Published in final edited form as:

Life Sci. 2018 September 15; 209: 236–241. doi:10.1016/j.lfs.2018.08.020.

Regulation of blood pressure is influenced by gender: A study in obese Zucker rats

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Abstract

Aim: The present study determined the role of renin-angiotensin system (RAS), endothelin system, and eicosanoid system in the blood pressure (BP) regulation in male and female Zucker rats, and whether the pressor response change similarly in lean and obese animals.

Material and methods: In female (f) and male (m), lean (L) and obese (O) Zucker rats (ZR) at 22 weeks old, we evaluated the role of the 3 mentioned systems using the following treatments: 1) enalapril (angiotensin I converting enzyme inhibitor), 2) the ABT-627 (endothelin receptor A (ET_A) antagonist), and 3) the 1-aminobenzotriazol (1-ABT: eicosanoid synthesis inhibitor).

Key findings: MAP by radiotelemetry was similar and significantly higher in mOZR (120 ± 2 mm Hg) and fOZR (116 ± 4 mm Hg) ($p < 0.05$ vs. m-, fLZR), than mLZR (105 ± 3 mm Hg) and fLZR (106 ± 1 mm Hg), that were also similar. Enalapril reduced MAP more in mOZR (23%) and mLZR (26%), than fLZR (20%, $p < 0.905$ vs. mLZR) or fOZR (9%; $p < 0.05$ vs. other groups). After 10 days of drug-free and recovery period, ABT-627 reduced MAP in fLZR and mLZR by similar amounts (102 ± 4 to 92 ± 3 mm Hg, $n = 6$; $p < 0.05$ and 105 ± 2 vs. 92 ± 3 mm Hg, $n = 6$; $p < 0.05$, respectively), but did not affect either fOZR or mOZR. After another 10 days of drug-

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

free and recovery period, 1-ABT reduced MAP in fOZR (116 ± 4 to 95 ± 2 , $n = 6$; $p < 0.05$), and did not affect all other groups.

Significance: We show that the mechanisms responsible for elevated BP in male and female OZR and LZR are different, and suggest that obesity may cause an increase in BP via different mechanisms in men and women as well.

Keywords

Hypertension; Obesity; Renin-angiotensin system; Endothelin; 20-HETE

1. Introduction

The obesity-related hypertension is a significant risk factor for cardiovascular disease in women and men. The Framingham Study showed that up to 70% of the newly diagnosed hypertensive cases are attributable to obesity [1]. However, it is unknown whether there are gender differences in the blood pressure (BP) regulation in obese individuals. Consequently, the Joint National Committee on Blood Pressure Treatment (JNC8) has not delineated different treatment options for obese versus lean individuals, not for men versus women.

The obese Zucker rat (OZR) has been studied for its metabolic and endocrine disorders, and they have a high risk to develop cardiovascular diseases. There is evidence that sex hormones play a role in body weight and blood pressure regulation in this strain. In this sense, females Zucker rats have an increase in the leptin- R_L transcripts and oxidative stress resistance which confers cardiovascular protection through the age [2–5]. In contrast, Kurtz et al., reported that acute measurement of BP was higher in female OZR than LZR as early as 10 weeks of age [6]. In this sense, we reported previously that male OZR had similar BP as their lean littermates (LZR) until 22 weeks of age [7]. In male OZR, the increased activity of the renin-angiotensin system (RAS) is linked to hypertension. Thus, blockade of the RAS with either angiotensin I converting enzyme inhibitors (ACEI) or AT-1 receptor antagonists reduce BP in male OZR [8,9].

Also, endothelin-1 (ET-1) plays a role in BP regulation in men and experimental animal models, including the male OZR [2,10–12]. However, the evidence is controversial. On the one hand, obesity is associated with enhanced ET-1-mediated vasoconstriction that contributes to endothelial dysfunction and hypertension with increased adiposity [13]. On the other hand, some studies have reported the opposite in obese animals [14]. Therefore, whether ET-1 plays a role in the development of obesity hypertension is unclear.

Another possible mechanism that may contribute to the elevated BP in obesity is the eicosanoid system (ES). Laffer et al. reported that obesity and insulin resistance was associated with increased urinary 20-HETE excretion in obese patients, contributing to the progression of hypertension [15]. Furthermore, this association was not observed in lean or overweight individuals who were insulin sensitive [16]. Peterson et al., have shown that obese women with BMI > 30 had elevated plasma oxidized-HDL and isoprostane levels along with increases in angiotensin II and 20-HETE [17]. Finally, there is evidence that the

vasodilator eicosanoids, epoxyeicosatrienoic acid (EET) system, may protect against hypertension and cardiovascular complications of obesity [18].

Thus, whether the RAS, ET-1, EET, or 20-HETE play roles in mediating BP in OZR versus LZR, whether there are sex differences in the BP or what mechanisms are responsible for the BP control, all remain to be determined. Therefore, in the present study we hypothesize that there is a sex difference in BP control in OZR and LZR, and thus there are different mediators that contribute to the elevated BP, such as RAS, ET-1 via the ET_A receptor, and/or the eicosanoid system (EET/20-HETE).

2. Materials and methods

2.1. Experimental animals

Female (f) and male (m) obese Zucker rats (OZR) (HsdHlr: ZUCKER-Lepr^{fa}) and age-matched lean Zucker rats (LZR) were obtained from the vendor at 8 weeks of age (Envigo, formerly Harlan SD, Indianapolis, IN). Rats were housed in a 12 h light:12-h dark cycle in a temperature-controlled (22 °C) room in the Laboratory Animal Facility at the University of Mississippi Medical Center. Rats were randomly divided into 4 groups (n = 5–8 rats/group) and were given ad libitum access to standard rat chow (Teklad 8630) and water throughout the study. Water intake was daily measured when rats were given drugs (Female: fLZR: 14 ± 1 ml/24 h. vs. fOZR: 18 ± 1 ml/24 h. and Male: mLZR: 27 ± 1 ml/24 h. vs. mOZR: 38 ± 2 ml/24 h.). The studies were approved by the Animal Care and Use Committee of the University of Mississippi Medical Center (Approval protocol: #297E) and followed the *NIH Guidelines for the Care and Use of Laboratory Animals, 11th edition*.

2.1.1. Experimental design—Rats were placed in metabolism cages for 24 h for urine collection before the study. Radiotelemeters were implanted as described below, and rats were allowed to recover for 14 days. Then, mean arterial pressure (MAP) was measured for 5 days as a baseline period. After that, the rats were given enalapril (30 mg/kg/day) in drinking water for 5 days. Reckelhoff and colleagues showed previously that this dose of enalapril prevents angiotensin I (1 mg/kg/h IV) from changing blood pressure [19]. Rats were then subjected to a 10 days of drug-free and recovery period of the MAP back to the baseline levels. Rats were then given ABT-627 (5 mg/kg/d), an endothelin ET_A receptor antagonist (a kind gift from Abbott Laboratories) for other 5 days, as previously described [20,21]. Following 10 days of drug-free and recovery period, the baseline was again measured for 5 days, and then rats were given 1-aminobenzotrizole (1-ABT; 50 mg/kg/d), an inhibitor of eicosanoid synthesis for 5 days, as previously described [20,22]. After this, animals were placed in metabolism cages for 24 h for urine collection. Finally, the animals were euthanized, and the kidneys and blood samples were taken for metabolic studies.

2.1.2. Blood pressure measurement—At 20 weeks of age, male and female OZR and LZR were implanted with radiotelemetry transmitters (TA11PA-C40; Data Sciences International, St. Paul, MN) in the abdominal aorta below the renal arteries, as we previously described [6]. After 2 weeks of recovery, the mean arterial pressure was recorded as described above.

2.1.3. Plasma metabolic and inflammatory markers—Blood glucose was determined from blood obtained from the tail vein of the rats ($n = 4\text{--}5$ per group), using a glucometer (Accu-check Advantage; Roche). Plasma insulin was measured by radioimmunoassay ($n = 4\text{--}5$ /group), according to the manufacturer's recommendations using the commercially available kit (Linco Research, St. Charles, MO). Plasma leptin was measured using commercially available assay kits (Leptin: M0B00, R&D Systems, Inc., Minneapolis, MN), as we previously described [6].

2.1.4. Plasma estradiol and testosterone—Plasma total testosterone was measured using a commercially available radioimmunoassay kit (Coat-A-Count testosterone kit; Diagnostic Products Corporation, Los Angeles, CA; Diagnostic Systems Laboratories, Inc., Beckman Coulter, Indianapolis, IN), as we previously described [23]. Plasma estradiol was also measured using a commercially available radioimmunoassay kit (Ultrasensitive Estradiol kit - Siemens Medical Solutions, formerly Diagnostic Products, Los Angeles, CA), as we previously described [6].

2.1.5. Urinary protein—Urinary protein excretion was measured by the method of Bradford [24], using a commercially available reagent (Bio-Rad, Richmond, CA), with albumin, as standard, as we previously described [6].

2.2. Statistical analyses

All results are expressed as mean $\pm$ SEM. Multiple groups were analyzed by three-way analysis of variance (ANOVA). Time series BP data were analyzed by repeated-measures three-way ANOVA followed by Student-Newman-Keul's comparisons. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed with SigmaStat software package V3.1 (Systat Software, San Jose, CA).

3. Results

3.1. Metabolic studies

As shown in Table 1, by 32–34 weeks of age, body weight was significantly higher in male OZR than male LZR, female OZR, or female LZR. After drug treatment, body weight was increased in all animals and maintained the same pattern as at the beginning of the experiment (Table 1). Also, Fig. 1A and B shows food intake and fasting glucose levels at the end of the experiment. While food intake was higher in male OZR than the other groups, food intake was comparable between female OZR and male LZR (Fig. 1A). The same pattern was observed in fasting glucose levels (Fig. 1B). Plasma insulin and plasma leptin were higher in obese than in lean rats and higher in males than in females (Fig. 2A and B, respectively).

3.2. Urine protein and sex steroids

To determine if there was a progression in the renal injury which could increase blood pressure over time, we assessed urinary protein excretion at the beginning and the end of the study. As shown in Table 1, urinary protein excretion was higher in male OZR than male LZR or female OZR and LZR at the beginning of the study. By the end of the study, there

was no further increase in proteinuria in male OZR, male LZR or female LZR. However, female OZR excreted a modestly higher level of protein by the end of the study that was similar to the proteinuria in male LZR at the beginning of the study. These results suggest that there was a no significant progression in renal injury to affect the blood pressure in any of the groups.

Table 2 shows that serum testosterone was not different between female LZR and female OZR, but was lower in male OZR than male LZR ($p < 0.05$). Also, the estradiol levels were significantly lower in female OZR than female LZR; and estradiol was significantly higher in male LZR than male OZR ($p < 0.05$).

3.3. Blood pressure and drug response

As shown in Fig. 3A, there was no sex difference in the MAP in either LZR or OZR, but the MAP was significantly higher in female and male OZR than female and male LZR. Enalapril reduced MAP in all groups, but to a lesser extent in females than males, regardless of strain (Fig. 3B). For example, in male OZR and LZR, enalapril reduced their blood pressure by 23–26%. However, in females, enalapril reduced blood pressure in female LZR by 20% and in female OZR by only 9%.

After 10 days of drug-free and recovery period, rats were given the ET_A receptor antagonist ABT-627 for 5 days. ABT-627 reduced MAP to a comparable extent in male and female LZR, and to a lesser extent in male OZR. However, ABT627 did not affect blood pressure in female OZR (Fig. 3C).

After another 10 days of drug-free and recovery period, rats were given 1-ABT for 5 days. As shown in Fig. 3D, 1-ABT had no effect on blood pressure in female LZR, or male LZR or OZR. In contrast, 1-ABT significantly reduced blood pressure in female OZR.

4. Discussion

The main findings in the present study are: 1) the blood pressure was higher in obese than lean Zucker rats; however, no sex difference in the blood pressure was observed; 2) there are both sex differences and strain differences in the depressor responses to antagonists that suggest that different mechanisms contribute to blood pressure regulation in male and female Zucker rats. For example, the RAS has a greater contribution to blood pressure in male OZR and LZR than in females with female OZR having little response to ACEI compared to the LZR females. ET_A receptor antagonists caused a depressor response in male and female LZR and male OZR, but not in female OZR. Finally, blockade of eicosanoid synthesis only affected blood pressure in female OZR, but not the other groups of rats.

Obesity is associated with a higher risk of cardiovascular events, such as myocardial infarction, stroke, and death. In the past decade, a growing body of evidence showed that obesity is a major risk factor for hypertension as reported by Neter and colleagues [25], and there may be gender differences. For example, Kagan et al. found that obese men have higher diastolic blood pressure and heart rate than their lean counterparts, whereas, in

women, diastolic blood pressure and heart rate were not different in obese and lean subjects [26]. Thus little is known about the mechanisms responsible for blood pressure regulation in obesity as related to gender.

The Zucker rat is an experimental animal model extensively used to study the pathophysiology of obesity and cardiovascular diseases. This animal model is characterized by hyperglycemia, insulin resistance, dyslipidemia, and hyperleptinemia due to dysfunction of the leptin receptor, compared to lean rats [8]. Bennett et al., have been shown that female obese Zucker rats have differential regulation of the leptin receptor on its long and short isoforms (leptin- R_L and leptin- R_S). Estradiol may alter the balance between leptin- R_L and leptin- R_S increasing tissue sensitivity to leptin which can partially explain the sex difference finding in this particular strain [3].

In previous studies, we observed that the male OZR had similar blood pressure at 22 weeks of age compared to the male LZR [6]. However, Kurtz et al. showed that the female OZR had elevated blood pressure compared to female LZR when measured 4–6 h after femoral catheter placement as early as 9 weeks of age [8]. Since there were few studies on the sex differences in blood pressure in OZR or LZR, we tested the hypothesis that there would be both sex differences and strain differences in blood pressure and blood pressure control mechanisms. Not surprisingly, we found that LZR males and females had no differences in blood pressure, and had similar responses to all drugs they were given. Thus their blood pressure is controlled by the RAS and, to some extent, the ET-1 system. However, there was no response to 1-ABT. It was surprising that the ETA receptor antagonist affected blood pressure in the normotensive LZR. The effect of ETA receptor antagonism in females is limited. Future studies will be necessary to determine if ET-1 receptor expression is upregulated in LZR males and females.

Similar to the LZR, we did not find a sex difference in the blood pressure in OZR. However, we did identify sex differences in the responses to the antagonists we used. We found that in male OZR, the hypertension is mediated by RAS activation since enalapril reduced their blood pressure. Although enalapril also decreased the blood pressure in female OZR, the reduction was modest compared to the males. Another alternative is that the AT1 receptor affects the dopamine receptors regulating the extracellular fluid volume and BP through natriuresis. In this regards, obese female Zucker rats have a higher D1R and D3R expression than do the male rats, which may contribute to reducing the salt sensitivity of BP in females [27]. However, if this is the case in our study, it needs further investigation.

Also, we found that in male OZR, ABT627, the ET_A receptor antagonist, lowered the blood pressure but had no effect in females, suggesting that in males the ET-1 system contributes to the elevated blood pressure. It is possible that since the treatment period was only 5 days perhaps chronic treatment (> 2 weeks) with ABT627 in obese female Zucker rats may reduce the blood pressure.

In human studies, the contribution of endothelin in the blood pressure regulation in obese women depends on several factors, such as endothelin levels, ethnicity, race, and age [28]. Another possible factor that may be affecting the response to ABT627 is the intrarenal ET_A

receptor density. There is evidence that ET_A receptor is upregulated in the presence of insulin resistance [10,15,29]. Since the female OZR have normoglycemia but elevated plasma insulin and thus are insulin resistant, it is possible that the ET_A receptor is upregulated. This fact could partially explain why the blockade of ET_A receptor by ABT627 did not reduce the blood pressure and may suggest that more extended treatment or much higher dose of ET_A receptor antagonist is required in obese female rats may have its effect. However, ABT-627 did not affect the blood pressure in male OZR despite the significantly higher fasting glucose and plasma insulin levels. These data clearly show a sex difference in the response. Another possible explanation is that since Ang II has been shown to increase expression of ET-1 [30], it is possible that this may be the mechanism by which the ET-1 is activated in male OZR, but not in females. Finally, a study by Ajayi et al., determined the role of endogenous sex steroid hormones on the systemic and renal microvascular reactivity to ET-1. It has suggested that the effect of testosterone is directly through the vascular ETB receptor which abolishes the vasopressor response to ET-1 after castration. While spaying the female rats with an estrogen deficit caused much less discernible effect, however [2].

In obesity, CYP metabolites of arachidonic acid have biological actions on blood pressure regulation and contribute to renal and cardiovascular function. In our study, while 1-ABT did not affect blood pressure in male OZR, the blood pressure was significantly reduced in females, suggesting that 20-HETE likely does contribute to hypertension in these females. If EETs played a role, one would expect a pressor response to 1-ABT. Thus it is likely EETs do not contribute to the maintenance of blood pressure in these females. As occurred with enalapril, 1-ABT has an acute effect (24 h) reducing blood pressure in female OZR. However, in male OZR, the depressor effect took a longer time to reach steady state, suggesting heterogeneity in the kinetics in response to medication, or sex differences in levels of vasoconstrictor and vasodilator eicosanoids.

Estrogens are known to modulate the expression of RAS components such as AT1 and AT2 receptors, ACE/ACE2 [31–33], and the production of Ang-(1–7) [34], the vasodilator arm of the RAS. Since estradiol levels were significantly reduced in female OZR, this may explain in part the modest response to enalapril in the female OZR compared to the female LZR. The reduction in estrogens is somewhat surprising since adipose tissue produces sex steroids, and thus one might expect more estradiol in female OZR than LZR. However, it is likely that the reduced levels of estradiol reflect endogenous production of estradiol from the ovaries.

In male OZR, we showed previously that the androgen levels were decreased compared to male LZR. Androgens are known to increase expression of angiotensinogen and thus increase renin activity if the enzyme is not working at V_{max} [35,36] producing more Ang II. While this may be the reason why the RAS is stimulated in male LZR, the fact that the androgen levels are significantly lower in male OZR suggests that androgens may not be the mechanism by which the RAS is activated. Adipose tissue can produce an activated RAS [37], and this likely contributes at least in part to the blood pressure in male OZR. Tallam and Jandhyala reported that male OZR had higher plasma renin activity than LZR [9], supporting an upregulated RAS. In addition, Muller-Fielitz and colleagues found that chronic infusion of Ang II increased blood pressure more in male OZR than LZR and was

associated with 6-fold higher increases in aldosterone in OZR than LZR [38], perhaps also suggesting that the endogenous system including AT1 receptors and mineralocorticoid receptors may be primed to be upregulated in OZR. There is evidence supporting that the sex and body-type interact to contribute to the regulation of the renal sodium transporters levels and urinary excretion differently in male than female Zucker rats [39].

In conclusion, the data from the present study support that there are both strain differences and sex differences in the control of the blood pressure in OZR and LZR. These data suggest that there are also gender differences in blood pressure control in obese and lean men and women. Currently, there are no guidelines for treatment of hypertension that differ whether the subject is male or female or whether the subject is lean or obese. Future studies are necessary to evaluate potential mechanisms responsible for blood pressure control in men and women, whether they should have different therapeutic options based on their gender, and whether their body mass index should be taken into account for treatment options to maximize precision medicine.

Acknowledgments

The authors would like to thank Dr. Jane Reckelhoff for her critical reading of the manuscript and to Deborah D. Davis and Huimin Zhang for excellent technical support. This study was supported by National Institutes of Health Heart, Lung and Blood Institute HL69194, HL66072 and HL51971 (to JFR), AHA 14POST18640015 (to MM), American Heart Association (AHA) 14POST18640015, and by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM121334 (to ROM and JFR). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Fig. 1. Food intake (A) and fasting glucose (B) of male and female obese and lean Zucker rats. Females and males OZR and LZR (n = 5–8/group). Data are presented as mean $\pm$ SEM. *p < 0.05 vs. female LZR and OZR, and male LZR, & p < 0.05 vs. female LZR.

Fig. 2.

Plasma Insulin (A) and plasma leptin (B) of male and female obese and lean Zucker rats. Females and males OZR and LZR (n = 5–8/group). Data are presented as mean $\pm$ SEM. * $p < 0.05$ vs. female LZR and OZR, and male LZR, & $p < 0.05$ vs. female LZR, # $p < 0.05$ vs. female and male LZR.

Fig. 3.

Mean arterial pressure of male and female obese and lean Zucker rats. Baseline and treated with enalapril, ABT-627, 1-ABT (panels A–D). Rats ($n = 5\text{--}8/\text{group}$, controls and treated, OZR and LZR females and males) were implanted with radiotelemeters, and the MAP was measured chronically beginning at 22 weeks of age. There was no sex difference in the MAP in either lean or obese. After 10 days of drug-free and recovery period, rats received ABT-627 (5 mg/kg/d, $n = 5\text{--}8/\text{group}$) in drinking water for 5 days, followed by another 10 days of drug-free and recovery period. The rats were given 1-Aminobenzotriazol (1-ABT; 50 mg/kg/d, $n = 5\text{--}8/\text{group}$) in drinking water for 5 days. Data are presented as mean $\pm$ SEM. * $p < 0.05$ vs. baseline period; # $p < 0.05$ vs. LZR. No symbols are included for OZR control vs. LZR control since they are significantly different ($p < 0.05$) and complicate the graph.

Table 1

Body weight and proteinuria pre- and post-treatment in male and female Zucker rats.

	Pre-treatment body weight (g)	Post-treatment body weight (g)	Pre-treatment proteinuria (mg/24 h)	Post-treatment proteinuria (mg/24 h)
fLZR (n = 9)	236 ± 8	256 ± 3	1.18 ± 0.18	2.86 ± 0.3
fOZR (n = 8)	511 ± 13 ^{&}	574 ± 15 ^{&}	4.71 ± 0.76 ^{&}	13.6 ± 3 ^{&}
mLZR (n = 6)	484 ± 5 ^{&}	641 ± 2 [#]	13.9 ± 5 [#]	11.96 ± 4 [#]
mOZR (n = 6)	575 ± 15 [*]	785 ± 36 [*]	131.5 ± 9.7 [*]	141 ± 20 [*]

^{*}
p < 0.05 vs. mLZR, fOZR, and fLZR.

[#]
p < 0.05 vs. fOZR and fLZR.

[&]
p < 0.05 vs. fLZR.

Table 2

Sex steroid measurements in female and male obese and lean Zucker rats at 32–34 weeks of age.

	Plasma testosterone (ng/dL)	Plasma estradiol (pg/mL)
fLZR (n = 9)	0.041 ± 0.02 [#]	20.6 ± 4 [*]
fOZR (n = 7)	0.040 ± 0.03 [#]	8.47 ± 3 [#]
mLZR (n = 6)	15.7 ± 1 [*]	1.61 ± 0.74
mOZR (n = 6)	2.2 ± 0.21	0.91 ± 0.14 [#]

^{*} p < 0.05 vs. mLZR, fOZR, and fLZR.

[#] p < 0.05 vs. mOZR.