Original Research

Effects of a Diet High in Salt, Fat, and Sugar on Telemetric Blood Pressure Measurements in Conscious, Unrestrained Adult Yucatan Miniature Swine (*Sus scrofa*)

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Radiotelemetry was used to evaluate diet-related elevation of blood pressure in adult Yucatan miniature swine. Systolic arterial blood pressure (SAP), diastolic atrial blood pressure (DAP), heart rate, and locomotor activity were assessed in 9- or 11-mo-old Yucatan miniature pigs fed a standard diet or a North American-type diet high in salt, fat, and sugar (HSFS). Compared with pigs fed standard diet, pigs fed HSFS diet showed markedly elevated SAP (132 ± 3 compared with 156 ± 6 mm Hg), whereas DAP was unchanged (92 ± 2 compared with 99 ± 5 mm Hg). In addition, all pigs were modestly sensitive to short-term changes in dietary salt, as indicated by a 6% to 7% response in blood pressure parameters. According to these data, the increase in SAP for pigs on the HSFS diet was too large to be explained by the NaCl content of the diet alone. We found no evidence of endothelial dysfunction, and the relaxation responses of isolated coronary arteries actually were enhanced in the HSFS group. In conclusion, in a Yucatan miniature pigs model chronically fed a HSFS diet, DAP did not increase, but SAP and pulse pressure appeared to be affected by high dietary levels of fat or sugar (or both).

Abbreviations: BP, blood pressure; DAP, diastolic arterial pressure; HSFS diet, diet high in salt, fat, and sugar; MAP, mean arterial pressure; SAP, systolic arterial pressure.

Dietary factors have prominent effects on cardiovascular parameters, including blood pressure (BP), a major risk factor for cardiovascular disease and mortality.[2,15] BP often is sodium-sensitive, rising and falling with the level of dietary salt intake,[16] and high dietary intakes of various fats and sugars have been associated with hypertension.[29,40] In addition, dietary factors can directly affect the vasculature, with increased intakes of NaCl, fats, and sugars each being linked with endothelial dysfunction.[4,11,12,21]

The factors that contribute to hypertension most often are studied by using rodent models. However, swine are an excellent animal model to study these factors, including diet-induced hypertension, because of the similarities in cardiovascular structure and function between swine and humans. For instance, like humans,[16] pigs[7] are susceptible to developing hypertension in response to high dietary intake of sodium. Swine are increasingly being used in cardiovascular research overall.[8,19,24,32,34,36,43] Although radiotelemetry is a preferred method for measurement of hemodynamics, only a few studies have incorporated this approach in assessing BP in swine. In the present study, our main objective was to use telemetry to evaluate BP, heart rate, and ac-

tivity level in Yucatan miniature swine under normal and unhealthy dietary conditions (short-term exposure to high dietary salt and long-term exposure to a diet high in salt, fat, and sugar [HSFS diets]). A secondary objective was to use isolated coronary arteries from these swine to investigate the concurrent effects of these diets on endothelium-mediated vascular reactivity as an index of early changes to the health of the coronary circulation.

Materials and Methods

The study population comprised 12 Yucatan miniature pigs (6 females and 6 noncastrated males) from the Memorial University swineherd. This herd is *Brucellosis*-free, *Pseudorabies*-negative, and vaccinated against *Erysipelothrix rhusiopathiae*; pigs also were tested and found negative for *Bordetella bronchiseptica, Corynebacteria kutscheri, Haemophilus* spp., *Klebsiella oxytoca, Klebsiella pneumoniae, Pasteurella* spp., *Pseudomonas aeruginosa, Streptococcus, Listeria monocytogenes, Salmonella, Clostridium,* and *Campylobacter*. All animal procedures were approved by Memorial University of Newfoundland Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.[5] At 4 wk old, the pigs were weaned from the sow, and 6 of the pigs (3 female and 3 male) were fed a standard pig grower diet (Figure 1). The pigs were housed with 2 of their siblings of the same sex (these siblings were not used in the present study) but were separated from them for 5 h daily (1200 to 1700) for ad libitum feeding and

	Standard diet		HSFS diet	
	Low salt	High salt	Low salt	High salt
Energy distribution (% total energy)				
Carbohydrates	67	67	40	40
Complex	67	67	30	30
Sugar	—	—	10	10
Fat	12	12	50	50
Protein	21	21	10	10
Ingredients (g/kg dry matter)				
Wheat shorts	400.5	400.5	264.3	264.3
Canola	49.0	49.0	32.3	32.3
Meat meal	19.0	19.0	12.5	12.5
Limestone	13.0	13.0	8.6	8.6
Corn gluten feed	40.0	40.0	26.4	26.4
Ground barley	297.0	297.0	196.0	196.0
Oats	175.0	175.0	115.5	115.5
Vitamin mix	0.8	0.8	0.5	0.5
Mineral mix	1.0	1.0	0.7	0.7
Lard	—	—	150.0	150.0
Margarine	—	—	50.0	50.0
Sugar	—	—	100.0	100.0
Sodium chloride	4.7	40.0	4.7	40.0

Figure 1. Composition of the diets used in the current study. Standard diet contained approximately 2900 kcal digestible energy/kg and 154 g protein/kg. HSFS diet (3230 kcal digestible energy/kg and 131 g protein/kg) was made by adding lard, hydrogenated margarine, granulated sugar, and NaCl to ground standard pig grower diet.

quantification of daily feed intake by individual pigs. All animals had 24-h ad libitum water access and were maintained on a 12:12-h light:dark cycle (lights on, 0700 to 1900) in a temperature-controlled (20 to 22 °C) environment. At approximately 9 mo of age (body weight [mean ± SEM], 69.2 ± 2.6 kg), the first group of pigs underwent surgery for implantation of telemeter and blood sampling catheters. The second group of 6 pigs (3 female and 3 male) followed the same protocol as outlined above, except these pigs were weaned onto HSFS diet (Figure 1) at 4 wk of age. The HSFS diet was made by adding NaCl (40 g/kg; Windsor Table Salt, The Canadian Salt Company, Pointe Claire, Quebec, Canada), hydrogenated margarine (50 g/kg; Central Dairies, St John's, Canada), lard (150 g/kg; Loblaw, Toronto, Ontario, Canada) and granulated sugar (100 g/kg; Lantic, Montreal, Quebec, Canada) to ground standard pig grower diet, thus providing 50% of caloric intake from fat, 40% from carbohydrate, and 10% from protein.

Because of the higher energy density of the HSFS diet (Figure 1) and because pigs eat only enough to meet their energy requirements,[27] this group consumed less diet overall and less protein in particular, which restricted their growth. As a result, to match final body weights, HSFS pigs underwent surgery at approximately 11 mo old (body weight, 69.7 ± 5.4 kg) instead of at 9 mo of age, as for pigs on standard diet.

Telemetry and catheter implantation. At approximately 9 mo (standard diet) or approximately 11 (HSFS diet) mo of age, pigs underwent surgical implantation of an arterial BP telemeter (TA-11PA-D70; Data Sciences International, St Paul, MN) and blood sampling catheters. Anesthesia was induced with ketamine (22 mg/kg IM) and xylazine (2 mg/kg IM) and maintained with 1.0% to 1.5% halothane and 3:2 oxygen:nitrous oxide mixture. The BP telemeter body was implanted subcutaneously in the inner hindlimb, with the telemeter catheter inserted 10 cm into the left femoral artery. This investigation was part of a larger series of studies; therefore, 2 blood sampling catheters (inner diameter, 1.0 mm; outer diameter, 1.8 mm; Tygon Medical Tubing, Saint

Gobain Performance Plastics, Akron, OH) were inserted into the left femoral vein, tunnelled under the skin, and exteriorized between the shoulder blades. Pigs were housed individually after surgery.

Pigs were given trimethoprim and sulfadoxine (40 and 20 mg/mL, respectively; total dose, 0.07 mL/kg IV; Borgal, Intervet, Whitby, Ontario, Canada) during the first 2 d after surgery and buprenorphrine hydrochloride (300 µg; Temgesic, Schering–Plough, Hertfordshire, UK) immediately and 1 d after surgery. Adequacy of analgesia was determined by monitoring behavior (that is, absence of 'guarding,' active in environment). General recovery was assessed by daily monitoring of body temperature and food intake. The 5-h daily feeding regimen described earlier was reestablished the day after surgery. Typically food intake had returned to presurgery levels by 3 d after surgery. As part of the larger series of studies conducted, these pigs were also tested for intravenous glucose tolerance, insulin sensitivity, and fat tolerance; no more than 50 mL blood per test was withdrawn during these 3 tests over 5 d. BP recording began 2 d after fat tolerance testing, so that all pigs were allowed at least 8 d of recovery from surgery before any BP recordings.

Telemetry system. Telemetry equipment (Data Sciences International) was set up to monitor hemodynamics and spontaneous locomotor activity in the pigs. The BP telemeter signal was received by a network of 6 receivers (RLA1020) positioned 3 on either side of the holding pen (1.9 m × 1.2 m × 2.0 m). The strongest signal was selected by a multiplexer (RMX10) and passed to an analog adapter (R11CPA), which provided a calibrated voltage output after correcting for atmospheric pressure using an ambient pressure monitor (APR-1). The calibrated signal was then recorded using a computerized data acquisition system and processed as previously described.[25] Pressure calibrations of the telemeters were assessed before implantation and after removal, and corrections were made to the data for any deviation from the original factory calibration.[38] The BP signal was sampled for 10 s at 30-s

intervals, and systolic, diastolic, and mean arterial BP (SAP, DAP, and MAP, respectively) and heart rate during this sample period were computed and stored for offline analysis. Locomotor activity data were derived from the activity signal reported by the telemetry equipment and were expressed in arbitrary units, with 0 corresponding to inactivity.

Salt challenge protocols. After at least 8 d of recovery from surgery, BP was recorded continuously for 48 h. After the 48-h baseline BP recordings, the pigs on the standard pig grower diet (0.5% NaCl) were placed on a high-salt (4.5% NaCl) version of the diet for 7 d, with continuous BP recordings during the last 48 h. After this 48-h recording period, the pigs were returned to standard pig grower diet (0.5% NaCl) for 5 to 7 d until necropsy.

After the 48-h baseline BP recordings while the pigs were on the HSFS diet (4.5% NaCl), they were challenged with a low-salt (0.5% NaCl) version of the HSFS diet for 7 d, with continuous recording of BP during the last 48 h. After the recording session, the pigs returned to HSFS (4.5% NaCl) diet for 5 to 7 d until necropsy.

Hemodynamic data analysis. A modified version of the Microsoft Excel 2000 template (HdStats; www.med.mun.ca/Medicine/Faculty/Van-Vliet,-Bruce.aspx) was used for routine analysis of 24-h telemetry data sets. We also computed several indices of locomotor activity including: (1) the mean value of the raw activity signal (arbitrary units), (2) the mean value of activity for values of activity greater than 0 (arbitrary units; an index of the intensity of activity when pigs were active), (3) the mean of the logarithm of activity values greater than 0 (similar to no. 2, but data were log-transformed to normalize the otherwise highly skewed distribution of values), and (4) inactive time, calculated as the percentage of raw activity values that were 0. Furthermore, we computed the mean values for BP and heart rate that were associated with sustained periods of activity and inactivity. For these calculations, we included only data for which the activity signal was consistently greater than 0 for at least 3 consecutive samples (sustained activity) or for which the value was 0 for at least 3 consecutive samples (sustained inactivity).

Circadian variations in BP were not analyzed due to the potential for posture-dependent artifacts, a potential limitation associated with BP measurements made in large animals by using fluid-filled catheters. Changes in the posture of pigs from the standing to lying position were associated with as much as a 10-cm change in the elevation of the telemeter catheter tip relative to the telemeter body, resulting in a posture-dependent pressure artifact of as much as 8 mm Hg (pressure lower when standing). Although this artifact is modest, it is similar in magnitude to anticipated circadian variations of BP. Therefore, we conservatively chose to forego analysis of day and night values of BP or other circadian indices. We expect that this posture-dependent artifact has a negligible effect on 24-h BP averages (effects of standing and lying tending to cancel, with any residual effect being small and consistent across treatment groups) and lacks any influence whatsoever on heart rate and activity data.

Necropsy. Approximately 5 to 7 d after salt challenge, pigs were anesthetized with sodium pentobarbital (105 mg/kg IV; Euthanyl, Biomeda-MTC, Cambridge, Ontario, Canada), ventilated, and maintained with 0.5% to 1% halothane in oxygen. The pigs were euthanized by exsanguination after removal of the liver.

Biochemical and histologic analyses. Plasma glucose concentrations were measured by analyzer (Rapid Lab 865; Bayer Diagnostics, Toronto, Canada); porcine insulin concentrations were evaluated by radioimmunoassay (coefficients of variation: intraassay, 7%; interassay, 9%; Linco Research, St Charles, MO). We used gross anatomic staining with Sudan IV to assess fatty streak formation within the pig aorta.[18] Briefly, at necropsy, the entire intact aorta (that is, from the ascending aorta to the abdominal aorta) was removed, rinsed with saline, trimmed of arteries, opened longitudinally along the ventral surface, pinned flat, and fixed with neutral-buffered formalin (10% v/v). After fixation, to determine whether atherosclerotic lesions were present, the intact aorta was stained with Herxheimer solution (5 mg/mL Sudan IV Red in 70% [v/v] ethyl alcohol and acetone [1:1, v/v]) and then rinsed with 70% (v/v) ethyl alcohol.

Coronary artery isometric tension measurements. At necropsy, whole hearts were removed from pigs and placed in ice-cooled Krebs physiologic salt solution (114 mM NaCl, 4.7 mM KCl, 0.8 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 11 mM D-glucose), which was aerated with a mixture of 95% O_2 and 5% CO_2. Left anterior descending coronary arteries were isolated from whole hearts and cut into 4 to 8 rings that were 1 to 2 mm in length. Coronary artery rings were mounted on 200-µm diameter hooks in myograph chambers (DMT 610M, 620M) containing Krebs physiologic salt solution (pH 7.4) at 37 °C. Under isometric conditions, the initial resting tensions of artery rings were set to 90% of the internal circumference estimated to produce wall stress equivalent to 13.3 kPa (100 mm Hg). After a 1-h equilibration period, tissue viability was tested by addition of high K^+-containing, osmotic balanced Krebs solution (Na^+ substituted with equimolar amounts of K^+).[6] Arteries having contractions greater than 2 mN per millimeter passed the test. Artery rings were contracted submaximally by using the thromboxane receptor agonist U46619 and then exposed to increasing cumulative concentrations of bradykinin (0.001 nM to 3 µM).[6] Relaxation is reported as the reversal of the contractions by U46619; 100% represents complete reversal of tension.

Statistical analyses. Data were analyzed by using Prism 4 (GraphPad Software, San Diego, CA) and are reported as mean ± SEM. Growth data were analyzed by using 2-way repeated-measures ANOVA, accounting for treatment and time effects, with Bonferroni posthoc tests. To account for potential sex-associated differences, we analyzed the hemodynamic data by using 2-way ANOVA, in which the dependent variables included diet, sex, and the interaction between the 2. However, this analysis indicated no significant sex effects for any parameter. As a result, hemodynamic parameters were analyzed by using one-way ANOVA. Responses to salt challenge and within-groups comparisons between values obtained during the light and dark phases were analyzed by using paired t tests. Linear regression analysis was used to assess whether age was associated with hemodynamic parameters. For blood vessel data, bradykinin concentration–relaxation relationships for each pig were determined in replicate (2 to 4 rings). The maximal response, pD_2 value (negative logarithm base 10 of the molar concentration of drug needed for 50% of maximum effect), and hill slope were determined by fitting a sigmoidal dose–response curve to the averaged data points for relaxations compared with bradykinin concentration from each group. An F test was used to determine whether the variables (pD_2, hill slope, maximal response) describing the best-fit curves ($r^2 > 0.90$) were the same between the 2 groups. Pearson correlations were performed to assess relationships between some of the

variables. Statistical significance was set at a *P* value of less than 0.05 for all analyses.

Results

Animal performance. Both groups (*n* = 6; 3 male, 3 female) of Yucatan minipigs were similar in mean birth weight (standard diet group, 1.00 ± 0.07 kg; HSFS diet group, 1.01 ± 0.05 kg; *P* = 0.830) and weaning weight (standard, 6.04 ± 0.49 kg; HSFS, 5.82 ± 0.30 kg; *P* = 0.714). However, the pigs on the HSFS diet grew at a slower rate than did the control pigs (Figure 2); that is, because of the higher energy density and higher energy-to-protein ratio of the diet, HSFS-fed pigs consumed less protein and grew slower (Figure 2), particularly between 1 to 4 mo of age, than did standard-fed pigs. Average relative feed intake was lower in HSFS pigs between 1 and 4 mo old (standard, 46.6 ± 1.2 g/kg body weight/d; HSFS, 42.5 ± 1.1 g/kg body weight/d; *P* < 0.05) but not between 4 and 7 mo old (standard, 27.4 ± 0.9 g/kg body weight/d; HSFS, 27.0 ± 0.4 g/kg body weight/d) or from 7 mo to necropsy (standard, 21.6 ± 0.6 g/kg body weight/d; HSFS: 21.6 ± 0.7 g/kg body weight/d). Therefore, the HSFS group was maintained on the diet 2 mo longer before hemodynamic recordings, and body weights at surgery (standard: 69.15 ± 2.62 kg, 9 mo old; HSFS: 69.67 ± 5.39 kg, 11 mo old; *P* = 0.932) and at necropsy (standard: 72.20 ± 2.62 kg, 10 mo old; HSFS: 72.72 ± 4.44 kg, 12 mo old; *P* = 0.922) were similar between groups. Moreover, at the end of the study, relative backfat thickness (dorsal midline, caudal to the last rib), a measure of subcutaneous fat deposition, was similar between groups (standard, 0.85 ± 0.08 mm backfat per kilogram body weight; HSFS, 0.86 ± 0.06 mm backfat per kilogram body weight; *P* = 0.811). Therefore, body weight was not a confounding factor in this study, thereby supporting direct comparison of dietary factors between the standard and HSFS diets.

We did not observe any gross evidence of aortic atherosclerosis (fatty streak) formation in any of the pigs (data not shown). Fasting plasma glucose (standard, 5.4 ± 0.3 mmol/L; HSFS, 5.8 ± 0.1 mmol/L; *P* = 0.34) and insulin (standard, 10.1 ± 1.7 μU/mL; HSFS, 14.0 ± 1.6 μU/mL; *P* = 0.12) concentrations were not different between groups.

Circadian hemodynamics and locomotor activity in Yucatan miniature pigs. Yucatan miniature pigs exhibited circadian variation in heart rate and locomotor activity (Figure 3), with both variables being significantly (*P* < 0.05) lower during the dark phase of the 24-h period (Table 1). Furthermore, regardless of diet, heart rate values and activity levels were greater during light compared with dark phases. For instance, pigs fed the standard diets had 8% to 10% higher heart rate (*P* < 0.05) and approximately 80% to 83% higher mean activity (*P* < 0.05) during the day than during the night. Similarly, pigs fed the HSFS diets had 5% to 9% higher heart rate and approximately 72% higher mean activity during the daytime compared with night. Overall, heart rate and activity tended to rise and fall in parallel and were highly correlated (Pearson *r* = 0.74, *P* = 0.009). Circadian variations in BP were not analyzed due to the potential for posture-dependent artifacts (see Materials and Methods).

Hemodynamic indices and sodium sensitivity. Comparison of BP parameters in Yucatan miniature pigs chronically fed a control diet (standard grower diet, 0.5% NaCl) compared with HSFS diet (4.5% NaCl) revealed that SAP was markedly elevated (by 24 mm Hg; *P* = 0.013) in pigs fed the HSFS diet. However, the tendency toward increased DAP (by 7 mm Hg) was not significant
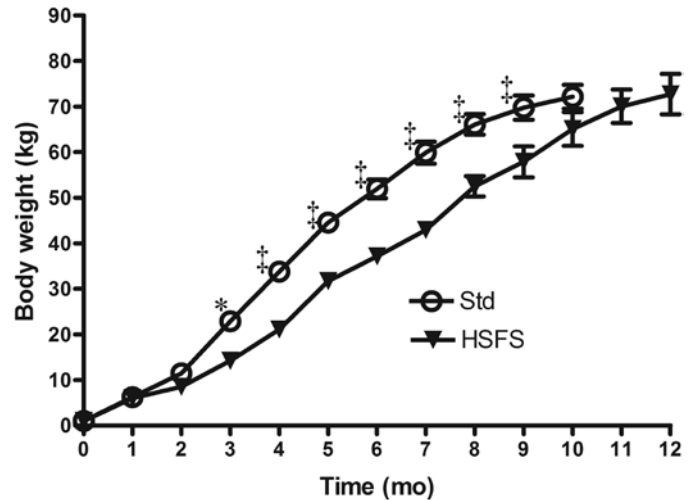
Figure 2. Growth (kg; mean ± SEM, *n* = 6 pigs per group) over time for Yucatan miniature swine on standard grower pig diet (Std) compared with those fed a diet high in salt, fat, and sugar (HSFS). Differences (*, *P* < 0.05; ‡, *P* < 0.001) between dietary treatments were assessed by 2-way repeated-measures ANOVA with Bonferroni posthoc tests.
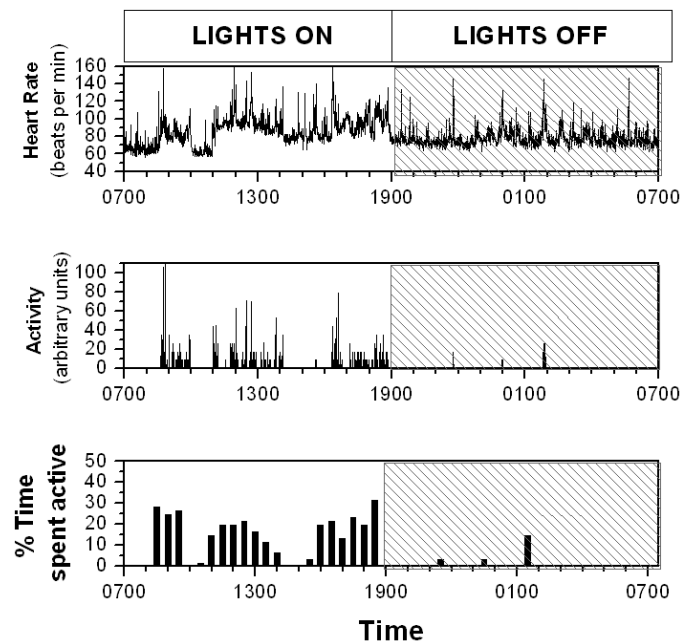
Figure 3. Representative 24-h recordings of heart rate (beats per min) and locomotor activity in a representative individual Yucatan miniature swine on standard grower pig diet. Day (lights son, 0700 to 1900) is represented by the unshaded section of graphs; night (lights off, 1900 to 0700) is represented by the shaded sections.

(*P* = 0.229; Table 2). To assess whether hemodynamic parameters were affected by age (in days) at this stage of development, linear regression analyses of data from the control pigs were performed. However, no significant correlations with age were noted for any of the parameters (*P* > 0.22).

The NaCl content of the diets clearly affected several hemodynamic indices (Figure 4). All pigs were moderately salt-sensitive (*P* < 0.05), as indicated by a 6% to 7% change in BP when the salt content of the diets was switched from 0.5% to 4.5% NaCl or

Table 1. Circadian heart rate and locomotor activity (mean ± SEM; n = 6 pigs) in adult Yucatan miniature pigs

			Dark period	Light period	Dark – light	P^a
Standard diet						
	0.5% NaCl, long-term[b]	Heart rate (beats/min)	82 ± 4	90 ± 2	−8 ± 3	0.047
		Inactive time (%)	93.5 ± 2.2	70.9 ± 4.1	22.6 ± 4.7	0.005
		Activity (arbitrary units)	0.5 ± 0.2	2.6 ± 0.7	−2.1 ± 0.7	0.025
		Activity >0 (arbitrary units)	17.1 ± 3.2	23.6 ± 2.9	−6.6 ± 3.9	0.155
		$\log_{activity > 0}$	1.17 ± 0.08	1.27 ± 0.04	−0.09 ± 0.08	0.308
	4.5% NaCl, short-term[b]	Heart rate (beats/min)	76 ± 2.3	84 ± 4	−9 ± 2	0.008
		Inactive time (%)	94.2 ± 0.9	69.1 ± 2.5	25.1 ± 2.2	<0.0001
		Activity (arbitrary units)	0.5 ± 0.1	3.0 ± 0.5	−2.5 ± 0.5	0.004
		Activity > 0 (arbitrary units)	20.5 ± 3.1	25.2 ± 3.4	−4.7 ± 2.4	0.109
		$\log_{activity > 0}$	1.25 ± 0.05	1.26 ± 0.06	−0.01 ± 0.05	0.835
HSFS diet						
	4.5% NaCl, long-term[b]	Heart rate (beats/min)	86 ± 2	95 ± 1	−8 ± 2	0.003
		Inactive time (%)	91.1 ± 1.9	67.8 ± 3.8	23.3 ± 3.6	0.001
		Activity (arbitrary units)	0.9 ± 0.2	3.2 ± 0.8	−2.3 ± 0.7	0.019
		Activity > 0 (arbitrary units)	20.2 ± 3.7	25.3 ± 2.0	−5.1 ± 2.3	0.081
		$\log_{activity > 0}$	1.21 ± 0.06	1.29 ± 0.03	−0.08 ± 0.04	0.067
	0.5% NaCl, short-term[b]	Heart rate (beats/min)	86 ± 4	91 ± 3	−5 ± 2	0.090
		Inactive time (%)	92.6 ± 2.0	71.4 ± 2.9	21.2 ± 2.7	0.001
		Activity (arbitrary units)	0.6 ± 0.2	2.5 ± 0.3	−1.8 ± 0.3	0.002
		Activity > 0 (arbitrary units)	16.4 ± 2.7	23.5 ± 2.3	−7.1 ± 3.3	0.081
		$\log_{activity > 0}$	1.15 ± 0.07	1.25 ± 0.03	−0.09 ± 0.06	0.179

[a]Significant ($P < 0.05$) differences between values for dark and light periods within dietary treatments were assessed by paired t test.
[b]Long-term feeding regimens were 9 mo for standard diet and 11 mo for HSFS diet. Short-term feeding regimens were 7 d for both diets.

Table 2. Summary of 24-h basic hemodynamic parameters (mean ± SEM; n = 6 pigs per group) for adult Yucatan miniature pigs fed a postweaning standard grower diet or HSFS diet

	Standard diet	HSFS diet	P^a
Systolic arterial pressure (mm Hg)	132 ± 3	156 ± 6	0.013
Mean arterial pressure (mm Hg)	111 ± 2	125 ± 5	0.035
Diastolic arterial pressure (mm Hg)	92 ± 2	99 ± 5	0.229
Pulse pressure (mm Hg)	40 ± 3	57 ± 2	0.005
Heart rate (beats/min)	86 ± 2	91 ± 2	0.109
Activity (arbitrary units)	1.6 ± 0.3	2.1 ± 0.5	0.486

Standard diet (0.5% NaCl) was fed for 9 mo after weaning; HSFS diet (4.5% NaCl) was fed for 11 mo after weaning.
[a]Significant ($P < 0.05$) differences between dietary treatments were assessed by one-way ANOVA.

vice versa (Figure 4). The magnitude of the responses to the salt content of the diets was similar between groups ($P > 0.05$). The acute high-salt challenge for the pigs on standard diet resulted in increases of 9 ± 3 mm Hg and 6 ± 2 mm Hg in SAP and DAP values, respectively. Similarly, the acute low-salt intervention in the pigs on HSFS diet resulted in decreases of 10 ± 3 mm Hg and 7 ± 3 mm Hg in SAP and DAP, respectively.

The average DAP in pigs fed the 0.5% NaCl HSFS diet for 7 d was 92 ± 3 mm Hg, similar to that in pigs chronically fed the 0.5% NaCl standard diet (that is, 92 ± 2 mm Hg). In contrast, although SAP and MAP were decreased by lowering the NaCl content from 4.5% to 0.5%, SAP remained approximately 14 to

15 mm Hg higher in HSFS-fed pigs than in those fed standard diet, regardless of the dietary NaCl content. This persistent difference of SAP resulted in an elevation of approximately 15 mm Hg in the pulse pressure of pigs fed HSFS diets, regardless of NaCl content.

Endothelial function: bradykinin-induced relaxation. To assess the vascular reactivity of coronary arteries, we measured bradykinin-induced relaxation in arteries that were contracted submaximally by exposure to U46619. The bradykinin concentration–relaxation curve (Figure 5) in HSFS-fed pigs ($r^2 = 0.97$) was shifted leftward compared with that of pigs that received standard diet ($r^2 = 0.99$). This finding indicates increased sensitivity to
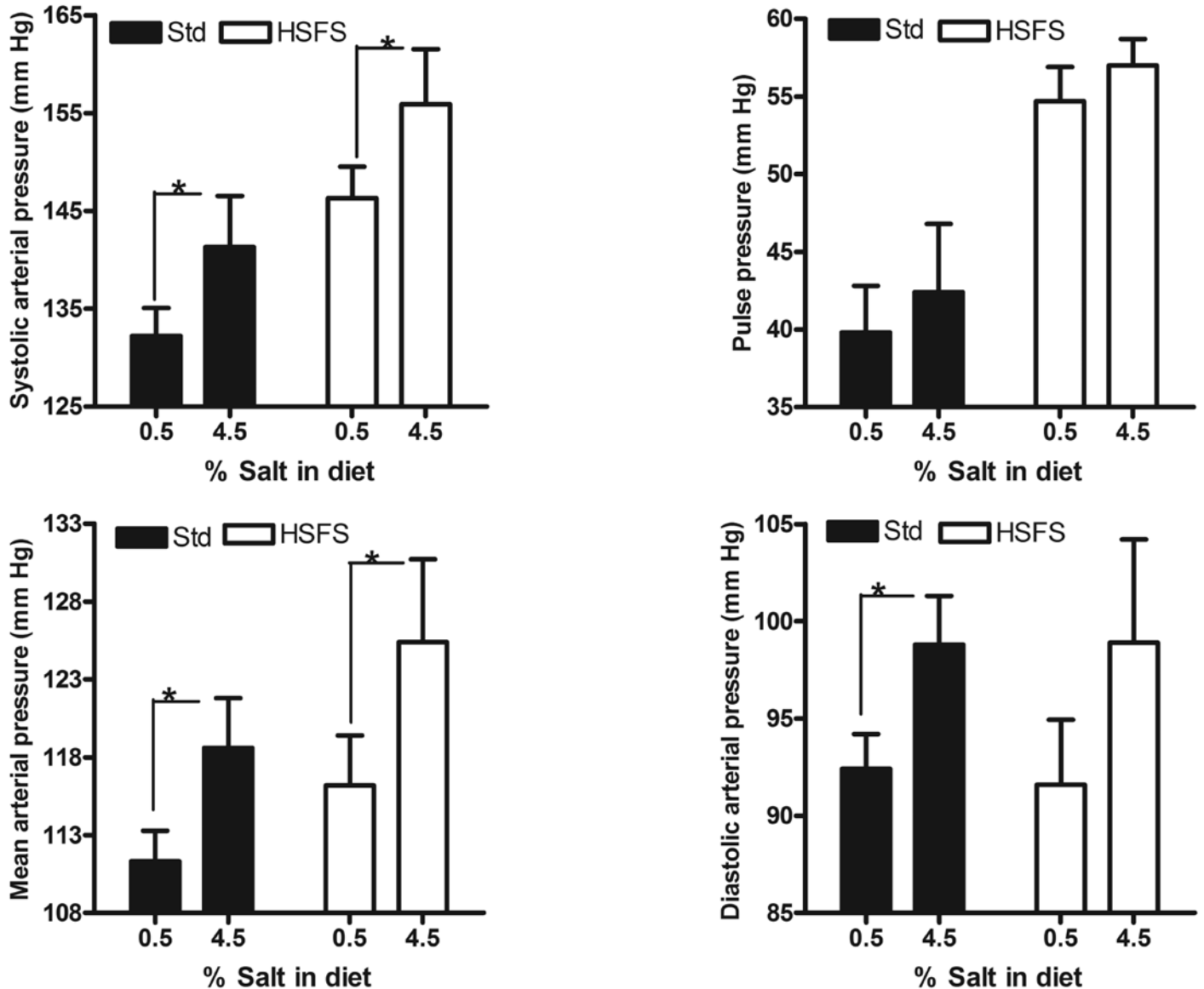
Figure 4. Effects of short-term salt challenge on blood pressure parameters in adult Yucatan miniature swine. Pigs on standard grower diet (Std) first received the typical version of the diet (0.5% NaCl) and then (7 d later) a high-salt (4.5% NaCl) version of the standard diet. Pigs on the HSFS diet first received the typical version (4.5% NaCl) of the HSFS diet and then (7 d later) a low-salt (0.5% NaCl) version. Data are given as mean ± SEM (n = 6 pigs per group). Significant (*, $P < 0.05$; paired t test) difference between the 2 dietary levels of salt within a group.

bradykinin in HSFS pigs (pD$_2$: standard, -8.44 ± 0.06; HSFS, -9.01 ± 0.07; $P = 0.003$). The maximum effect (standard, $100\% \pm 2\%$; HSFS, $97\% \pm 2\%$) and steepness (hill slope) of the bradykinin–relaxation relationship (standard hill, 0.63 ± 0.05; HSFS, 0.76 ± 0.09) did not differ between HSFS and standard diet groups (Figure 5).

Discussion

Swine have long been used for studying the basic mechanisms and pathophysiology of cardiovascular diseases.[8,19,24,32,34,36,43] The similarities in nutritional requirements and metabolism between swine and humans[36] make the swine an excellent model to study the effect of diet on the cardiovascular system. The typical North American diet is high in saturated fat, simple sugars, and NaCl and contributes to more than 30% of the heart attacks that occur worldwide.[1,42] In our current study, we found the BP of Yucatan miniature swine was significantly elevated in pigs chronically fed a North American-style diet. Although the increase in their BP was partly due to the moderate sodium sensitivity of their BP, other constituents of the diet (that is, fat and sugar) also appeared to have contributed to the effect on BP.

Sodium sensitivity of BP has been associated with increased risk of cardiovascular disease and premature death in hypertensive and normotensive humans.[26,41] In the current study, comparison of the responses to acute salt challenge in the context of both diets (standard compared with HSFS) suggests that any modest effect of the HSFS diet on DAP could be attributable, at least in part, to the NaCl content of the HSFS diet (Figure 4). That is, DAP values were similar between pigs that were acutely fed 0.5% NaCl
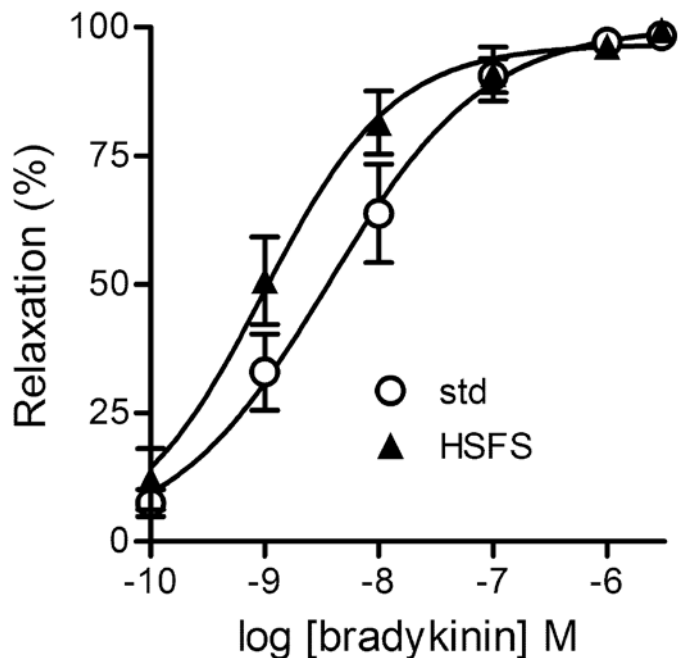
Figure 5. Bradykinin-induced relaxation (%; mean ± SEM, n = 6 pigs per group) of epicardial coronary artery rings in adult Yucatan miniature swine. Left anterior descending coronary arteries from pigs fed either the standard diet (Std) or HSFS diet first were exposed to U46619 for submaximal contraction and then increasing concentrations of bradykinin. 100% relaxation is equivalent to complete reversal of U46619-induced tone. The best-fit variable slope sigmoidal dose response curves (solid lines) differed significantly ($P < 0.005$, F test) between groups.

HSFS diet and those chronically fed 0.5% NaCl standard diet. Moreover, DAP values were similar between pigs fed the 4.5% NaCl HSFS and 4.5% NaCl standard diets. SAP and pulse pressure were considerably higher in pigs fed the HSFS diets, with the increase in SAP on HSFS diet amounting to approximately 24 mm Hg compared with that in pigs on a standard diet (Table 2). In contrast, acute (7 d) low-salt treatment (HSFS, 0.5% NaCl) reduced SAP by less than half of this amount (approximately 10 mm Hg).

These results suggest that both the fat and sugar contents of the diet affected SAP and pulse pressure. Indeed, this conclusion is consistent with studies in rats[33] and mice,[44] in which the effects of dietary fat, NaCl, sugar, and combinations of these components on systemic BP were evaluated and showed that NaCl had the most dramatic effect on BP, followed by the fat content of the diet. Overall, as in a North American-style diet, these nutrients exert synergistic harmful effects on blood pressure and long-term risks for cardiovascular diseases. Alternately, the persistently high SAP values that were not reversed completely by salt restriction may represent irreversible effects produced by chronic intake of the high salt content of the HSFS diet. In analyses of human cross population datasets, populations with higher levels of sodium intake have an increased rate of rise in SAP with age.[14,23] An increased rise in BP with age also occurred in chimpanzees[10] that were fed a high-sodium diet for almost 2 y (approximately 5% of their lifespan) and in regular outbred rats[9] that received a high-sodium diet for at least 1 y (approximately 50% of their lifespan). In the case of the chimpanzee study,[10] the progressive increases

in systolic BP appeared to be fully reversible with salt restriction, whereas the effects in rats were not, possibly because treatment was sustained for a far greater proportion of the lifespan. Overall, our current results provide a strong rationale for examining each of the constituents of the HSFS diet, including its high sodium content, to determine the precise cause of the persistent increase in BP associated with this North American-style diet.

Systolic hypertension is attributed chiefly to stiffening of the major arteries and is increasingly common with advanced age.[37] In humans, an increase of pulse pressure by approximately 10 mm Hg has been shown to be associated with an increase in cardiovascular complications and mortality of about 20%.[3] Although atherosclerosis appeared to be absent in the aorta of the pigs in this study, it is feasible that chronic exposure to the fat components of the diet, in addition to its high salt and sugar, could contribute to increased SAP and pulse pressure in pigs fed the HSFS diet due to another diet-related impairment in the vascular system.[17] High NaCl intake has been linked with stiffening of the central vasculature[31] and endothelial dysfunction,[11,12] in addition to increases in the BP level.[16]

Dietary sugar has also been linked with impaired endothelial function[4,21] as well as with increased BP itself. For example, results from one study[4] suggested that the sucrose component of a high-fat, high-sucrose diet fed to rats enhanced the deleterious effect of fat on BP and endothelial function in those animals. In contrast, a high-sucrose diet impaired muscle vasodilation but had no effect on BP in rats.[4]

In our current study, we hypothesized that a HSFS diet may lead to endothelial dysfunction evident as a reduced relaxation response to bradykinin in isolated coronary arteries. However, our results showed that the vascular relaxation response not only was intact in the HSFS group but was in fact enhanced. These observations are similar to those of other researchers who also reported increased coronary relaxation in Sinclair miniature pigs fed a high-fat, high-cholesterol diet.[13] Although the cause of the enhanced endothelial response is unknown, we speculate that increased sensitivity to bradykinin may reflect an upregulation of bradykinin type 2 receptors by proinflammatory mediators; this hypothesis that can be investigated in future studies.

A large body of cardiovascular data from pigs is available, particularly in relation to establishing models for studying metabolic syndrome.[20,28,30,35,39] Notably, in contrast to our data, other studies have shown that blood pressure is unchanged in Yucatan pigs fed high-fat diets.[28,30] However, in these cited studies, blood pressure was measured by using noninvasive, indirect techniques (that is, tail cuff), which may be less sensitive than assessment of 24-h average hemodynamic parameters by radiotelemetry.[22] Alternatively, the high-fat diet we used was also high in salt, which itself has the potential to account for the discrepant blood pressure outcomes. According to the definition of metabolic syndrome,[39] impaired glucose tolerance and insulin resistance are critical components in animals models of metabolic syndrome. Like us, several other researchers[28,30] have shown that Yucatan miniature pigs do not develop glucose intolerance or insulin resistance in response to high-fat, high-cholesterol diets, despite the development of dyslipidemia and hypertension.

In conclusion, our current data show that Yucatan miniature swine are a good experimental model to investigate hypertension associated with a North American style diet containing high levels of fat, sugar, and salt. Additional studies are required to

determine the contributions of individual components to the effects of this diet on BP.

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