br. J. Pharmacol. 40 (1970) 257–267, https://doi.org/10.1111/j.1476-5381.1970.tb09919.x.

[27] R.E. Shipley, J.H. Tilden, Pithed rat preparation suitable for assaying pressor substances, Exp. Biol. Med. 64 (1947) 453–455, https://doi.org/10.3181/00379727-64-15828.

[28] L.I. Kleinman, E.P. Radford, Ventilation standards for small mammals, J. Appl. Physiol. 19 (1964) 360–362, https://doi.org/10.1152/jappl.1964.19.2.360.

[29] R.E. Klabunde, Cardiovascular physiology concepts, Lippincott Williams & Wilkins, Philadelphia, 2005.

[30] F. Akar, E. Sumlu, M.E. Alçığır, A. Bostancı, G. Sadi, Potential mechanistic pathways underlying intestinal and hepatic effects of kefir in high-fructose-fed rats, Food Res. Int. 143 (2021), 110287, https://doi.org/10.1016/j.foodres.2021.110287.

[31] L.O. Batista, V.W. Ramos, M.A. Rosas Fernández, C.M. Concha Vilca, K.T. de Albuquerque, Oral solution of fructose promotes SREBP-1c high-expression in the hypothalamus of Wistar rats, Nutr. Neurosci. 22 (2019) 648–654, https://doi.org/10.1080/1028415X.2018.1427659.

[32] M.A. Mayer, C. Höcht, J.F. Giani, M.C. Muñoz, A. Carranza, C.A. Taira, F. P. Dominici, A.M. Puyó, B.E. Fernández, Central insulin–angiotensin II interaction in blood pressure regulation in fructose overloaded rats, Regul. Pept. 185 (2013) 37–43, https://doi.org/10.1016/j.regpep.2013.06.001.

[33] Y.-H. Chan, G.-J. Chang, Y.-J. Lai, W.-J. Chen, S.-H. Chang, L.-M. Hung, C.-T. Kuo, Y.-H. Yeh, Atrial fibrillation and its arrhythmogenesis associated with insulin resistance, Cardiovasc. Diabetol. 18 (2019) 125, https://doi.org/10.1186/s12933-019-0928-8.

[34] G. García, E.J. Gutiérrez-Lara, D. Centurión, V. Granados-Soto, J. Murbartián, Fructose-induced insulin resistance as a model of neuropathic pain in rats, Neuroscience 404 (2019) 233–245, https://doi.org/10.1016/j.neuroscience.2019.01.063.

[35] M.C. Petersen, G.I. Shulman, Mechanisms of insulin action and insulin resistance, Physiol. Rev. 98 (2018) 2133–2223, https://doi.org/10.1152/physrev.00063.2017.

[36] H. Kolb, K. Kempf, M. Röhling, S. Martin, Insulin: too much of a good thing is bad, BMC Med. 18 (2020) 224, https://doi.org/10.1186/s12916-020-01688-6.

[37] P. Bjornstad, R.H. Eckel, Pathogenesis of lipid disorders in insulin resistance: a brief review, Curr. Diab. Rep. 18 (2018) 127, https://doi.org/10.1007/s11892-018-1101-6.

[38] S.H. Tawfik, B.F. Mahmoud, M.I. Saad, M. Shehata, M.A. Kamel, M.H. Helmy, Similar and additive effects of ovariectomy and diabetes on insulin resistance and lipid metabolism, Biochem. Res. Int. 2015 (2015) 1–8, https://doi.org/10.1155/2015/567945.

[39] V.J. Vieira-Potter, J. Padilla, Y.-M. Park, R.J. Welly, R.J. Scroggins, S.L. Britton, L. G. Koch, N.T. Jenkins, J.M. Crissey, T. Zidon, E.M. Morris, G.M.E. Meers, J. P. Thyfault, Female rats selectively bred for high intrinsic aerobic fitness are protected from ovariectomy-associated metabolic dysfunction, Am. J. Physiol. Integr. Comp. Physiol. 308 (2015) R530–R542, https://doi.org/10.1152/ajpregu.00401.2014.

[40] G.B. Marchand, A.-M. Carreau, S.J. Weisnagel, J. Bergeron, F. Labrie, S. Lemieux, A. Tchernof, Increased body fat mass explains the positive association between circulating estradiol and insulin resistance in postmenopausal women, Am. J. Physiol. Metab. 314 (2018) E448–E456, https://doi.org/10.1152/ajpendo.00293.2017.

[41] S. Handgraaf, R. Dusaulcy, F. Visentin, J. Philippe, Y. Gosmain, 17-β Estradiol regulates proglucagon-derived peptide secretion in mouse and human α- and L cells, JCI Insight. 3 (2018), https://doi.org/10.1172/jci.insight.98569.

[42] S. Soriano, A.B. Ropero, P. Alonso-Magdalena, C. Ripoll, I. Quesada, B. Gassner, M. Kuhn, J.-A. Gustafsson, A. Nadal, Rapid regulation of K(ATP) channel activity by 17{beta}-estradiol in pancreatic beta}-cells involves the estrogen receptor {beta and the atrial natriuretic peptide receptor, Mol. Endocrinol. 23 (2009) 1973–1982, https://doi.org/10.1210/me.2009-0287.

[43] L. Xu, G. Hu, J. Qiu, Y. Fan, Y. Ma, T. Miura, M. Kohzuki, O. Ito, High fructose-induced hypertension and renal damage are exaggerated in dahl salt-sensitive rats via renal renin-angiotensin system activation, J. Am. Heart Assoc. 10 (2021), https://doi.org/10.1161/JAHA.120.016543.

[44] L. Béghin, I. Huybrechts, E. Drumez, M. Kersting, R.W. Walker, A. Kafatos, D. Molnar, Y. Manios, L.A. Moreno, S. De Henauw, F. Gottrand, High Fructose intake contributes to elevated diastolic blood pressure in adolescent girls: results from The HELENA Study, Nutrients 13 (2021) 3608, https://doi.org/10.3390/nu13103608.

[45] V. Ha, J.L. Sievenpiper, R.J. de Souza, L. Chiavaroli, D.D. Wang, A.I. Cozma, A. Mirrahimi, M.E. Yu, A.J. Carleton, M. Dibuono, A.L. Jenkins, L.A. Leiter, T.M. S. Wolever, J. Beyene, C.W.C. Kendall, D.J.A. Jenkins, Effect of fructose on blood pressure, Hypertension 59 (2012) 787–795, https://doi.org/10.1161/HYPERTENSIONAHA.111.182311.

[46] L. Tappy, K.-A. Lê, Metabolic effects of fructose and the worldwide increase in obesity, Physiol. Rev. 90 (2010) 23–46, https://doi.org/10.1152/physrev.00019.2009.

[47] J. Fan, L.Y. Liu, X.Z. Liu, Hyperinsulinemia negatively affects the association between insulin resistance and blood pressure, Nutr. Metab. Cardiovasc. Dis. 31 (2021) 3359–3366, https://doi.org/10.1016/j.numecd.2021.08.029.

[48] U. Scherrer, C. Sartori, Insulin as a vascular and sympathoexcitatory hormone, Circulation 96 (1997) 4104–4113, https://doi.org/10.1161/01.CIR.96.11.4104.

[49] M.C. Honigberg, S.M. Zekavat, K. Aragam, P. Finneran, D. Klarin, D.L. Bhatt, J. L. Januzzi, N.S. Scott, P. Natarajan, Association of premature natural and surgical menopause with incident cardiovascular disease, JAMA 322 (2019) 2411, https://doi.org/10.1001/jama.2019.19191.

[50] G.L. Shimojo, R.K. Palma, J.O. Brito, I.C. Sanches, M.C. Irigoyen, K. De Angelis, Dynamic resistance training decreases sympathetic tone in hypertensive ovariectomized rats, Braz. J. Med. Biol. Res. 48 (2015) 523–527, https://doi.org/10.1590/1414-431x20154387.

[51] C. Buitrago, V. Massheimer, A.R. de Boland, Acute modulation of Ca2+ influx on rat heart by 17β-estradiol, Cell. Signal. 12 (2000) 47–52, https://doi.org/10.1016/S0898-6568(99)00066-2.
[52] S. Mahmoodzadeh, E. Dworatzek, The Role of 17β-estradiol and estrogen receptors in regulation of Ca2+ channels and mitochondrial function in cardiomyocytes, Front. Endocrinol. (Lausanne) 10 (2019), https://doi.org/10.3389/fendo.2019.00310.
[53] Y. Li, X. Sun, L. Zang, Q. Zhang, J. Li, S. Zou, Correlation between steroid hormonal levels and cardiac function in women during controlled ovarian hyperstimulation, Endocrine 44 (2013) 784–789, https://doi.org/10.1007/s12020-013-9953-7.