Brief Communication

Sex differences in cardio-metabolic and cognitive parameters in rats with high-fat diet-induced metabolic dysfunction

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Impact statement

Excessive dietary fat intake plays important roles in the process of metabolic dysfunction and increases susceptibilities to chronic diseases such as hypertension. Few previous studies, however, have accurately reflected real-world medical conditions. In addition, studies performed to date have not examined detailed sex-differences in cardio-metabolic and cognitive parameters, precluding the development of sex-tailored interventions for patients with metabolic dysfunction who are susceptible to hypertension and cognitive impairment. In this study, using rats with HFD-induced metabolic dysfunction that made them susceptible to hypertension and cognitive impairment, we demonstrate that male rats show greater impairment of acetylcholine-induced vasorelaxation of the carotid artery and systolic blood pressure compared to female rats. These findings may provide a basis for the early detection of carotid artery dysfunction and systolic blood pressure increase, especially in males.

Abstract

Excessive dietary fat intake is related to metabolic dysfunction and enhances susceptibility to hypertension and cognitive impairment. Although there are sex differences in the prevalence and progression of these diseases, few studies have investigated sex differences in cardio-metabolic and cognitive parameters in rats with high-fat diet-induced metabolic dysfunction. To better reflect actual clinical conditions, sex-differences in rats with high-fat diet-induced metabolic dysfunction were evaluated. Male and female Sprague-Dawley rats were fed a high-fat diet to induce metabolic dysfunction and intraperitoneally injected with N-nitro-L-arginine methyl ester and scopolamine to model vulnerability to hypertension and cognitive impairment, respectively, whereas control rats were fed a regular diet and treated with distilled water and 0.9% saline. Male experimental rats showed significantly higher systolic blood pressure than female experimental animals. More importantly, acetylcholine-induced relaxation of carotid arteries was decreased only in the male experimental rats, revealing a significant difference compared with female experimental rats. These findings provide evidence for individualized sex-based management of patients with metabolic dysfunction and susceptibilities to hypertension and cognitive impairment.

Keywords: Sex-differences, high-fat diet, systolic blood pressure, acetylcholine-induced vasorelaxation

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Introduction

Excessive dietary fat intake plays an important role in increasing the risk of cardiovascular diseases,[1] type 2 diabetes,[2] and cognitive impairment.[3] Evidence has shown that a high-fat diet (HFD) itself is associated with the pathophysiology of hypertension,[4] diabetes,[5] and cognitive decline.[6] In real clinical settings, however, susceptibilities to hypertension and cognitive impairment interact with each in people with metabolic dysfunction who consume an HFD. For example, higher intake of dietary fat was shown to be related to more severe cognitive decline in subjects with than without type 2 diabetes,[7] and higher intake of dietary cholesterol and saturated fat was found to be associated with a greater risk of cardiovascular diseases in patients with than without type 2 diabetes.[8] Intake of fried foods was also shown to increase the incidence of metabolic syndrome.[9] Therefore, animal models reflecting these actual clinical conditions can more accurately help investigators to develop patient-tailored interventions.

One of the most important factors affecting metabolic dysfunction, hypertension, and cognitive impairment is sex. For example, the prevalence of diabetes mellitus was

higher in middle-aged men than in middle-aged women,[10] and the prevalence of hypertension was significantly higher in white men than in white women aged 20–51 years.[11] Cortical tau deposition was found to be more frequent in clinically normal adult women than men.[12] Few studies to date, however, have assessed whether sex affects cardio-metabolic and cognitive parameters in rats with HFD-induced metabolic dysfunction. This study therefore analyzed sex differences in cardio-metabolic and cognitive parameters in a rat model of HFD-induced metabolic dysfunction making them susceptible to hypertension and cognitive impairment.

Materials and methods

Animals and experimental protocol

This study was approved and conducted in accordance with guidelines of the committee of Korea University for institutional research and animal care (KUIACUC-2016–153). Six-week-old male and female Sprague-Dawley rats were purchased from Youngbio (Seongnam, Korea). Rats were acclimated to a 12:12-h dark:light cycle at 21–23°C with free access to standard chow and tap water for one week. After acclimation, male and female rats were separately assigned to two groups each (total of four groups) as follows: female controls ($n = 8$), female experimental rats ($n = 8$), male controls ($n = 6$), and male experimental rats ($n = 8$). Rats in experimental groups were intraperitoneally injected with 25 mg/kg N-nitro-L-arginine methyl ester (L-NAME) once daily[13] and fed an HFD ad libitum for two weeks. The HFD (Research Diets, Inc., New Brunswick, NJ, USA) was composed of 60% fat, 20% protein, and 20% carbohydrate (% of total kcal). In addition, 1 mg/kg scopolamine was intraperitoneally injected once daily for the last one week period.[14] Control rats were treated with the same volume of distilled water and 0.9% saline and fed standard chow, consisting of 13% fat, 27% protein, and 60% carbohydrate (% of total kcal). After measuring blood sugar (BS) and hematocrit, rats were anesthetized with isoflurane and decapitated. Serum was obtained by centrifuging blood at $3000 \times g$ for 20 min. The brain was removed, after which hippocampal and prefrontal tissues were separated immediately in ice-cold saline. The carotid artery and thoracic aorta were dissected for assessment of vascular tone. All samples were stored at −70°C for subsequent molecular assay. A detailed protocol is illustrated in Figure 1(a).

Measurement of blood pressure and heart rate

Systolic and diastolic BP and HR were measured 30 min after intraperitoneal injection of L-NAME and scopolamine using a noninvasive tail cuff recording system (CODA-6; Torrington, CT, USA).

Measurement of BS and hematocrit

BS was assessed using a glucometer (Barozen, Seoul, Korea). Hematocrit was determined by centrifuging heparinized blood in a capillary tube filled with blood to 75% of its length and sealed with plasticine. Centrifuging at $1500 \times g$ for 5 min separated blood into compacted blood cell and plasma layers, from which hematocrit (compacted layer/total) was calculated.

Measurement of serum corticosterone and cholesterol levels

Serum concentrations of corticosterone and cholesterol were assessed by sandwich enzyme-linked immunosorbent assay (ELISA) using a corticosterone ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) and a high-density lipoprotein/low-density lipoprotein cholesterol assay kit (Cell Biolabs, San Diego, California, USA), respectively, according to the manufacturers' instructions.

Measurement of acetylcholinesterase activity

AChE activity in the hippocampus was assessed using an AChE colorimetric assay kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. Absorbance at 540 nm was measured immediately using a spectrophotometer (BMG Labtech, Ortenberg, Germany).

Measurement of S100B and brain-derived neurotrophic factor

S100B, a biomarker for neuronal distress,[15] and BDNF, an important regulator of synaptic plasticity,[16] were assessed by Western blotting. Prefrontal tissue from the rat brain was homogenized, and protein concentrations in the lysate were adjusted to 30 μg. Samples were separated by SDS-PAGE on 12% gels, and then electrophoretically transferred to a nitrocellulose membrane which was incubated overnight with primary anti-S100B (Thermo-Fisher, San Jose, CA, USA), anti-BDNF (Abcam, Cambridge, UK), and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) primary antibodies. After incubation with anti-rabbit IgG HRP-linked secondary antibody for 1 h, signals were visualized using an ECL Plus Western blot detection kit (Bio-Rad, Hercules, CA, USA), then analyzed using NIH Image J software.

Wire myography

For measuring vascular tone, carotid arteries and thoracic aortas from rats were cut into 2-mm segments and mounted on vascular rings in an organ bath (Danish Myo Technology, Aarhus, Denmark) with continuously oxygenated fresh Krebs solution. Vascular rings were equilibrated at a resting tension of 0.8–1.0 g, after which vasoconstriction was evoked by bath application of 10 μM phenylephrine (PE). After reaching maximum contraction, maximal vasorelaxation was induced by adding 10 μM ACh. ACh-induced vasorelaxation was calculated using the formula: ACh-induced vasorelaxation (%) = absolute value of ACh-induced vasorelaxation/absolute value of PE-induced vasoconstriction × 100.

Chemicals

L-NAME, scopolamine, PE, and ACh were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-NAME was

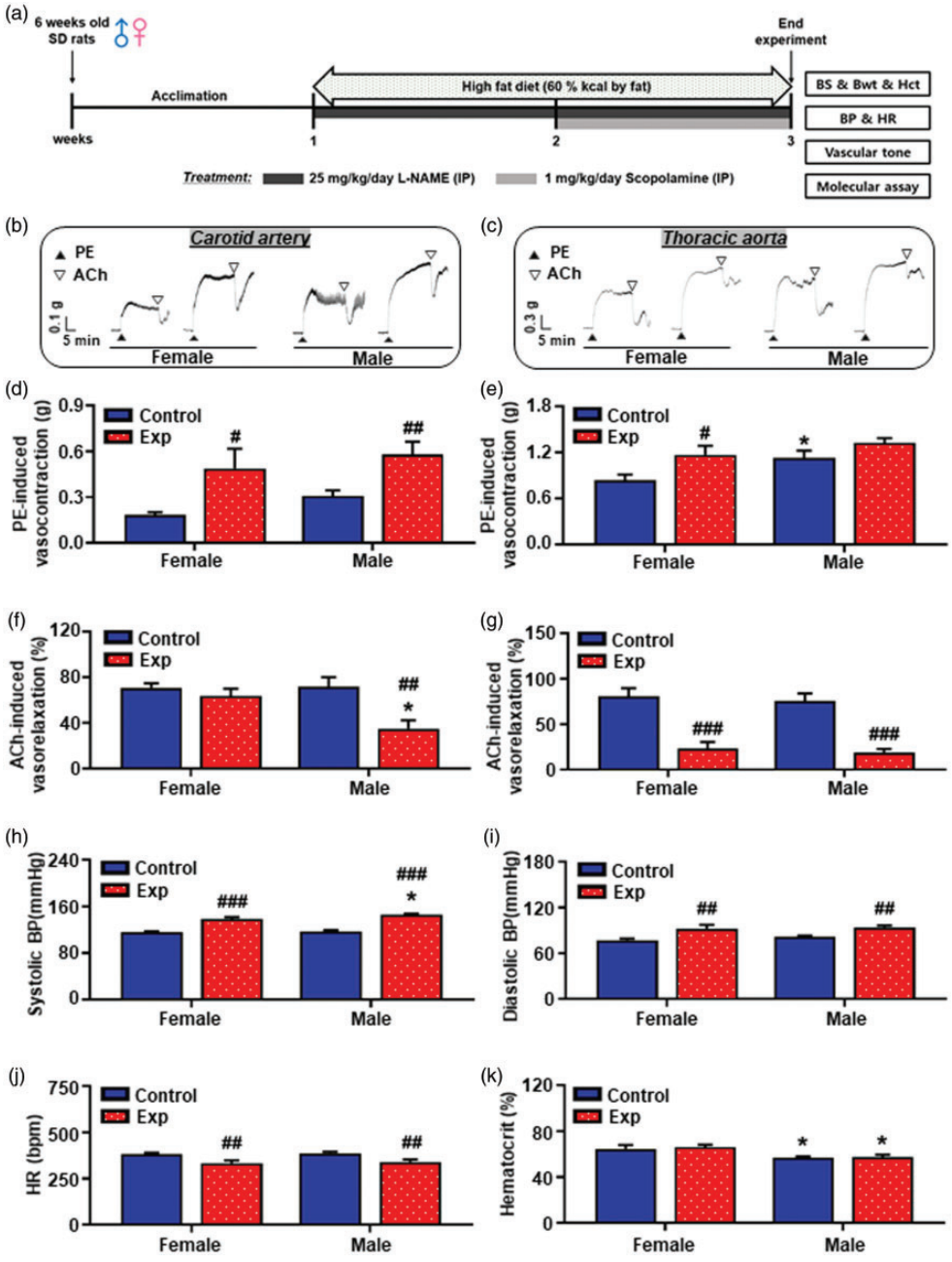

Figure 1. (a) Experimental scheme. (b) and (c) Representative wire myography traces. (d) and (e) PE-induced contraction of the carotid artery and thoracic aorta, respectively. (f) and (g) ACh-induced vasorelaxation responses to PE-induced vasoconstriction of the carotid artery and thoracic aorta, respectively. (h) Systolic BP. (i) Diastolic BP. (j) HR. (k) Hematocrit. Results are presented as means ± SEM ($n = 3$–8 per group). $^{*}P < 0.05$ vs. respective females; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. respective controls by one-way ANOVA followed by post hoc LSD. Abbreviations: ACh: acetylcholine; BP: blood pressure; Exp: experimental; HR: heart rate; PE: phenylephrine. (A color version of this figure is available in the online journal.)

dissolved in distilled water, whereas scopolamine was dissolved in 0.9% saline.

Statistical analysis

Results are reported as means ± SEM. Variables were analyzed by one-way analysis of variance (ANOVA) followed by a *post hoc* LSD test. All statistical analyses were performed using IBM SPSS Statistics version 22 (Chicago, IL, USA). A P-value < 0.05 was considered statistically significant.

Results and discussion

This study aimed to identify the sex-specific pathophysiological features in rats with HFD-induced metabolic dysfunction and susceptibility to hypertension and cognitive impairment. Rats in the experimental group were exposed to HFD to mimic metabolic dysfunction, and were treated with L-NAME and scopolamine to model vulnerability to hypertension and cognitive impairment, respectively. Endothelial dysfunction contributes to vascular remodeling and stiffening, resulting in hypertension, which has

deleterious effects on brain health and the neurovascular network.[17] We found that ACh-induced relaxation of the carotid artery was significantly lower in experimental male rats than in experimental female rats, suggesting a protective effect of female sex (Figure 1(f)). This is consistent with previous reports that 17β-estradiol increases the activity of endothelial nitric oxide synthase and enhances the release of nitric oxide in cerebral and peripheral endothelial cells.[18] Interestingly, in male rats, ACh-induced vasorelaxation was decreased in both the thoracic aorta and carotid artery, whereas in females, ACh-induced vasorelaxation was decreased only in the thoracic aorta. As the number of cardiovascular risk factors increases, the correlation between aortic stiffness and carotid artery stiffness becomes weaker, demonstrating that the aorta stiffens more than the carotid artery.[19] Systolic BP was also significantly higher in male experimental than in female experimental rats (Figure 1(h)). Therefore, we speculated that female rats

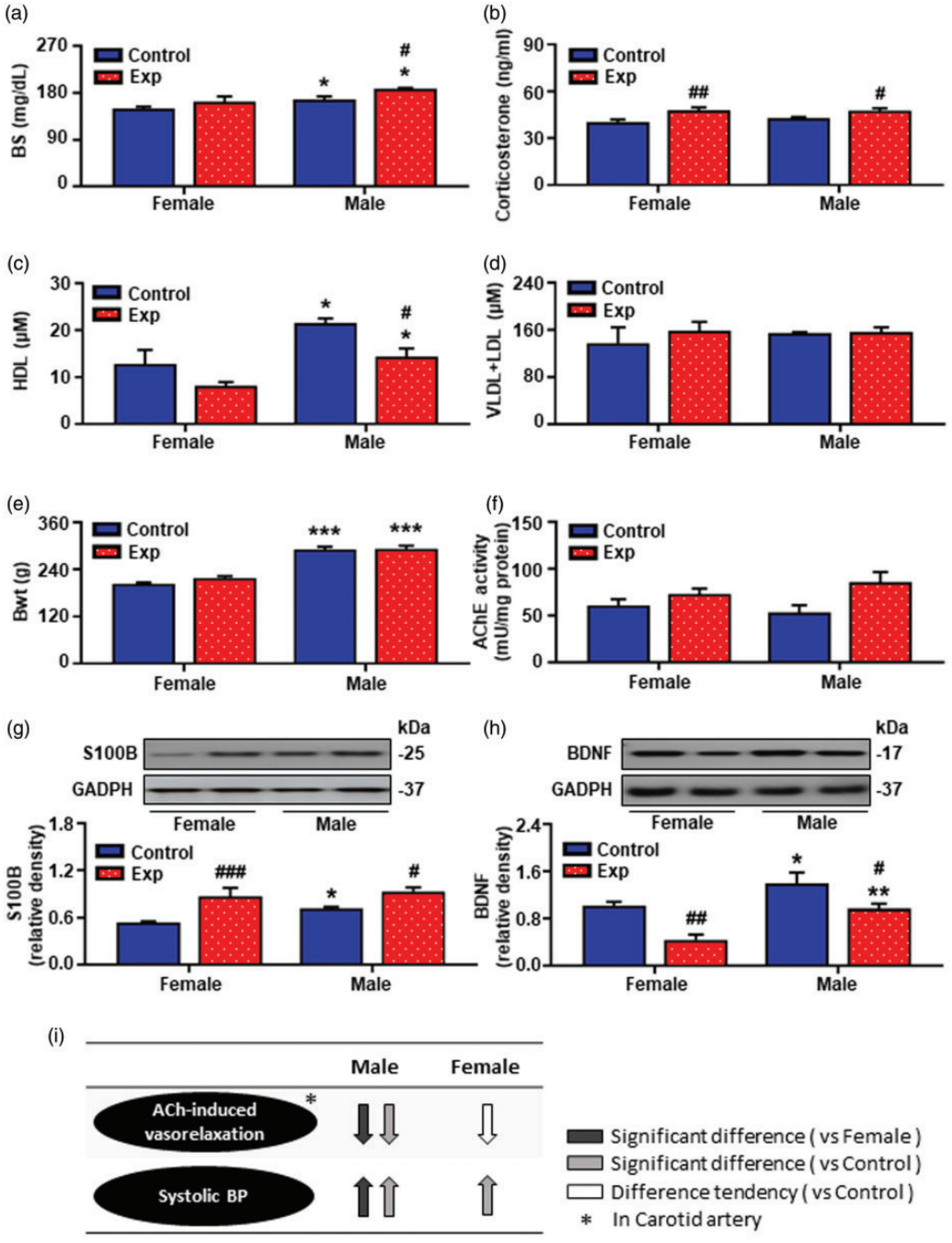

Figure 2. (a) BS. (b) Serum corticosterone. (c) Serum HDL. (d) Serum VLDL + LDL. (e) Body weight. (f) AChE activity. (g) Expression of S100B relative to GADPH. (h) Expression of BDNF relative to GADPH. (i) Summary of key findings in rats with HFD-induced metabolic dysfunction. Results are presented as means ± SEM (n = 4–8 per group). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. respective females; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. respective controls by one-way ANOVA followed by *post hoc* LSD. Abbreviations: AChE: acetylcholinesterase; BDNF: brain-derived neurotrophic factor; BS: blood sugar; Exp: experimental; GADPH: glyceraldehyde 3 phosphate dehydrogenase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein. (A color version of this figure is available in the online journal.)

showed a decrease in ACh-induced vasorelaxation only in the aorta because their systolic BP was lower than that in male rats.

BP is regulated by numerous factors. It is well known that premenopausal women have a lower risk of hypertension compared to men because estrogen prevents against BP increase. This suggests that vasodilating factors in female rats, such as estrogen and progesterone, were probably sufficient to protect carotid arteries. Because hematocrit, an indicator of whole blood viscosity, did not differ in the control and experimental groups (Figure 1(k)), the significant increase in BP in male experimental rats could be partly attributable to changes in vascular tone. HR was significantly lower in experimental animals than in controls (Figure 1(j)), possibly reflecting compensatory mechanisms that protect against hypertension.

Serum corticosterone levels were higher in experimental than in control rats (Figure 2(b), indicating elevated stress levels despite the absence of an imposed stress. In addition to physical/psychological stress, dietary lipids[20] have been reported to alter circulating corticosterone levels. Cortisol concentrations were found to be significantly higher in patients with major depression than in healthy subjects, suggesting that cortisol concentration is a strong predictor of cognitive function.[21] Moreover, urinary free cortisol levels were higher in diabetic patients with complications than in diabetic patients without complications and in non-diabetic subjects. These findings suggest that the susceptibility of experimental rats in our study to metabolic dysfunction, hypertension, and cognitive impairment may result from a stress response.[22]

In contrast to previous studies, which reported increased body weight after intake of a HFD, we observed no significant difference in body weight between control and experimental rats (Figure 2(e)). This discrepancy may have been due to the relatively short duration of experiment in our study. Body weight of five-week-old rats was found to increase significantly after feeding with a diet containing 58.3% fat,[23] with body weight gain being greater in males than in females,[24] a finding consistent with our results. We also observed a difference in baseline HDL concentrations in males and females (Figure 2(c)), consistent with animal studies reported that circulating HDL concentrations are higher in males than in females.[25] Although HDL concentrations tended to be lower in female experimental than in female control rats, HDL concentrations were significantly lower in male experimental than in male control rats. This finding may be clinically meaningful, because reduced HDL can affect inflammatory responses associated with the pathogenesis of atherosclerosis.[26]

We also investigated factors related to cognitive function. Both male and female experimental rats showed a tendency toward increased AChE activity, although this difference did not reach statistical significance (Figure 2 (f)). We observed differences between male and female rats in baseline S100B (Figure 2(g)) and BDNF (Figure 2 (h)) levels, findings consistent with reports showing that baseline *BDNF* gene expression was significantly higher in male than in female rats.[27] Moreover, serum S100B levels were found to be higher in healthy male than female participants.[28] Regardless of sex, S100B was significantly increased in experimental animals compared with controls, whereas BDNF was significantly decreased. Circulating S100B levels were significantly higher in patients with essential hypertension than in healthy subjects,[29] and chronic administration of corticosterone to rats has been reported to significantly reduce mature BDNF levels in the hippocampus.[30] Therefore, these results suggest that neuronal vulnerabilities in the brains of experimental rats may be affected by changes in hemodynamic factors or corticosterone levels.

In summary, we sought to identify sex-differences in cardio-metabolic and cognitive parameters in a rat model of HFD-induced metabolic dysfunction and susceptibility to hypertension and cognitive impairment. We found that male experimental animals were more vulnerable to changes in ACh-induced vasorelaxation of the carotid artery and systolic BP than female experimental animals, suggesting the importance of earlier intervention in males to manage these factors compared with females. These results highlight the importance of sex-tailored care in patients with metabolic dysfunction and susceptibility to hypertension and cognitive impairment.

Authors' contributions: YKS, SK, and GHS conceived and designed the experiments; YKS, YSH, AYH, SK performed the experiments; YKS, YSH, AYH, SK and GHS analyzed the data; YKS, YSH, AYH, and GHS wrote the paper; GHS supervised the project. All authors have read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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