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Effects of high-fat diet on sympathetic neurotransmission in mesenteric arteries from Dahl salt-sensitive rat

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ABSTRACT

Obesity hypertension is driven by sympathetic neurotransmission to the heart and blood vessels. We tested the hypothesis that high-fat diet (HFD)-induced hypertension is driven by sympathetic neurotransmission to mesenteric arteries (MA) in male but not female Dahl salt-sensitive (Dahl ss) rat. Rats were fed a control diet (CD; 10 kcal% from fat) or HFD (60 kcal% from fat) beginning at 3 weeks (wk) of age; measurements were made at 10-, 17- and 24-wk. Body weight increased with HFD, age and sex. Mean arterial pressure (MAP) was higher in HFD versus CD rats from both sexes at 17- and 24-wk. MA constriction measured using pressure myography, and electrical field stimulation (EFS, 0.2–30 Hz) was greater in HFD versus CD in males at 17-wk; this was not due to changes in α2 autoreceptor or norepinephrine transporter (NET) function. Prazosin (α1-AR antagonist) and suramin (P2 receptor antagonist) inhibited neurogenic MA constriction equally in all groups. Arterial reactivity to exogenous norepinephrine (NE; 10^{-8} - 10^{-5} M) was lower in HFD versus CD at 10-wk in males. Female MA reactivity to exogenous ATP was lower at 24-weeks compared to earlier time points. HFD did not affect tyrosine hydroxylase (TH) or the vesicular nucleotide transporter (VNUT) nerve density in MA from both sexes. NE content was lower in MA but higher in plasma at 24-wk compared to 10- and 17-wk in both sexes. In conclusion, HFD-induced hypertension is not driven by increased sympathetic neurotransmission to MA in male and female Dahl ss rats.

1. Introduction

Obesity is a risk factor for hypertension (Hall et al., 2015) and increased sympathetic nervous system (SNS) activity can contribute to obesity-associated hypertension (da Silva et al., 2009). Mechanisms responsible for SNS effects on obesity-associated hypertension include impaired regulation of sympathetic nerve activity (da Silva et al., 2009; Esler et al., 2006), increased vascular reactivity to sympathetic neurotransmitters (Jerez et al., 2012; Sivitz et al., 2007) and increased sympathetic neurotransmission (Julius et al., 2000; Kalil and Haynes, 2012). Obese Sprague Dawley (Haddock and Hill, 2011; Mui et al., 2018), Deoxycorticosterone acetate (DOCA)-salt (Kandlikar and Fink, 2011; Luo et al., 2004; Park et al., 2010; Mui et al., 2018), and spontaneously hypertensive (SHR) rats (Bencze et al., 2016; Judy et al., 1976) are commonly used to study sympathetic neurotransmission in hypertension. Similarly, Dahl salt-sensitive (ss) rats are widely used to study ss-hypertension (Gillis et al., 2015; Takahashi et al., 2017). Recent studies describe the effect of a high fat diet (HFD) on blood pressure in Dahl ss rats (Fernandes et al., 2018; Nagae et al., 2009; Zhang et al., 1999). However, there have been no studies of sympathetic neurotransmission in resistance arteries from Dahl-ss rats.

Sympathetic nerves regulate vascular tone in the mesenteric blood vessels. This conclusion is based on studies showing that celiac ganglionectomy (King et al., 2007; Li et al., 2010) and ganglionic blockade (Fink et al., 2000) can reduce blood pressure. Sympathetic neurotransmission in mesenteric arteries (MAs) is mainly mediated by norepinephrine (NE) and ATP co-released from periarterial sympathetic nerve varicosities. Transmitter binding to adrenergic and purinergic receptors causes arterial constriction (Townsend et al., 2016). NE and ATP release is regulated through negative feedback inhibition by prejunctional α2-AR. Impaired α2-adrenergic receptor (AR) in DOCA-salt rats is known to increase NE and ATP release from sympathetic nerves (Park et al., 2010; Mui et al., 2018) resulting in a greater vasoconstriction. Moreover, the availability of NE in the neuroeffector junction

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depends on its clearance by the norepinephrine transporter (NET) located at the sympathetic nerve varicosities, and blockade of NET leads to greater vasoconstriction (Park et al., 2006). Nevertheless, the specific impact of HFD on these control mechanisms, and on vascular reactivity, is not established in Dahl ss hypertensive rats.

Sex differences in the impact of SNS activity on obesity-associated hypertension have been studied previously (Faulkner and Belin de Chantemele, 2018), with most results supporting a larger impact in male compared to female rats. While obesity-associated changes in SNS activity and vascular reactivity have been studied extensively, only one published study specifically explored sympathetic neurotransmission in obesity-associated hypertension (Haddock and Hill, 2011) and none have addressed possible sex differences. A recent study from our group showed that sympathetic nerves contribute to hypertension in HFD-fed male rats but not in HFD-fed hypertensive female rats. These studies demonstrated a sex difference in sympathetic nervous system control of HFD-induced hypertension (Fernandes et al., 2018). Therefore, the goal of the work reported here was to evaluate sympathetic neurotransmission in MA from Dahl ss rat. We studied sympathetic neurotransmission in arteries from the mesentery (neurovascular transduction) because of the importance of the splanchnic circulation in overall regulation of blood pressure. Specifically, we tested the hypothesis that HFD-induced hypertension is driven by sympathetic neurotransmission to MA in male but not female Dahl ss rats.

2. Materials and methods

2.1. Chemicals

NE, ATP disodium salt hydrate, prazosin, and suramin were acquired from Sigma-Aldrich (St. Louis, MO, USA). Tetrodotoxin (TTX) was bought from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Animals

All animal use protocols were approved by the Institutional Animal Care and Use Committee at Michigan State University (AUF# 10/17-179-00). Dahl ss rats are genetically salt-sensitive but in our study all rats were fed normal salt diet as we focused on the effects of HFD on blood pressure. Three week old male and female Dahl ss rats (initial weight = 60–75 g, Charles River Laboratories, Portage, MI) were fed either a CD (Research Diets #D12450J: 10 kcal% fat, 0.24% NaCl, 0.36% K^+, 70 kcal% carbohydrate, and 20 kcal% protein) or a HFD (Research Diets #D12492: 60 kcal% fat, 0.33% NaCl, 1% K^+, 20 kcal% carbohydrate, and 20 kcal% protein) with ad libitum access to food and tap water. Rats were randomly assigned to either diet. All rats were housed in groups of two or three in transparent plastic cages in a controlled humidity and temperature room with 12:12-h light-dark cycles. Animals were used after 10, 17 or 24-wk on the control or HFD and were fasted for 12 h overnight before euthanasia. These time-points were chosen because telemetrically measured blood pressure in our previous studies began to separate between CD and HFD rats at 10-wk, and exhibited significant differences at 17-wk which continued until 24-wk when blood pressure no longer changed at high rate (Fernandes et al., 2018). Rats were euthanized with a lethal dose (4%) of isoflurane by inhalation. Tail and leg pinch were used to confirm unconsciousness of rats. Tail vein blood samples and MA were harvested from euthanized rats. Blood was collected in heparin-coated tubes and centrifuged at 4000 rpm for 15 min at 4 °C. Then, plasma was collected and stored at −80 °C until further use.

2.3. Body weight (BW) and mean arterial pressure (MAP)

Body weight was measured weekly and right before euthanasia. MAP was measured weekly using the tail-cuff plethysmography method (CODA High Throughput, Kent Scientific, CT, USA).

2.4. Tissue preparation and video monitoring of MA diameter

Third order small intestinal MA (inner diameter; male = 275–350 μm, female = 245–315 μm) were isolated from euthanized rats and were pinned flat on a Sylgard®-lined petri dish (Dow Corning, Midland, MI) filled with Krebs solution (in mmol, pH 7.4: 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 11 glucose and 25 $NaHCO_3$). Surrounding perivascular fat and connective tissue were carefully removed and an arterial segment was transferred to the recording chamber where each end was tied to micropipettes in a pressure myograph chamber (Danish Myo Technology, 114P, DMT, Denmark) containing Krebs solution. The myograph system kept MA at a constant pressure (60 mmHg), temperature (37 °C) and tissue oxygenation (95% O_2 and 5% CO_2). A video camera was positioned underneath the chamber, and the pressure myograph was connected to a computer. Myoview II software (DMT, Denmark) allowed continuous monitoring of arterial diameter changes in response to drugs or EFS. At the beginning of each experiment, the viability of MA was tested with NE (10^{-5} M) that produced close to 100% constriction where the arterial lumen was almost completely closed.

2.5. Transmural electrical field stimulation (EFS) of periarterial sympathetic nerves

Mounted MA were positioned in the myograph chamber between two parallel wire electrodes which were connected to a stimulator (Grass Instruments S48, Quincy, MA, USA). Stimulation parameters were 100 V, 0.5 ms pulse duration and 30 stimuli per train with a frequency range of 0.2–30 Hz. The neurogenic response was validated by applying TTX (3×10^{-7} M) to block the electrically evoked maximum constriction to 20 Hz stimulation at the end of each experiment. TTX was added to the Krebs solution flowing through the recording chamber.

2.6. Adrenergic and purinergic transmission

Maximum constriction to 20 Hz stimulation was compared with response to the same stimulation at 20 Hz in the presence of: 1) prazosin (0.1 μM) to assess for adrenergic contributions to arterial constriction; 2) 0.1 μM prazosin and 100 μM suramin to assess purinergic component; and 3) 0.3 μM TTX to differentiate neurogenic from non-neurogenic constrictions.

2.7. Vascular reactivity to exogenous NE and ATP

Concentration-response studies were performed by cumulative addition of NE (10^{-8}–10^{-5} M) to flowing Krebs solution to assess adrenergic constriction mediated by α1-ARs in MA SMCs. Similar studies were performed by adding ATP, non-cumulatively, (10^{-6}–3×10^{-3} M) to the Krebs solution. This was to determine the purinergic constriction mediated by P2X and P2Y receptors expressed by arterial SMCs. There was a 10 min wash between addition of increasing concentrations of ATP to the Krebs solution. Responses are reported as a percent constriction determined by measuring the arterial outside diameter change during NE or ATP treatment divided by the baseline diameter multiplied by 100.

2.8. Immunohistochemistry and confocal imaging

We used a modified immunostaining procedure for MA as described previously (Mui et al., 2018). Briefly, connective tissue and perivascular fat were carefully removed from third-order MA harvested from euthanized rats. MA were fixed with Zamboni fixative (4% paraformaldehyde in 0.1 M phosphate buffer (PB) solution, pH 7.4) for 24 h. Tissues were transferred to 70% ethanol and stored at 4 °C before use. Tissues were washed with 0.1 M PB solution (pH 7.4) and incubated for

Table 1
Change in body weight and blood pressure with HFD, sex and age.

		Male			Female		
		10-wk	17-wk	24-wk	10-wk	17-wk	24-wk
BW (g)$^{\&}$	CD	**347** ± 5.3 (n = 19)	**409** ± $3.5^{\&}$ (*n* = 32)	**466** ± $5.9^{\&}$ (*n* = 29)	**224** ± 2.5 (*n* = 19)	**250** ± $4.7^{\&}$ (*n* = 20)	**273** ± $4.0^{\&}$ (n = 20)
	HFD	**374** ± 6.1^{*} (n = 19)	**458** ± $4.6^{*,\&}$ (*n* = 30)	**499** ± $6.5^{*,\&}$ (n = 32)	**242** ± 3.8^{*} (n = 19)	**272** ± $5.1^{*,\&}$ (n = 20)	**293** ± $3.9^{*,\&}$ (n = 20)
MAP (mmHg)	CD	**137** ± 2.5 (n = 21)	**125** ± 4.1 (*n* = 25)	**124** ± 5.6 (*n* = 18)	**122** ± 4.6 (n = 20)	**115** ± 4.6 (*n* = 21)	**142** ± $8.1^{\#,@}$ (*n* = 15)
	HFD	**138** ± 4.7 (n = 21)	**145** ± $4.0^{*,\#}$ (*n* = 26)	**155** ± $4.7^{*,\#,\%}$ (n = 20)	**121** ± 4.6 (n = 18)	**138** ± $5.5^{*,\#}$ (n = 19)	**162** ± 5.7^{*} (*n* = 16)

*Indicates significant difference between CD- and HFD groups. $^{\&}$Indicates increase in BW with age irrespective of diet in both sexes. $^{\#}$Indicates higher MAP at 17- and 24-wk compared to 10-wk in males on CD; $^{\%}$indicates higher MAP at 24- compared to 10-wk in males on HFD; $^{@}$indicates higher MAP at 24-wk compared to 10- and 17-wk in females on CD. *increased MAP with age in females on HFD.

10 min in dimethyl sulfoxide (DMSO). Tissues were washed again with 0.1 M PB solution and incubated in blocking solution (0.1 M PB solution, 4% goat serum, 4% donkey serum) for 1 h at room temperature. Tissues were further incubated for 12 h at 4 °C in blocking solution with primary antibodies targeting TH (1:200; MAB318, Millipore, Burlington, MA, USA) and the VNUT (1:1000; SC-86312, Santa Cruz Biotechnology, Dallas, TX, USA) to localize sympathetic nerve fibers. Tissues were washed with 0.1 M PB solution and incubated in blocking solution consisting of secondary antibodies (goat anti-mouse Alexa Fluor 488; 1:1000, Thermo Fisher Scientific, Waltham, MA, USA and donkey anti-rabbit Alexa Fluor 594; 1:1000, Thermo Fisher Scientific, Waltham, MA, USA) for 1 h at room temperature in a covered chamber to prevent photobleaching. Tissues were washed with 0.1 M PB solution and with de-ionized water before mounting them with a mounting media (B0730, Vector Laboratories, Burlingame, CA, USA). Finally, confocal *Z*-series images were acquired with 40× objective (0.75 N.A.) and combined using a C2 laser scanning microscope and NIS-Elements software (Nikon Instruments, Melville, NY, USA). Pictures were sampled in a non-systematic way from slides.

We also used a blocking peptide to verify the specificity of the VNUT antibody. The VNUT antibody was incubated on a shaker at a 1:200 dilution in 0.1 M phosphate buffer with the blocking peptide (SCBT, SC-86312 P) at 1:5 ratio in blocking buffer (0.1 M phosphate buffer, 0.2% Tx-100 with 4% donkey serum) overnight at 4 °C. The next day, MA were immunostained with the VNUT alone or with VNUT antibody with the blocking peptide overnight at 4 °C. Tissues were washed the next day with 0.1 phosphate buffer (4 × 5 min each) and then incubated with the donkey anti-rabbit Alexa 594 antibody (1:1000 dilution) for 1 h at room temperature. Samples were then washed in 0.1 M phosphate buffer (4 × 5 min each) and then mounted on slides with mounting media. Supplemental Fig. 1 shows that the blocking peptide prevented labelling of periarterial sympathetic nerve fibers, confirming selectivity of the VNUT antibody.

2.9. Nerve fiber counts

Five images were randomly taken from different regions of arteries obtained from each rat. ImageJ was used to lay a grid over each image of the same area (1262 × 1262 pixels). The nerves that cross the vertical and horizontal lines were counted and totaled for the images obtained from each rat. We than averaged the total fiber count obtained from each rat so the "n" values are mean + s.e.m. of the number of rats from which the images were obtained.

2.10. Tissue and plasma NE content

Briefly, MA were weighed, homogenized in 0.1 M perchloric acid in 4 to 1 ratio (perchloric acid/tissue or plasma sample by volume) and centrifuged at 15,000 *g* for 10 min. The supernatant was removed into a new tube and analyzed by HPLC. The HPLC electrochemical detector potential was set at −300 mV. Plasma samples were mixed with activated aluminum oxide solution that bonds to NE in Tris-EDTA buffer at a pH of 8.1 pH. After repeated washes to remove contaminants, 0.2 M acetic acid was added to recover NE. High-pressure liquid chromatography (HPLC) was used to measure NE level in MA and plasma from tail vein (under brief isoflurane anesthesia) using protocols described previously (Ayala-Lopez et al., 2015).

2.11. Data and statistical analysis

Frequency and concentration response graphs were curve-fitted using a log agonist vs response (3 parameter) dose response non-linear curve fitting routine. Grouped data were analyzed using a two-way ANOVA with Sidak's multiple comparison post hoc test was performed when comparing CD- and HFD-fed rats. One-way ANOVA was used to compare groups on the same diet but at different age. Data are presented as mean ± SEM. $P < .05$ was considered statistically significant. Data were analyzed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. Effects of HFD, age, and sex on BW and MAP

Body weight was greater in HFD compared to CD rats at 10-, 17-, and 24-wk in both sexes. It also increased with advancing age in both sexes on CD and HFD. Males gained more weight than females regardless of diet at all time points. MAP was greater in HFD compared to CD rats at 17- and 24-wk in both sexes. It was also higher at 24- vs. 10-wk in males on HFD. In addition, there was a significant increasing trend in MAP in female rats with age (Table 1).

3.2. Effects of HFD, age, and sex on nerve-mediated vasoconstriction

Frequency response curves for EFS (0.2–30 Hz) were used to compare nerve-mediated constriction of MA from male (Fig. 1a) and female (Fig. 1b) rats. Neurogenic constriction was greater in HFD compared to CD rats in 17-wk males but not females (Fig. 1c, d). Female (but not male) rats exhibited higher maximum response (Emax) and lower half maximal frequency (S_{50}) at 17-wk compared to 10- and 24-wk, but this was not affected by diet.

3.3. Effects of HFD, age, and sex on adrenergic and purinergic vasoconstriction

Adrenergic and purinergic components of neurovascular coupling were determined by comparing constrictions in the presence of prazosin and suramin to the baseline response at 20 Hz EFS (Fig. 2a, b, c). Almost 90% of the constriction was blocked by TTX confirming that the constriction was neurogenic (Fig. 2d). The proportion of adrenergic constriction was not different between HFD and CD rats. Furthermore, there was no age difference in adrenergic proportion (not shown). The proportion of purinergic constriction was also similar between HFD and CD rats. However, it was lower at 24-wk compared to 10- and 17-wk in

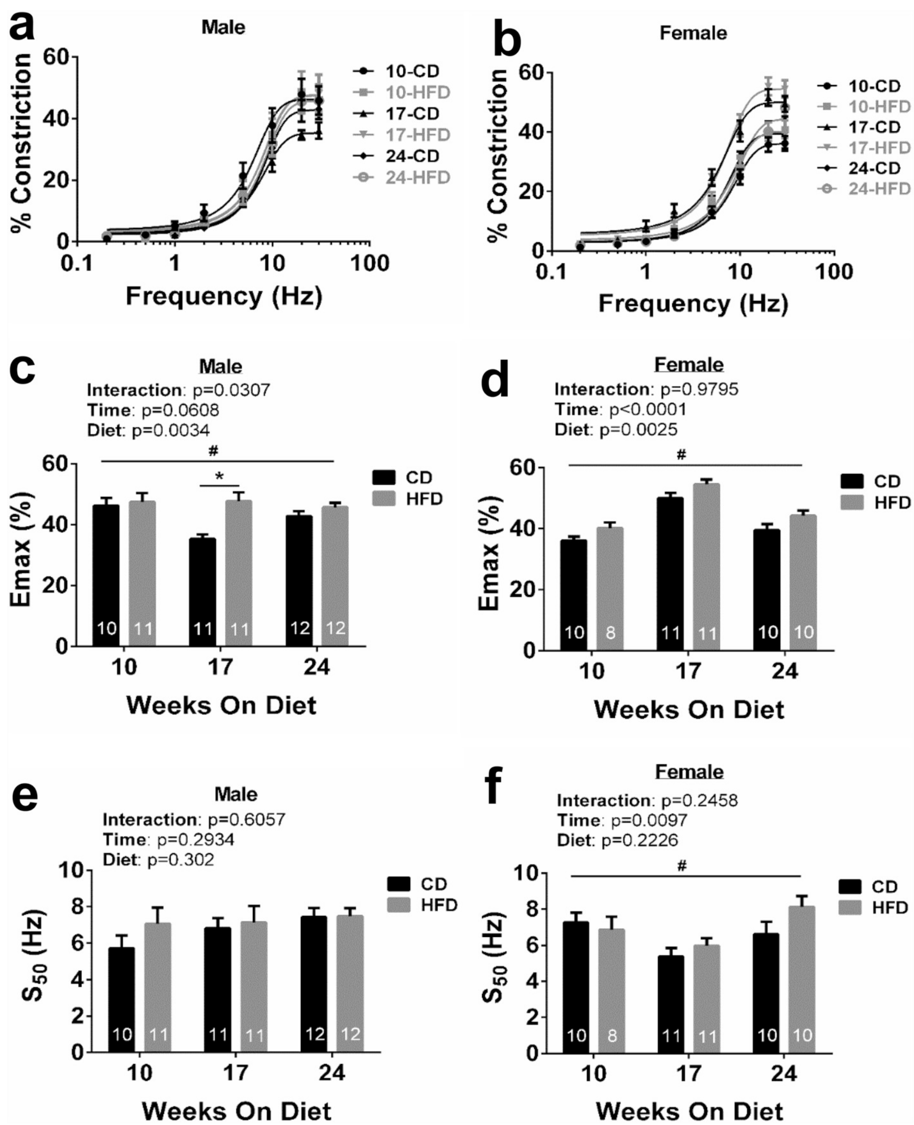

Fig. 1. Vasoconstriction in response to nerve stimulation. Frequency-response curves for a) male and b) female rats. c) *Emax was greater in 17 wk. HFD vs CD fed male rats. #Emax was lower in 17-wk vs 10- and 24-wk CD male rats. d) #Emax was higher in 17-wk compared to 10- and 24-wk female rats on CD and HFD. e) S_{50} was not affected by diet or age in MA from male rats. f) #S_{50} was lower in 17-wk compared to 10- and 24-wk female rats on CD and HFD.

males in both diets. In females, it was lower at 17-wk HFD compared to 10-wk in HFD (Fig. 2e, f).

3.4. Effects of HFD, age, and sex on vascular reactivity to exogenous NE and ATP

Concentration-response curves were plotted to determine the *E*max and concentration for half-maximum constriction (EC_{50}) in MA from male and female rats (Fig. 3a, b and 4a, b). *E*max was not different between HFD and CD rats in both sexes (not shown). EC_{50}, on the other hand, was higher in HFD compared to CD rats at 10-wk in males (Fig. 3c). EC_{50} was lower at 17-wk compared to 10- and 24-wk in males indicating a decrease in vascular reactivity to exogenous NE at mainly 24-wk. In addition, EC_{50} was greater at 24-wk compared to 17- and 10-wk in females (Fig. 3d). EC_{50} for exogenous ATP was greater in HFD- versus CD rats in 24-wk females. EC_{50} was greater at 24- compared to 10- and 17-wk in in both sexes (Fig. 4c, d) indicating a decrease in vascular reactivity to exogenous ATP with advancing age.

3.5. Effects of HFD, age, and sex on TH nerve density

TH-ir nerve fibers were counted in MA using confocal microscopy (Fig. 6a, b). There was no difference in TH nerve density in HFD and CD (Fig. 6c) in males. Similarly, the was no difference in TH nerve density between HFD and CD in females. However, TH nerve density was greater in CD at 24-wk compared to CD at 10-wk in females (Fig. 5d).

3.6. Effect of HFD, age, and sex on VNUT nerve density

VNUT-ir nerves were imaged and counted to assess nerve density in MA (Fig. 6a, b). VNUT nerve count was greater at 24-wk compared to 17-wk in HFD-fed male rats (Fig. 6c). In female rats, VNUT-ir nerve density is higher at 24-wk compared to 10- and 17-wk in CD. Moreover, VNUT-ir nerve density is greater at 24-wk compared to 17-wk in HFD (Fig. 6d).

3.7. Effect of HFD, age, and sex on NE level in MA and plasma

NE levels in MA were lower at 24-wk compared to 10- and 17-wk in male and female rats. NE plasma levels were higher at 24-wk compared

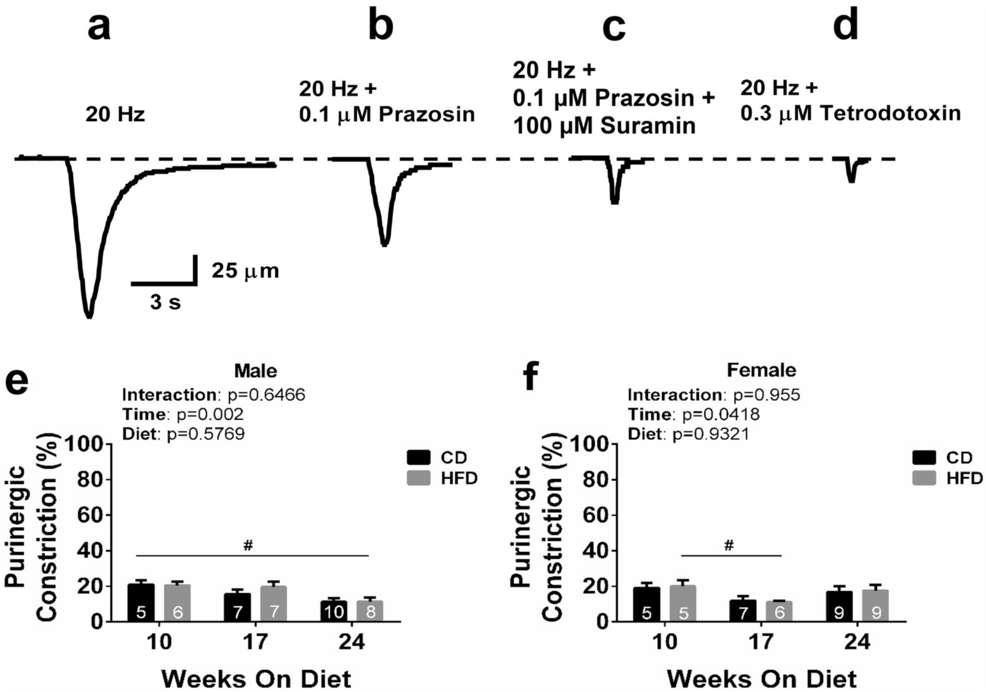

Fig. 2. Contribution of purinergic constriction. Representative nerve stimulation responses: a) 20 Hz. b) 20 Hz + 0.1 μM Prazosin (α1-AR antagonist). c) 20 Hz + suramin (100 μM)(P2X/P2Y antagonist). d) 20 Hz + tetrodotoxin (0.3 μM, (Na^+ channel blocker). e) Purinergic proportion was not different between HFD and CD male rats. #Purinergic constriction was lower at 24- compared to 10-wk in males on both diets. f) Purinergic proportion was not different between HFD and CD female rats. #Purinergic constriction was lower at 17- vs. 10-wk in HFD females.

to 10- and 17-wk in male and female rats (Table 2). There was no significant effect of HFD NE levels in either sex.

4. Discussion

4.1. Summary of findings

Age, sex, and obesity affect blood pressure regulation and the development of hypertension (Buford, 2016; Leggio et al., 2017; Sandberg and Ji, 2012), but the mechanisms responsible are still being debated. Although one widely proposed mechanism is differences in sympathetic nervous system activity (da Silva et al., 2009; Straznicky et al., 2016), the objective of this study was specifically to investigate the possible differences in the effectiveness of sympathetic neurovascular coupling as an explanation. Relatively little attention has been given to possible changes in the effectiveness of neurovascular coupling in cardiovascular regulation because the process is difficult to evaluate in humans (Fink, 2018). We used a relatively new experimental model of hypertension which allowed us to analyze, separately and together, the impact of age, sex and obesity on blood pressure and sympathetic neurovascular coupling in resistance arteries. Our main findings were: 1) BW and MAP were significantly higher in HFD- compared to CD-fed rats in male and female rats; 2) neurogenic MA constriction was transiently higher in HFD- versus CD-fed males during hypertension development, but not in females; however, it was greater at 17-wk compared to 10- and 24-wk in female rats regardless of diet; 3) adrenergic and purinergic vasoconstriction were not affected by HFD, age or sex; 4) TH- and VNUT-ir nerve densities were not changed in HFD-

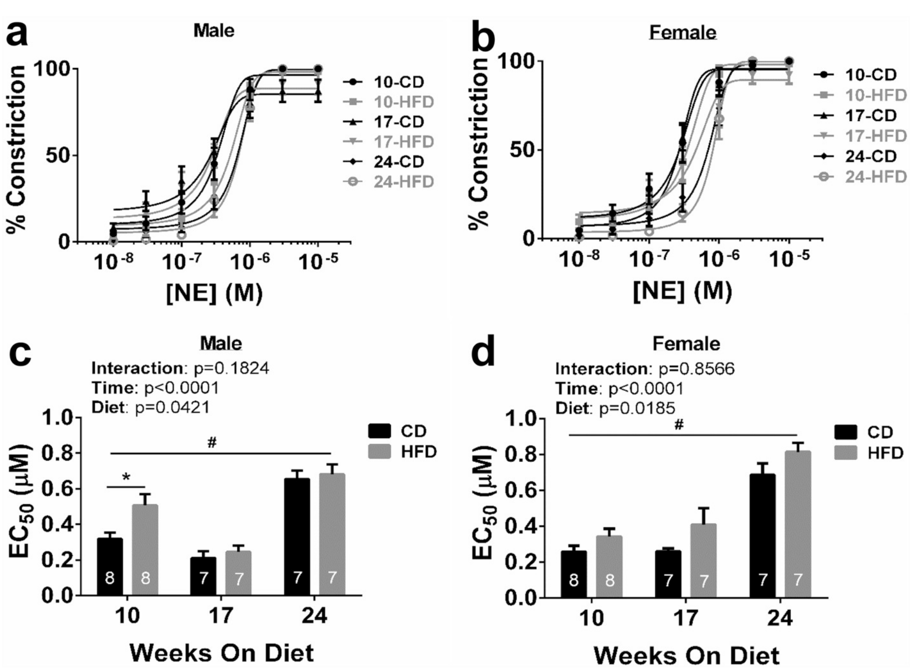

Fig. 3. Vascular reactivity to exogenous NE. a) NE concentration-response curves for males. b) NE concentration-response curves for females. c) *EC_{50} was higher in HFD compared to CD rats at 10-wk in males. #EC_{50} was higher at 24-wk contrasted with 17-wk regardless of diet. d) #EC_{50} was higher at 24-wk compared to 10- and 17-wk in female rats.

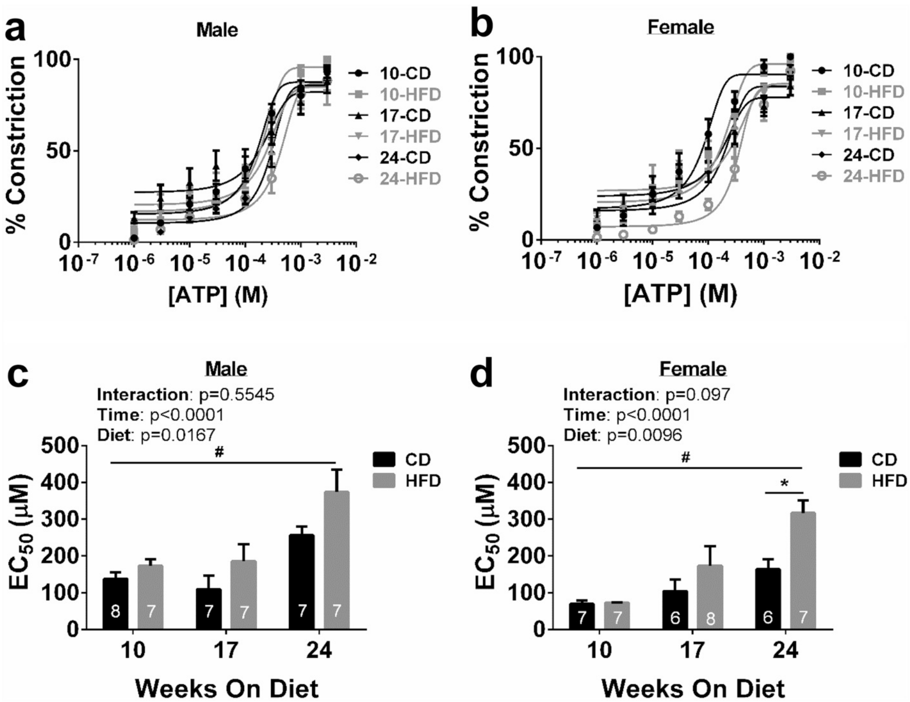

Fig. 4. Vascular reactivity to exogenous ATP. a) ATP concentration-response curves for males. b) ATP concentration-response curves for females. c) #EC_{50} was higher at 24-wk contrasted with 10- and 17-wk in male rats. d) *EC50 was greater in HFD compared to CD at 24-wk in females. #EC_{50} was higher at 24-wk compared to 10- and 17-wk in female rats.

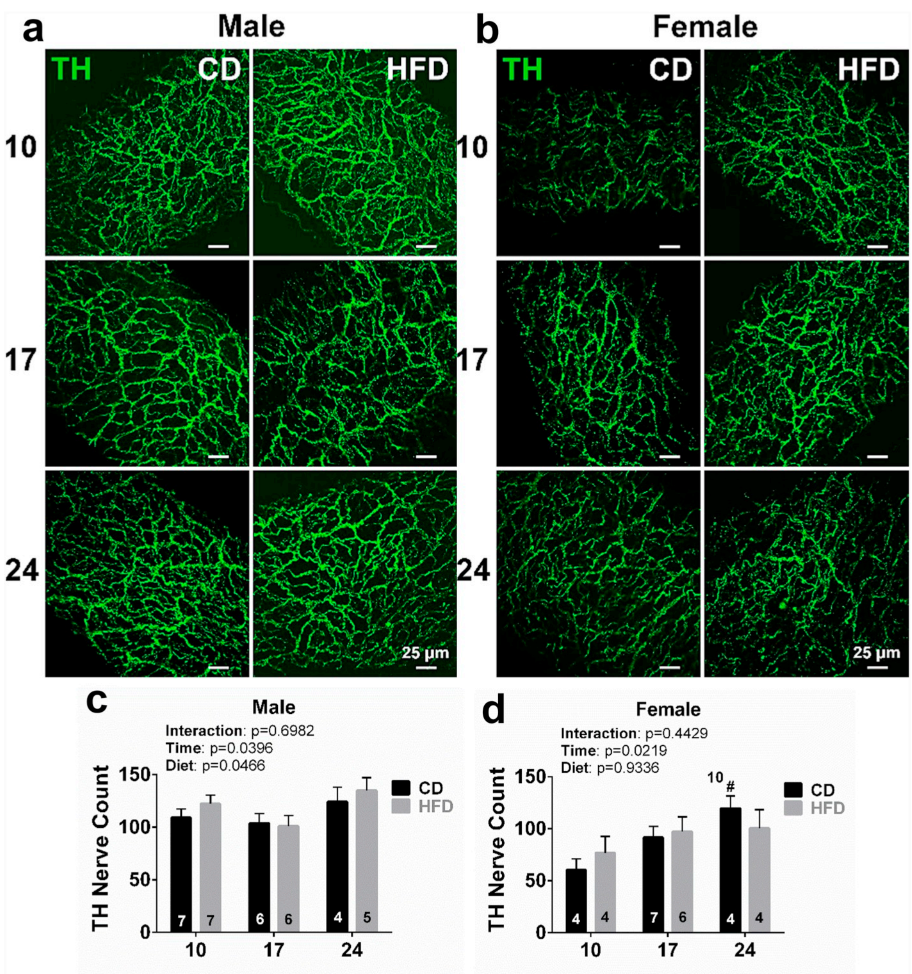

Fig. 5. Periarterial TH nerve density. a) TH-ir nerve fiber immunofluorescent images from male rats. b) TH-ir nerve fiber immunofluorescent images from female rats. c) TH-ir nerve fiber density from CD and HFD fed male rats and d) female rats at 10-, 17-, and 24-wks. #TH-ir nerve density is greater in CD at 24-wk compared to CD at 10-wk.

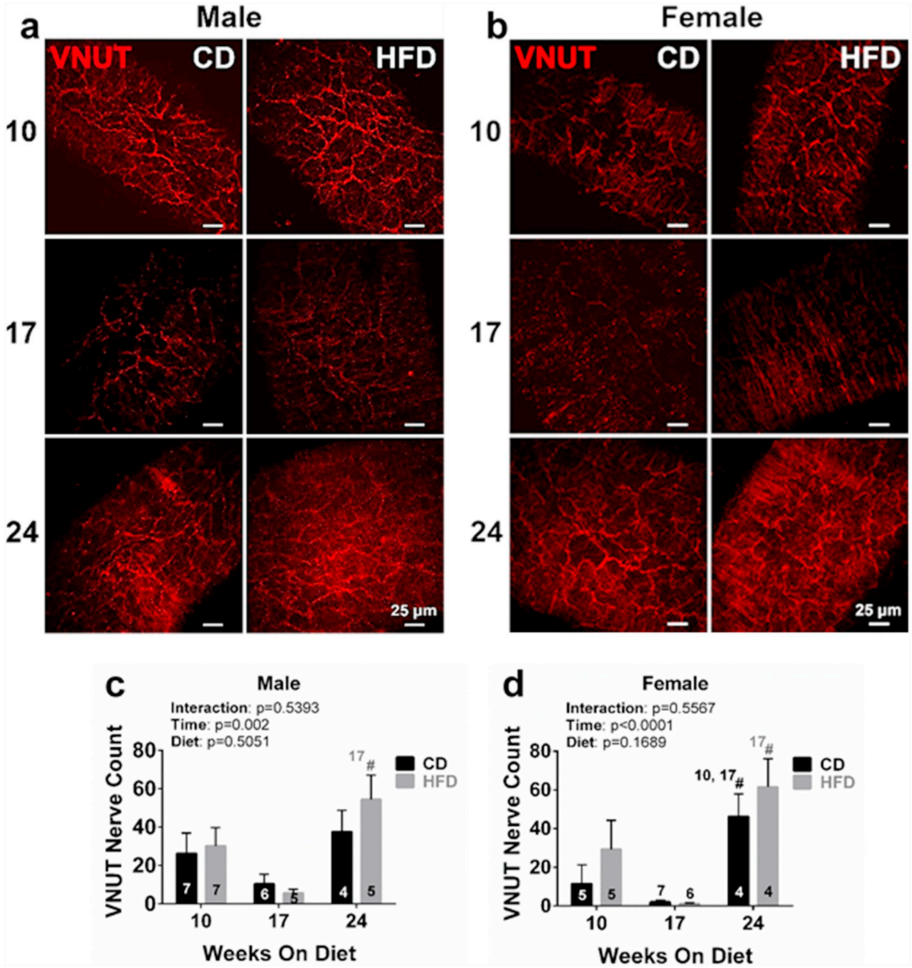

Fig. 6. Periarterial VNUT nerve density. VNUT-ir nerve fiber immunofluorescent images from a) male and b) female rats. c) VNUT-ir nerve fiber density MA from CD and HFD male rats at 10-, 17-, and 24-wks. # (grey)VNUT-ir nerve density is lower in HFD at 17-wk compared to HFD at 24-wk in males. d) VNUT-ir nerve fiber density in MA from CD and HFD fed female rats. #(black)VNUT-ir nerve density is higher at 24-wk compared to 10- and 17-wk in CD. # (grey)VNUT-ir nerve density is greater at 24-wk compared to 17-wk in HFD.

Table 2
HPLC measurement of tissue and plasma NE contents for male and female Dahl SS rats on CD and HFD for 10-, 17-, and 24-wk.

		Male			Female		
		10-wk	17-wk	24-wk	10-wk	17-wk	24-wk
Tissue NE (ng/g)	CD	**5127** ± 439 (n = 9)	**5519** ± 462 (*n* = 9)	**4259** ± 169 (n = 15)	**7036** ± 707 (*n* = 8)	**5788** ± 526 (*n* = 7)	**3608** ± 368* (*n* = 12)
	HFD	**4998** ± 457 (*n* = 13)	**5198** ± 261 (n = 12)	**3603** ± 199 (*n* = 11)	**5317** ± 243 (*n* = 10)	**4897** ± 225 (*n* = 6)	**3677** ± 348* (n = 13)
Plasma NE (pg/ml)	CD	**348** ± 19.6 (n = 10)	**362** ± 23.9 (n = 12)	**472** ± 20.4 (n = 7)	**571** ± 35.0 (n = 13)	**486** ± 36.6 (n = 7)	**597** ± 68.8* (*n* = 8)
	HFD	**286** ± 9.9 (n = 12)	**347** ± 16.0 (n = 13)	**498** ± 35.9 (n = 7)	**471** ± 20.9 (*n* = 16)	**478** ± 50.5 (n = 8)	**642** ± 47.1* (n = 7)

* Indicates significant difference between 24- and 17-wk, and 24- and 10-wk of the same diet.

compared to CD-fed rats of either sex; 5) NE content in MA was not affected by HFD, but there was a slight decrease at 24-wk compared to 10- and 17-wk in both sexes; 6) Plasma NE levels were higher at 24-wk compared to 10- and 17-wk in male and female rats and 7) vascular reactivity to exogenous NE and ATP was decreased with age in both sexes regardless of diet. In general, the data do not support the hypothesis that alterations in vascular sympathetic neurotransmission are responsible for obesity- or age- associated hypertension in our model in male or female rats.

4.2. Neurogenic constriction

In a previous study, a HFD was associated with increased nerve-mediated vasoconstriction in MA from male Sprague Dawley rats (Haddock and Hill, 2011). Female rats were not studied. Another study showed that EFS-induced adrenergic constriction was higher in obese male Wistar rats (Blanco-Rivero et al., 2011). Again, females were not studied. In our model, we showed amplified sympathetic neurotransmission to MA in HFD-fed male rats during the developmental phase of hypertension (17-wk), but not after hypertension was established. No differences between HFD versus CD fed rats were detected in female rats. Although it is by no means certain that changes in neurovascular coupling affect hypertension development in HFD-fed male Dahl ss rats, it is worth considering possible mechanisms underlying these changes.

4.3. Adrenergic nerves

TH-ir nerve fiber density was quantified as an additional way of assessing adrenergic neurotransmission in MA from control and HFD fed rats. Previous studies using HFD-fed Sprague Dawley rat showed an increase in renal TH expression (Matthews et al., 2017), and sympathetic nerve density in MA (Haddock and Hill, 2011). A similar outcome was reported in studies of MA from young and old SHR (Kawamura et al., 1989). Moreover, increased sympathetic nerve density in the brain from stroke-prone SHR male rats is associated with severe HTN (Smeda, 1990). However, we did not find a diet-related difference in TH nerve fiber density in the present study. Lack of effect of HFD on the adrenergic (TH) nerve density is consistent with the absence of HFD effect on NE content in MA. In contrast, NE content from MA is lower in other hypertensive rat models (Luo et al., 2003). To our knowledge the TH-ir nerve density was not previously studied in female MA. However, others have shown increased TH expression in the hypothalamus in males and in testosterone treated female rats compared to female rats fed a cafeteria diet (Plut et al., 2002). It's worth noting that total NE content was not higher in HFD-fed versus CD-fed rats. Taken together, both TH-ir nerve density which marks adrenergic nerves and the NE content within the adrenergic nerves were not changed with HFD in male or female rats. These data suggest that hypertension in the Dahl ss rat is not associated with changes in adrenergic neurotransmission.

4.4. Purinergic nerves

Purinergic neuroeffector transmission plays an important role in maintaining vascular tone (Burnstock and Ralevic, 2014; Townsend et al., 2016). A previous study demonstrated that increased sympathetic transmission is mediated partly by enhanced purinergic neurotransmission in obese male Sprague Dawley rats (Haddock and Hill, 2011). Furthermore, they showed that both adrenergic and purinergic components were augmented in obese rats at 3 and 5 Hz EFS. Interestingly, at a higher frequency (10 Hz) only the purinergic proportion was increased in obese rats indicating that purinergic transmission dominates at high frequency in obese male rats. However, in our study the purinergic component in obese male rats was stimulated at a higher EFS frequency (20 Hz) than what was used in the previous study (10 Hz). Despite this difference our study showed no differences in the adrenergic or purinergic components of the contractions between the HFD and control rats at any time. Similar results occur in female rats.

4.5. Vascular reactivity to exogenous NE and ATP

A study of the tail artery from diabetic rats showed decreased vascular reactivity to NE and ATP (Speirs et al., 2006), which is similar to our data. However, these data disagree with the increase in adrenergic reactivity previously reported in obese male Zucker rats (Stepp and Frisbee, 2002). Other studies showed no difference in MA reactivity obtained from male DOCA-salt versus sham treated rats or mice (Galligan et al., 2001; Luo et al., 2003; Perez-Rivera et al., 2004). Similarly, there was no difference in vasoconstriction (obese vs. control) to exogenous NE in MA from male Wistar rats (Blanco-Rivero et al., 2011). In regard to ATP, there are studies of the renal vasculature showing increased reactivity in male Wistar and SHR hypertensive rats that is inconsistent with our data (Fernandez et al., 2000; Harris, 1972). However, there are no similar studies in female rats. The discrepancy in the vascular response to direct nerve stimulation and to exogenous NE and ATP could be due to the following differences. First, when periarterial nerves are stimulated they release neuropeptide Y (NPY) along NE and ATP, and NPY is another vasoconstrictor known to be involved in HTN (Westfall, 2006). Thus, NPY enhances the vascular response to nerve stimulation compared to exogenously applied NE and ATP. Second, exogenous NE and ATP are dispersed throughout the tissue including to extra junctional receptors (Hotta, 1969; Itoh et al., 1983). Therefore, they will not have as quick and intense response as the neurotransmitters released within a very short distance from the post-junctional receptors.

4.6. Effect of age and sex on sympathetic neurotransmission

With regard to sex and aging effects on neurovascular transduction, a recent thorough study in humans demonstrated that neurovascular transduction increases with age in women but decreases in men (Briant et al., 2016). Another study showed no relationship between resting sympathetic activity and MAP or vascular conductance in young females in comparison to males (Robinson et al., 2019). In general, human studies support a gradual increase in the effectiveness of neurovascular coupling with age in women and a decrease in men; we did not observe this pattern in our model. This could represent a species-related effect. For example, EFS-induced adrenergic constriction was shown to decline with age in Fisher 344 female rats (Sullivan and Davison, 2001). Alternatively, methodological differences could be a factor, since all experimental studies were in isolated vessels in vitro, whereas human experiments were in vivo.

4.7. Indirect measure of sympathetic activity

We did not assess sympathetic control of blood pressure directly in these studies, but Dahl ss rats are generally considered to exhibit high sympathetic activity (Bayorh et al., 1998; Mark, 1991). Our only measure of sympathetic activity itself was plasma NE concentration, a relatively insensitive indicator. That measure showed no differences between HFD and CD rats, with similar small increments during aging in male and female animals. Thus, global sympathetic overactivity per se may contribute to hypertension development in Dahl ss rats but does not likely account for the difference in blood pressure between HFD and CD groups in males or females.

5. Conclusion

Development of HFD-induced hypertension in Dahl ss rats is similar in males and females, being of similar magnitude, gradual in onset and accompanied by relatively modest increments in body weight in both sexes. In males, but not females, HFD-induced hypertension may be driven in part by a transient and mild increase in neurovascular transmission. In females, however, a transient increase in neurogenic response is diet-independent. The mechanism by which HFD augments neurovascular coupling in males despite an aging related reduction in vascular reactivity to adrenergic transmitters cannot be identified with certainty. Overall, our findings do not support the hypothesis that HFD-induced hypertension is entirely driven by increase in sympathetic neurotransmission in MA in Dahl ss rat.

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Declaration of competing interest

None.

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