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Short-term hyper-caloric diet induces blunted aortic vasoconstriction and enhanced vasorelaxation via increased NOS3 activity and expression in Dahl salt-sensitive rats

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Abstract

Aim—To test the hypothesis that hyper-caloric diet on mechanisms of angiotensin (Ang)II-induced vasoconstriction in Dahl salt-sensitive (SS) rats and genetic control SS-13^{BN} rats.

Methods—Aortic function was assessed using wire myography in 16-week-old rats maintained on a normal diet or started on a 4-week hyper-caloric diet at 12-weeks-old.

Results—On normal diet, AngII vasoconstriction was greater in SS rats than SS-13^{BN} rats. Intriguingly, the AngII response was reduced in aortic rings from SS rats on hyper-caloric diet versus normal diet, whereas this response was not altered by hyper-caloric diet in SS-13^{BN} rats. We probed whether O₂[−], H₂O₂, or NO mediate the AngII response. PEG-SOD reduced the AngII response in all 4 groups. Catalase treatment of aortic rings did not alter aortic AngII response from SS rats on hyper-caloric but reduced the aortic AngII response of SS rats on normal diet; the exact opposite finding was observed with catalase in SS-13^{BN} rats. L-NAME increased AngII response in SS rats on hyper-caloric but was without effect in the normal diet group. In stark contrast, L-NAME did not alter the AngII response in SS-13^{BN} rats on hyper-caloric diet, yet L-NAME enhanced AngII responsiveness in the normal diet group. In SS rats, hyper-caloric diet increased aortic NOS3 activity and expression corresponding with increased endothelial-dependent vasorelaxation.

Conclusion—A short-term hyper-caloric diet elicits a vasodilatory phenotype in SS rats, but not in SS-13^{BN} rats, by increasing NOS3 expression and function as well as reducing H₂O₂ function.

Keywords

Angiotensin; Dahl salt-sensitive; nitric oxide synthase; SS-13^{BN}; reactive oxygen species

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Disclosures

None

Introduction

Angiotensin (Ang)II plays a dual role in regulating vascular tone (Zhang et al., 2003). Classically, AngII mediates vasoconstriction via the AngII type 1 receptor (AT1R) (Loria et al., 2011b, Oddie et al., 1993, Ryan et al., 2004, Zhou et al., 2003b, Moltzer et al., 2010, Sparks et al., 2011) and vasorelaxation when signaling through the AT2R (Tsutsumi et al., 1999, Loria et al., 2011b). Regulation of nitric oxide synthase (NOS) function links the vasoconstrictor and vasodilator actions of AngII. AT1R activation produces reactive oxygen species (ROS) i.e., superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) from the endothelium, vascular smooth muscle cells, and fibroblasts (Rueckschloss et al., 2002, Zafari et al., 1998), which induces a pro-oxidant environment in the vessel wall that subsequently quenches NO-mediated vasodilation (Dikalova et al., 2010). Both the AT1R (Suzuki et al., 2006) and AT2R (Zhang et al., 2003) activate NOS. Because NOS functionally buffers vasoconstriction in healthy blood vessels (Loria et al., 2011b, Sauzeau et al., 2010), it is critical to understand the functional regulation of AngII-NOS signaling on vascular homeostasis in the pathogenesis of cardiovascular disease.

The large artery disease, atherosclerosis, is a causative factor in cardiovascular disease, the leading cause of death worldwide (Pepine, 2001, Vaartjes et al., 2009). In experimental settings of pro-atherosclerosis models, such as hyper-caloric diet feeding in ApoE^{-/-} or LDLr^{-/-} mice, AT1R promotes (Koga et al., 2008, Wassmann et al., 2004) and the AT2R protects against atherosclerosis (Iwai et al., 2005, Tesanovic et al., 2010). Additionally in these models, the expression of anti-atherosclerotic endothelial NOS (NOS3) is reduced (Wever et al., 1998, Tesanovic et al., 2010). Reports of hyper-caloric feeding in non-genetically manipulated animal models demonstrate a pro-contractile vascular phenotype. In Sprague-Dawley rats, 4 weeks of hyper-caloric diet increased AngII-mediated aortic vasoconstriction (Ghatta and Ramarao, 2004). In dogs, 6 weeks of hyper-caloric diet enhances coronary arteriolar AngII response with no change in AT1R or AT2R expression (Zhang et al., 2005). Moreover, C57BL/6 mice on hyper-caloric diet for 15–30 weeks have increased aortic AngII response linked to increased AT1R expression and reduced NOS buffering capacity (Yang et al., 1998, Mundy et al., 2007, Zhang et al., 2005, Bhattacharya et al., 2008, Barton et al., 2000).

The Dahl salt-sensitive (SS) rat strain is a model of polygenetic cardiovascular disease (Mattson et al., 2008) and mimics the progression of salt-sensitive hypertension in humans (Herrera et al., 2001, Wendt et al., 2009). This rat strain presents heightened cardiovascular disease risk under normal diet conditions (Poyan Mehr et al., 2003, Garrett et al., 2003). On a normal diet, we found greater AngII reactivity along with a lack of NOS buffering of AngII response in aortas from SS rats compared to SS-13^{BN} genetic control rats. Based on this finding, we hypothesized that hyper-caloric diet induces an increase in the vasoconstrictive action of AngII via increased ROS function and/or reduced NOS buffering capacity in SS rats but not SS-13^{BN} rats. Contrary to this hypothesis, a 4-week hyper-caloric diet drastically blunted AngII-mediated aortic constriction in SS rats but no change in SS-13^{BN} rats. This finding changed the scope of our work. Our hypothesis was redirected to examine if reductions in pro-contractile ROS and anti-contractile NOS mediate blunted aortic AngII reactivity in SS rats on a hyper-caloric diet.

Methods

Animals

Our studies conformed to the *Good Publishing Practice in Physiology* (Persson and Henriksson, 2011). Male Dahl salt-sensitive (SS) rats and SS-13^{BN} rats were purchased from Charles River Laboratories (Wilmington, MA). Upon arrival at our institution at 10–11

weeks old, both rat strains were started on Teklad 8604 rodent diet and water provided *ad libitum*. The Teklad diet was used as the normal diet in our study and consisted of calories from: 33% protein (source: soy, fish meal, wheat, corn, yeast, molasses, whey), 53% carbohydrates (source: corn, wheat, soy, molasses, whey, yeast), and 14% fat (source: soy, corn, wheat, fish) with 3.93 kcal/g gross energy and 0.4% sodium. At 12 weeks old, rats from each strain were randomly placed on hyper-caloric (BioServ F2685, Frenchtown, NJ) or selected to remain on the normal diet. The hyper-caloric diet consisted of calories from: 15% protein (source: casein), 27% carbohydrates (source: corn and sucrose) and 59% fat (source: lard) with 5.45 kcal/g gross energy and 0.4% sodium.

At 16 weeks old, rats were euthanized with pentobarbital sodium (Nembutal; 0.5 mg/kg; Abbott Laboratories, Abbott Park, IL). Whole blood was collected in ice-cold syringes primed with EDTA (Sigma, St. Louis, MO), centrifuged at 3000xg at 4°C for 10 minutes, and plasma was snap-frozen in liquid N₂ and stored at -80°C until analyzed. Thoracic aortas were isolated for vascular reactivity studies or snap frozen for Western blotting as detailed below.

Systolic blood pressure measurements

Tail-cuff plethysmyography was utilized in 16-week old SS-13^{BN} rats and SS rats on normal diet and hyper-caloric diet groups as previously described (Kang et al., 2011).

Plasma assays

Colorimetric assays were used to assess plasma total cholesterol (Cayman Chemical, Ann Arbor, MI), triglycerides (Cayman), and free fatty acids (Zen-Bio, Inc., Research Triangle Park, NC). Plasma renin activity (PRA) was assessed via radioimmunoassay (DiaSorin, Saluggia, Italy).

Aortic vascular reactivity

Thoracic aortas were prepared for wire myography (Danish Myo Technology A/S, Aarhus, Denmark) as previously described (Spradley et al., 2011). Rings were incubated for 15 minutes in the presence or absence of polyethylene glycol superoxide dismutase (PEG-SOD; 200 U/mL; Sigma), catalase (1000 U/mL; Roche Diagnostics, Indianapolis, IN) or the non-selective NOS inhibitor N_ω-Nitro-L-arginine methyl ester (L-NAME; 100 μM; Sigma) followed by generation of cumulative-concentration response curves to AngII (1 x 10⁻¹¹ to 1 x 10⁻⁷ M; Phoenix Pharmaceuticals, Inc., Burlingame, CA). We previously demonstrated this concentration response range elicits a maximal response to AngII-mediated aortic vasoconstriction (Loria et al., 2011a), and a pilot study for our present investigation showed this concentration response range elicits maximal AngII response without provoking tachyphylaxis (data not shown). Vessel segments were washed and stabilized for 30 min with PSS and KCl (8 x 10⁻³ to 100 x 10⁻³ M) response curves performed as previously described (Spradley et al., 2011). Separate rings were incubated in the presence or absence of L-NAME and response curves generated to acetylcholine (ACh; 1 x 10⁻⁹ to 3 x 10⁻⁵ M; Sigma); vessel segments were washed and stabilized for 30 min with PSS and sodium nitroprusside (SNP; 1 x 10⁻¹⁰ to 3 x 10⁻⁵ M) response curves performed.

AT1R and NOS3 expression and NOS activity measurements

In a different set of SS rats on normal diet or hyper-caloric diet, whole thoracic aortas were used for Western blotting as previously described (Spradley et al., 2011). Membranes were probed using antibodies for anti-NOS3 (1:500; BD Biosciences, San Jose, CA), anti-AT1R (1:5000; Santa Cruz Biotechnology, CA), and anti-β-actin (1:20,000; Sigma). Secondary antibodies were used to detect AT1R and NOS3 antibody (goat anti-mouse 1:1000;

Invitrogen, Carlsbad, CA) and the β -actin antibody (goat anti-rabbit 1:10,000; Invitrogen). When analyzed, AT1R and NOS expression was normalized to corresponding β -actin densities.

In a different set of SS rats on normal diet or hyper-caloric diet, thoracic aortas were processed for the L-[3 H]arginine-to-L-[3 H]citrulline conversion assay and NOS isoform activities defined as described previously (Loria et al., 2011b).

Statistical analyses

Data are expressed as mean $\pm$ standard error. Data were graphed and statistically analyzed using GraphPad Prism (La Jolla, CA). The metabolic parameters in Table 1 were analyzed using 2-way ANOVA. The remainder of the data was analyzed using Student's t-test or 2-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

Metabolic parameters

Table 1 details the metabolic parameters of SS-13^{BN} rats and SS rats maintained on a normal diet until 16 weeks old or a 4-week hyper-caloric diet started at 12 weeks old. Body weight increased similarly in SS-13^{BN} and SS rats with hyper-caloric diet and was not different under normal diet conditions in both rat strains. Epididymal fat mass was increased with hyper-caloric diet in both rat strains but fat mass was greater in SS-13^{BN} rats than SS rats in both diet groups. Hyper-caloric diet increased plasma total cholesterol only in SS-13^{BN} rats, whereas free fatty acids and triglycerides were increased in both rat strains. Hyper-caloric diet increased plasma renin activity in SS-13^{BN} rat but not SS rats. Food intake was less in hyper-caloric diet groups but was not different between rat strains or in normal diet groups.

Systolic blood pressure

SBP was assessed at 16 weeks old in SS-13^{BN} rats and SS rats on normal diet or hyper-caloric diet (Table 1). In both rat strains, SBP was similar in normal diet and hyper-caloric groups; however under normal diet conditions, SBP was greater in SS rats than SS-13^{BN} rats.

Assessment of AngII function using aortic rings

Maximum response ($E_{\max}$) to AngII was similar in SS-13^{BN} rats on normal diet or hyper-caloric diet (Figure 1, Table 2). In contrast, $E_{\max}$ was reduced in aortas from SS rats on hyper-caloric diet compared to normal diet counterparts (Figure 1, Table 2). On normal diet, $E_{\max}$ response to AngII was greater in SS rats than SS-13^{BN} rats (Figure 1, Table 2). Sensitivity ($\log EC_{50}$) to AngII was similar in all 4 groups (Table 2).

When KCl responses were assessed, $E_{\max}$ was reduced solely in SS-13^{BN} rats with hyper-caloric diet, whereas $E_{\max}$ was similar between rat strains on normal diet (Table 2). Sensitivity to KCl was similarly increased in both rat strains with hyper-caloric diet with no difference between rat strains on normal diet (Table 2).

Aortic ROS function during AngII vasoconstriction

Treatment of aortic rings with PEG-SOD significantly reduced $E_{\max}$ and $\log EC_{50}$ to AngII constriction in SS-13^{BN} rats on either diet (Figure 2A, Table 2), whereas, PEG-SOD treatment reduced $E_{\max}$ with no change in $\log EC_{50}$ to AngII in aortas from SS rats on either diet (Figure 2B, Table 2).

In SS-13^{BN} rats, catalase reduced $E_{\max}$ with no change in $\log EC_{50}$ to AngII in aortic rings from the hyper-caloric diet group but no effect on rings from the normal diet groups (Figure 2C, Table 2). In contrast, catalase had no effect on $E_{\max}$ or $\log EC_{50}$ to AngII in aortic rings from SS rats on hyper-caloric diet but reduced on $E_{\max}$ in rings from SS rats on normal diet (Figure 2D, Table 2).

Aortic NOS function during AngII vasoconstriction

L-NAME, a non-selective NOS inhibitor, increased $E_{\max}$ and reduced $\log EC_{50}$ to AngII in SS-13^{BN} rats on normal diet but not on hyper-caloric diet (Figure 3A, Table 2). In stark contrast, L-NAME increased $E_{\max}$ without altering $\log EC_{50}$ to AngII in SS rats on hyper-caloric diet but not on normal diet (Figure 3B, Table 2).

Aortic vasorelaxation

$E_{\max}$ and $\log EC_{50}$ to ACh-mediated vasorelaxation was similar in SS-13^{BN} rats on normal diet and hyper-caloric diet (Figure 4A, Table 3); ACh response was totally blocked with L-NAME (Figure 4A, Table 3). In contrast, $E_{\max}$ but not $\log EC_{50}$ to ACh-mediated vasorelaxation was greater in aortas from SS rats on hyper-caloric diet normal diet (Figure 4B, Table 3); ACh response in both diet groups of SS rats was totally blocked with L-NAME (Figure 4B, Table 3). SNP response was similar in SS-13^{BN} rat (Figure 4C, Table 3) and SS rat diet groups (Figure 4D, Table 3).

AT1R and NOS3 expression and NOS activity in aortas from SS rats

Aortic AT1R expression was similar in SS rats on normal diet or hyper-caloric diet (Figure 5A), whereas NOS3 protein expression was increased in SS rats on hyper-caloric diet (Figure 5B).

Total NOS activity and NOS1 specific activity were similar in aortas from SS rats on normal diet or hyper-caloric diet; however, hyper-caloric diet elicited a reduction in NOS2 activity and an increase in NOS3 activity in aortas from SS rats (Figure 5C).

Discussion

Our study elucidated the interaction between AngII and NOS signaling in the vasculature of Dahl salt-sensitive (SS) rats and the extent to which this interaction is modulated by a hyper-caloric diet. Preliminary studies showed under normal diet conditions that a lack of NOS function resulted in greater AngII reactivity in aortas from SS rats than SS-13^{BN} rats. Because a hyper-caloric diet elicits exaggerated AngII reactivity in large arteries when NOS function is reduced (Bhattacharya et al., 2008), we tested the hypothesis that a 4-week hyper-caloric diet promotes greater AngII reactivity in aortas from SS rats than SS-13^{BN} rats. Contrary to this hypothesis, hyper-caloric did not increase AngII reactivity in aortic rings from SS-13^{BN} rats and dramatically blunted this response in SS rats compared to rats on normal diet. Therefore, we redirected our hypothesis to examine if the apparent imbalance between AngII and NOS signaling in the SS rats on normal diet is attenuated by a hyper-caloric diet. Our main findings are congruent with this hypothesis as NOS3 expression and function was increased in SS rats on hyper-caloric diet.

Classical studies use the SS rat to model salt-sensitive cardiovascular disease in humans. Even on a normal-salt diet, SS rats are susceptible to cardiovascular-renal disease (Garrett et al., 2003). In fact, we showed on normal diet that SS rats but not salt-resistant SS-13^{BN} rats had greater aortic responsiveness to AngII linked to reduced NOS function. It is established that local AngII disrupts NO's regulation of vascular tone through reactive oxygen species (ROS) signaling in aortas from SS rats on high-salt diet (Zhou et al., 2003a); this had not

been examined under normal diet circumstances. Although $O_2^{\cdot-}$ similarly mediated aortic response to AngII in both rat strains, H_2O_2 -mediated AngII reactivity was greater in SS rats than SS-13^{BN} rats. These data highlight the susceptibility of cardiovascular disease via pro-oxidant mechanisms in SS rats under normal diet conditions.

In SS-13^{BN} rats, hyper-caloric diet did not significantly alter aortic AngII reactivity. However, the mechanisms of AngII reactivity were different between the SS-13^{BN} diet groups. It is well known in the blood vessel wall that AngII is a potent stimulator of ROS including $O_2^{\cdot-}$ (Berry et al., 2000) and H_2O_2 (Zafari et al., 1998). Many studies show these two ROS mediate AngII-induced vasoconstriction (Bagi et al., 2008, Puntmann et al., 2005, Hussain et al., 2006, de Groot et al., 2004, Shastri et al., 2002, Torrecillas et al., 2001, Kawazoe et al., 1999, Kim et al., 2010, Pfister et al., 2011). Quenching of $O_2^{\cdot-}$ with PEG-SOD blunted AngII reactivity similarly in SS-13^{BN} rats on normal diet and hyper-caloric diet, whereas H_2O_2 -mediated AngII reactivity was only present in aortas from SS-13^{BN} rats on hyper-caloric diet. Our data in SS-13^{BN} rats are reminiscent of studies utilizing salt-resistant Fisher rats whereby 7 months of hyper-caloric diet increased plasma levels of H_2O_2 without altering aortic catalase expression (Roberts et al., 2006, Roberts et al., 2005). Our data confirm that hyper-caloric diet promotes a pro-oxidant environment in the vasculature in salt-resistant rats.

In contrast to SS-13^{BN} rats, we propose that short-term hyper-caloric feeding promotes an anti-oxidant environment in the blood vessel wall in SS rats. Foremost, H_2O_2 -mediated AngII vasoconstriction in aortas from SS rats on a normal diet was not present following the hyper-caloric diet, possibly due to the scavenging effect of NO through the increased NOS3 activity. Hyper-caloric diet increased aortic NOS3-specific activity and expression in addition to reducing aortic NOS2 activity in SS rats. Studies show that activation of the peroxisome proliferator-activated receptors (PPARs) class of transcription factors, in particular PPAR- α , increases endothelial cell NOS3 expression (Wang et al., 2006) and activity (Okayasu et al., 2008). It has been shown that PPAR- α reduces NOS2 activity and expression in macrophages (Colville-Nash et al., 1998). Interestingly, a 12-week low-carbohydrate/high-fat diet increases PPAR- α protein expression in whole hearts from SS rats (Okere et al., 2006). Further research will examine if hyper-caloric diet-mediated enhancement of aortic endothelial function in SS rats is mediated by activation of PPAR- α .

AngII-mediated vasoconstriction in aortic tissue has been utilized as a surrogate measure of large artery disease. In aortic ring preparations from rabbits, a 10-week hypercholesterolemic diet increased AT1R mRNA and protein expressions corresponding to exaggerated AngII reactivity and endothelial dysfunction; importantly, these changes in aortic reactivity paralleled the formation of atherosclerotic plaques (Yang et al., 1998). Because circulating AngII levels are increased from picomolar to nanomolar concentrations in diet-induced obese mice (Gupte et al., 2008), we propose that SS rats are protected against endothelial pathologies linked to atherogenesis following a short-term hyper-caloric diet. This proposal is supported by findings from Lombard's group demonstrating that not only is middle cerebral arterial response to acetylcholine enhanced in SS rats fed a 16–20 hyper-caloric diet, but that AT1R protein expression is reduced in these arteries as well (Beyer et al., 2012).

The choice of utilizing Teklad 8604 as the normal diet in the present study was based on our previous studies (Spradley et al., 2011). SS rats were maintained on the American Institutes of Nutrition (AIN)-76A diet since weaning at 3-weeks-old at Charles River Laboratories but started on Teklad diet at 12-weeks-old at our institution. When assessing acetylcholine-mediated vasorelaxation and phenylephrine-mediated vasoconstriction, we showed this diet protocol did not alter aortic NOS function but promoted NOS dysfunction in small

mesenteric arteries (Spradley et al., 2011). We now demonstrate aortic NOS dysfunction in SS rats on this diet regimen in response to AngII. However, we can only speculate about using a conductance vessel as a surrogate measure of AngII function in systemic resistance blood vessels.

There are limitations to using the Teklad diet as the control diet in our hyper-caloric diet studies. As detailed in the Methods, the Teklad diet is proprietary and consists of multiple components for each macronutrient whereas the hyper-caloric diet is a purified diet. Because of the complex nature of the Teklad diet, future studies should use a proper control diet to solidify our findings. Furthermore, we highlight that both rat strains consumed approximately half as much of the hyper-caloric diet, and thus salt as well, when compared to the normal diet groups. Lombard's laboratory showed that endothelial dysfunction is present in cerebral arteries from SS rats not SS-13^{BN} rats when on a low salt (0.01% NaCl) or normal salt (0.4% NaCl) via reduced AngII signaling; in fact, a subpressor infusion of AngII for 3 days restored endothelium-dependent vasorelaxation in SS rats on normal-salt diet. (Drenjancevic-Peric and Lombard, 2005, Durand et al., 2010, Durand and Lombard, 2011). The reduced salt intake in the SS-13^{BN} rats on hyper-caloric diet was echoed by increased plasma renin activity, indicating that the renin-angiotensin system is increased in this diet group. In contrast, PRA was not altered with 4-week hyper-caloric diet in SS rats, indicating that systemic activation of the RAS is not increased in this diet group. We speculate that the increased vasodilator mechanisms in SS rats on hyper-caloric diet is not due to a reduction in salt intake.

In perspective, classic monogenetic models of hyper-caloric diet-induced cardiovascular diseases have yielded invaluable mechanistic insights, yet most cardiovascular diseases are polygenetic (Dominiczak et al., 2005). The SS rat is a polygenetic model of cardiovascular disease (Mattson et al., 2008). SS rats responded to the short-term hyper-caloric diet by reduced H₂O₂-mediated vasoconstriction and increased aortic NOS3 activity and endothelial function as well. Whereas, SS-13^{BN} rats showed reduced NOS function response to hyper-caloric diet. NO is protective against atherosclerosis, a slowly progressing large artery disease associated with high caloric intake (Mainous et al., 2011). Future research will be directed at elucidating the molecular mechanism(s) involved with a short-term hyper-caloric diet to induce vascular NOS3 activity and expression possibly revealing novel vasoprotective target(s) in the salt-sensitive population.

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Figure 1.

Angiotensin (Ang)II-mediated vasoconstriction in aortas from SS-13^{BN} rats normal diet (N=12) or hyper-caloric diet (N=10) or SS rats on normal diet (N=8) or hyper-caloric diet (n=14). *P<0.05 for E_{max} in aortic segments from SS rats on normal diet vs. hyper-caloric diet; †P<0.05 for E_{max} in aortic segments SS rats vs. SS-13^{BN} rats on normal diet.

Figure 2.

Angiotensin (Ang)II-mediated vasoconstriction in aortas from SS-13^{BN} rats (A: normal diet: untreated N=11, +PEG-SOD N=6; hyper-caloric diet: untreated N=10, +PEG-SOD N=3) (C: normal diet: untreated N=11, +CATALASE N=4; hyper-caloric diet: untreated N=10, +CATALASE N=4). AngII vasoconstriction in aortas from SS rats (B: normal diet: untreated N=8, +PEG-SOD N=6; hyper-caloric diet: untreated N=14, +PEG-SOD N=9). (D: normal diet: untreated N=8, +CATALASE N=5; hyper-caloric diet: untreated N=14, +CATALASE N=12). *P<0.05 for E_{max} in aortic segments in corresponding untreated groups.

Figure 3.

A: Angiotensin (Ang)II-mediated vasoconstriction in aortas from SS-13^{BN} rats on normal diet (untreated N=12, +L-NAME N=6) or hyper-caloric diet (untreated N=10, +L-NAME N=5). B: AngII vasoconstriction in aortas from SS rats on normal diet (untreated N=8, +L-NAME N=6) or hyper-caloric diet (untreated N=14, +L-NAME N=11). *P<0.05 for E_{max} in L-NAME-treated vs. untreated aortic segments from SS-13^{BN} rats on normal diet. †P<0.05 for E_{max} in L-NAME-treated vs. untreated aortic segments from SS rats on hyper-caloric diet.

Figure 4. Acetylcholine (ACh)- and sodium nitroprusside (SNP)-mediated vasorelaxation in SS-13^{BN} rats (A and B, respectively; normal diet: untreated N=10, +L-NAME N=10; hyper-caloric diet: untreated N=9, +L-NAME N=9) and SS rats (C and D, respectively; normal diet: untreated N=8, +L-NAME N=8; hyper-caloric diet: untreated N=8–14, +L-NAME N=14). *P<0.05 E_{max} vs. SS rats on normal diet.

Figure 5.

In aortic tissue from SS rats, A: AT1R expression (normal diet N=8; hyper-caloric diet N=8), B: NOS3 protein expression (normal diet N=7; hyper-caloric diet N=7), and C: NOS activity (normal diet N=3; hyper-caloric diet N=4). *P<0.05 vs. SS rats on normal diet.

Table 1

Metabolic parameters measured at 16 weeks old in SS-13^{BN} rats and SS rats on normal diet or 4 weeks of hyper-caloric diet.

Parameter	SS-13 ^{BN}		SS	
	Normal	Hyper-caloric	Normal	Hyper-caloric
Body Weight (g)	368±16 (6)	393±12 (6)*	366±7 (6)	402±12 (6) [†]
Epididymal Fat/BW (mg/g)	9±1 (4)	19±2 (6)*	3±0.1 (3)*	5±0.4 (5) [†]
Total Cholesterol (mg/dL)	127±12 (6)	171±6 (6)*	168±9(6)*	168±8 (6)
Triglycerides	120±12 (6)	211±27 (5)*	132±11 (5)	190±17 (5) [†]
Free Fatty Acids	15±1 (6)	42±8 (5)*	17±2 (6)	46±7 (6) [†]
PRA (ng Ang1/mL/h)	4.8±0.6 (4)	9.7±2.7 (4)*	6.9±1.0 (5)	5.5±0.4 (6)
Food Intake (g/day)	24±1 (6)	12±0.4 (6)*	23±1 (6)	12±1 (6) [†]
SBP (mmHg, Tail-Cuff)	135±6 (6)	136±3 (6)	174±5 (6)*	176±6 (6)

PRA = plasma renin activity; SBP = systolic blood pressure.

* $P < 0.05$ vs. SS-13^{BN} rats on normal diet;

[†] $P < 0.05$ vs. SS rats on normal diet. Number of rats is in parentheses.

Table 2

Maximum response ($E_{\max}$) and sensitivity ($\log EC_{50}$) to angiotensin II (ANGII)-, phenylephrine (PE)-, and KCl-mediated vasoconstriction in aortas from SS-13^{BN} rats and SS rats on normal diet or hyper-caloric diet.

	SS-13 ^{BN}		SS	
	Normal	Hyper-caloric	Normal	Hyper-caloric
<i>$E_{\max}$ % Increase in Force</i>				
AngII	71±10 (10)	53±11(10)	133±11 (8)*	43±5 (14) [†]
+PEG-SOD	55±6 (6) [‡]	27±7 (3) [‡]	53±17 (6) [‡]	16±2 (9) [‡]
+CATALASE	88±13 (4)	25±5 (4) [‡]	73±21 (5) [‡]	46±6 (12)
+L-NAME	114±15 (6) [‡]	51±6 (5)	117±13 (5)	91±11 (11) [‡]
KCl	140±4 (12)	114±9 (10)*	156±9 (9)	148±6 (14)
<i>$\log EC_{50}$ [M] for AngII; EC_{50} [mM] for KCl</i>				
AngII	-8.1±0.01 (11)	-8.2±0.1 (10)	-8.4±0.1 (11)	-8.1±0.1 (14)
+PEG-SOD	-7.8±0.2 (6) [‡]	-7.7±0.1 (3) [‡]	-8.0±0.2 (5)	-7.9±0.1 (9)
+CATALASE	-8.2±0.1 (4)	-8.1±0.2 (4)	-8.3±0.4 (5)	-8.1±0.1 (11)
+L-NAME	-8.4±0.1 (6) [‡]	-7.9±0.1 (5)	-8.7±0.3 (6)	-8.2±0.04 (11)
KCl	17.6±1.1 (10)	28.8±3.1 (6)*	20.3±1.3 (13)	25.9±1.4 (8) [†]

* $P < 0.05$ vs. untreated aortas from SS-13^{BN} rats on normal diet;

[†] $P < 0.05$ vs. untreated aortas from SS rats on normal diet;

[‡] $P < 0.05$ vs. corresponding untreated aortas. Number of rats is in parentheses.

Table 3

Maximum response ($E_{\max}$) and sensitivity ($\log EC_{50}$) to acetylcholine (ACh)- and sodium nitroprusside (SNP)-mediated vasorelaxation in aortas from SS rats on normal diet or hyper-caloric diet.

	SS-13 ^{BN}		SS	
	Normal	Hyper-caloric	Normal	Hyper-caloric
<i>E_{max} % of PE</i>				
ACh	85±3 (10)	86±3 (10)	56±7 (7) [*]	78±4 (14) [†]
+L-NAME	0.3±0.9 (10) [‡]	1.4±0.9 (10) [‡]	2.1±0.7 (8) [‡]	-3.7±2.3 (14) [‡]
SNP	95.0±1.5 (10)	96.0±0.8 (10)	91.2±3.5 (8)	94.8±2.3 (14)
<i>logEC₅₀ [M]</i>				
ACh	-7.1±0.1 (10)	-7.3±0.1 (10)	-6.9±0.1 (8)	-6.9±0.2 (13)
SNP	-8.37±0.1 (9)	-8.49±0.1 (9)	-8.0±0.2 (8)	-7.9±0.1 (13)

^{*} $P < 0.05$ vs. aortic artery segments from SS-13^{BN} rats on normal diet,

[†] $P < 0.05$ vs. aortic artery segments from SS rats on normal diet,

[‡] $P < 0.05$ vs. corresponding untreated aortic artery segments. Number of rats is in parentheses.