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# Differential biomechanics in resistance arteries of male compared with female Dahl hypertensive rats

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**Background:** Increase in vascular stiffness is associated with a higher risk of cardiovascular morbidity and mortality and is likely sex-specific.

**Method:** Our objectives were to compare structural and functional alterations in small resistance arteries as related to vascular stiffness from Dahl salt-sensitive male and female rats ($n = 8$, mean ± s.e.m.).

**Results:** Arterial blood pressure and pulse wave velocity were significantly ($P < 0.05$) elevated in males ($161 \pm 3$ mmHg; $6.4 \pm 0.2$ m/s) and females ($147 \pm 2$ mmHg; $5.5 \pm 0.1$ m/s) on a high (H) salt compared with regular (R) diets but were significantly higher in males (H) than in all others. Significant increases in collagen and smooth muscle cell areas were evident in ultrastructure of mesenteric arteries of hypertensive males compared to normotensive or corresponding females. There were no significant differences in composite Young's modulus (CYM) between groups. Vasoconstriction resulted in significantly higher CYM in male (H: $8.6 \pm 1$ KPa) than R ($4.5 \pm 0.8$ KPa), and the corresponding females (H: $5.6 \pm 0.6$ KPa and R: $5 \pm 0.9$ KPa). In contrast, vasodilation significantly reduced CYM in the male groups (H: $2.5 \pm 0.4$ KPa and R: $2.7 \pm 0.5$ KPa) compared with the corresponding values in females (H: $4.2 \pm 0.6$ KPa and R: $5 \pm 0.5$ KPa). Moreover, the slope of pressure-volume curves revealed significantly greater distended vascular compliance in male H than R, and the corresponding females.

**Conclusion:** Our findings are supportive of a link between high salt intake and elevated blood pressure as being sex specific, likely involving sex-dependent changes in ultrastructure of the vessels, which ultimately may alter the biomechanics, and thus, the haemodynamic functions of both macro-circulation and micro-circulations.

**Keywords:** high salt diet and hypertension, sex differences, small resistance arteries, vascular elasticity, vascular structure and function

**Abbreviations:** Cym, composite young modulus; EMC, extracellular matrix; FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet; PWV, pulse wave velocity; TEM, transmission electron microscopy; VSMC, vascular smooth muscle cell

## INTRODUCTION

Arterial stiffness is defined primarily in terms of the changes to the mechanical properties (i.e. stress/strain relationships) of the arteries, and the associated fundamental morphological changes to the wall [1,2]. It is the primary determinant of vascular impedance influencing the systemic pressure-flow relationship [3,4], resulting in changes in hemodynamic function such as pulse wave velocity (PWV) and arterial pulse pressure. It is believed that the underlying structural changes and modifications in large conduit arteries cause arterial stiffness, and can significantly increase cardiovascular risk factors and mortality [5–7]. Remodelling (i.e. stiffening) of the arteries is an initially adaptive response to the stress posed on the blood vessel. Causally, it involves shear stress from the flow of blood across the vessel lumen, longitudinal stress from the surrounding tissues and circumferential stress from the blood pressure in response to physiological and pathophysiological changes in the body [8–10]. The change eventually becomes maladaptive as it compromises vessel mechanics functionality and integrity, contributing to cardiovascular complications.

Arterial stiffness can be considered to have two distinct but interconnected components, which are structural, and dynamic [11]. The structural component consists of the collagen and elastin fibres and other associated molecular states of the extracellular matrix (ECM), while the dynamic component consists of the smooth muscle tone. The dynamic component of arterial stiffness is tone-dependent and is influenced mainly by the vasoactive substances released by the endothelium as well as the nerves innervating the blood vessels [11,12].

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Elevated arterial stiffness is recognized as a surrogate endpoint for cardiovascular diseases due to its association with subclinical atherosclerosis and cardiovascular diseases, including angina, myocardial infarction, stroke, and heart failure [13]. Chronic high sodium intake is also suggested to be associated with hypertrophy of the arterial wall and ECM development independent of blood pressure [14]. This results in a subsequent increase in vascular stiffness and modified secretory populations of vascular smooth muscle cells (VSMCs) [14–16].

Dahl salt-sensitive rats have been used as a model for hypertension over decades, while both abnormal vasoconstriction and vasodilation have been described in blood vessels of this strain [17]. Distinct rapid (days) and slow phases (5 weeks) for elevation in blood pressure in Dahl salt-sensitive rats have been described for this model of hypertension [18,19]. In addition, differential functional responses to sympathetic nerve stimulation were found to exist in vasculature such as the mesenteric bed in Dahl salt-sensitives rats [20]. Further, morphological and structural changes have been also described in blood vessels from this strain [21,22].

In general, the evidence in the literature seems to mainly be concerned with stiffness of the larger conduit arteries and there is less emphasis on regional and local changes in smaller arteries in hypertension. Nonetheless, there is evidence in the current literature that suggests increased stiffness and augmented pressure wave (e.g. abnormal wave reflection) could additively contribute to the onset of hypertension [23,24]. It is possible that the crosstalk between micro-circulation and macro-circulation and the inter-connection between them leads to additive detrimental effects on the circulation due to increased global vascular stiffness [25,26]. There are limited reports on the direct relationship between the changes in vascular elasticity due to high sodium intake in both males and females. Thus, the primary objectives of this study were to compare the effects of high salt consumption and elevation in systemic arterial pressure on vascular biomechanics and function in small resistant arteries in males and females, which has not been previously studied. Accordingly, in the current study, we made comparisons on the biomechanical and pharmacomechanical functions (i.e. composite modules and compliance) and ultrastructure in small resistance arteries (150–200 μm) using pressure myography.

# MATERIALS AND METHODS

## Animals

All procedures on animals were carried out in accordance with the guidelines of the Canadian Council on Animal Care, with the approval of the Institutional Animal Care Committee of Memorial University of Newfoundland and the Canadian Council of Animal Care (Guide to Care and Use of Experimental Animals, Vol 1, 2nd Edition). Male and female Dahl salt-sensitive rats (age 5–6 weeks) were purchased from Charles River Laboratories. (Saint Constant, Quebec, Canada), housed two per cage and were kept in a temperature-controlled environment ($22 \pm 2^{\circ}$ C) on a 12 h-12 h light-dark cycle. They were given access to normal tap water and standard chow (regular) or Japanese style stroke-prone high salt diet containing 4% NaCl (Zeigler Bros., Inc. Gardners, Pennsylvania, USA) *ad libitum* for 6–7 weeks.

## Experimental design

At 6–7 weeks, each animal was anesthetized (induction 5% isoflurane in 100% $O_2$, maintenance 1.5–1.25% isoflurane in 100% $O_2$), and were injected with the analgesic, buprenorphine (0.01 mg/kg, subcutaneously). The core body temperature was maintained at $37 \pm 1^{\circ}$ C using a heating lamp and monitored with a rectal thermometer. The external iliac and carotid arteries were isolated and catheterized using polyethylene tubing [I.D. 0.58 mm, O.D. 0.965 mm (9 cm) connected to I.D. 0.28 mm, O.D. 0.61 mm (7 cm)]. The catheters were advanced forward (approximately 2 cm) such that the catheter in the femoral artery was just at the distal end of the abdominal aorta, while the catheter in the carotid artery was just beyond the aortic arch and in the proximal end of the thoracic aorta [27]. All catheters were filled with heparinized physiological (0.9% NaCl) saline (25 iu/ml). Central (aortic) and peripheral (femoral artery) blood pressure, as well as heart rate were continuously recorded by AcqKnowledge (3.9.1.6) software (Biopac Systems Inc., Goleta, California, USA) with a pressure transducer (P23XL; Spectramed Statham; Viggo-Spectramed, Oxnard, California, USA) for 20–25 min. The signals were amplified (DA 100A; Biopac Systems Inc.), wherein the amplifier was connected to a universal interface module (UIM 100; Biopac Systems Inc.), and to an acquisition unit (MP100; Biopac Systems Inc.). The analogue output signal was then converted to a digital signal (USB1W; Biopac Systems Inc.), and displayed in AcqKnowledge (3.9.1.6). Animals were euthanized by anaesthetic overdose and thoracotomy. The mesenteric arteries were removed and prepared for functional and histological studies. As well, the heart of each animal was excised, and the right ventricle and left ventricle as well as septum were separated and weighed. In addition, the length between the carotid artery catheter and the femoral artery catheter was measured at postmortem, and PWV was then calculated with the following formula $PWV = d/\Delta t$ [27].

## Pressure myograph experiments

All chemicals used in the pressure myograph experiments were purchase from Sigma Aldrich (Montreal, Canada) unless otherwise stated. The mesenteric bed was placed in a dissecting dish containing modified Krebs buffer with the following composition (mmol/l): 120 NaCl, 4 KCl, 1.2 $MgCl_2.6H_2O$, 1.5 $CaCl_2H_2O$, 25 $NaHCO_3$, 1.2 $KH_2PO_4$ and 0.1 EDTA in an oxygenated (95% $O_2$ and 5% $CO_2$) environment. The third-order branch of the mesenteric artery was determined to be the third branch off the superior mesenteric artery of the gut. A length of approximately 5 mm was isolated and carefully cleaned of surrounding tissues under a dissecting microscope as described by Jadeja *et al.* [28]. The mechanical properties of isolated third-order mesenteric arteries were studied with a pressure myograph. Isolated vessels were mounted onto the Single Vessel Chamber component of the Pressure Servo System (Living System Instrumentations, Model CH-1-SH/CH-1-QT

P100; St. Albans City, Vermont, USA) for the pressure myograph studies. In detail, the mesenteric arteries were mounted on two glass micropipettes, secured with 0.2 metric (10–0) surgical nylon suture obtained from Covidien (Monosof, Covidien LLC, Mansfield, Massachusettss, USA). The vessel length was adjusted so that the vessel walls were parallel without stretch. Intraluminal pressure was then set to a baseline pressure of 3.9996 KPa (1.0 mmHg = 0.1333 KPa), and allowed to equilibrate for 20 min at $37 \pm 1^{o}$C in a modified Krebs buffer gassed with a mixture of 95% $O_2$ and 5% $CO_2$. Vascular response was imaged using an inverted microscope and measured using a Video Dimension Analyzer (Living Systems Instrumentation) and the iWORX Data Recording Software (Dover, New Hampshire, USA). Three different groups of experiments were undertaken in assessment of the mechanical function of the blood vessel using two vasoactive agents phenylephrine (0.3 μmol/l), sodium nitroprusside (0.3 μmol/l) or equivalent volume (24 μl) of vehicle (distilled water). Five minutes after the additions of the vasoactive agents or vehicle, intraluminal pressure was raised stepwise at an increasing transmural pressure of 5.3329, 7.9993, 10.6658 and 13.3322 KPa to obtain a pressure-diameter (D) curves. For each vessel, the left wall and right wall thickness was also measured. In another group of experiments without the presence of any vasoactive agents, isolated third-order mesenteric arteries were pressure-fixed (13.3322 KPa) with Karnovsky fixative at $37 \pm 1^{o}$C for 30 min for ultrastructure assessment using electron microscopy.

### Calculation of mechanical parameters

The luminal diameter at baseline $D_1$, left wall and right wall were measured at various intraluminal pressures (5.3329, 7.9993, 10.6658 and 13.3322 KPa) using a video frame capture and real-time edge-detection system available with the Video Dimension Analyzer. The wall thickness was calculated using the following formula, WT = (LW + RW)/2.

Vascular compliance (C), which is the ability of a vessel to distend and increase volume with an increasing transmural pressure, is equal to changes in vessel volume (ΔV) divided by changes in transmural pressure (ΔP) (i.e. $C = \Delta V/\Delta P$).

The following mechanical parameters were calculated according to the methods by Intengan and Schiffrin [29].

Circumferential wall strain $(\varepsilon) = (D - D_0)/ D_0$, where $D_0$ is the diameter at baseline transmural pressure, D is the observed luminal diameter for a given transmural pressure. Circumferential wall stress $(\sigma) = (PD)/(2WT)$, where P is the intramural pressure, D and wall thickness are the luminal diameter and wall thickness, respectively.

To estimate the arterial stiffness independent of vessel geometry, the composite elastic ($E_c$) modulus of the vessel was determined where $E_c$ = stress/strain. The stress/strain data from each vessel was fitted to an exponential curve $y = ae^{bx}$ ($\sigma = \sigma_O e^{x\beta}$) (plots of *ln* y vs. x) in order to compensate for the nonlinear nature of the stress/strain relationship. In the equation, $\sigma_0$ is the stress at baseline transmural pressure, and β is a constant directly proportional to $E_c$, related to the rate of increase of the stress/strain curve, that is an increase in β implies an increase in $E_c$ (increase in stiffness) [29].

### Preparation of tissues for morphometry ultrastructure

For transmission electron microscopy (TEM), blood vessels were fixed in Karnovsky fixative at 4°C overnight Karnovsky [30]. Tissues were then washed in 0.1 mol/l sodium cacodylate buffer pH 7.4 and postfixed in 1% Osmium tetroxide, dehydrated in increasing concentrations of ethanol and acetone followed by infiltration with EPON resin using a modified protocol by Hyam [31]. Resin blocks were polymerized in BEEM capsules (Electron Microscopy Sciences, EMS) overnight at 70° C and cut to 100 nm with a diamond knife (Diatome), mounted on 300 mesh copper grids, stained with uranyl acetate and lead citrate (EMS), and examined using a Tecnai Spirit transmission electron microscope with an accelerating voltage of 80 kV.

For light microscopy, the same processing protocol was used, but the sections are 1 μm and placed on a glass slide and stained with Toluidine Blue in 1% sodium borate solution. Each section was examined on an Olympus FV300 microscope with a SC50 5 MP digital colour camera (Olympus Canada, Richmond Hill, Ontario, Canada).

### Morphometry

The morphometric parameters were calculated using a test system (grid) consisting of a coherent square lattice of points generated by a JAVA-written stereological tool (STEPanizer). The cross-sectional area of the various vessel components determined was then estimated as the area of the component per unit containing area according to the method described by Lee *et al.* [32], cross-sectional area $(A_A) = \left(\frac{\Sigma a}{\Sigma A}\right)t$.

Where, $t$ = thickness of serial sections, $a$ = area of the profiles for a and $A$ = area of the section. In order to compensate for eccentricity due to sectioning, the correction factor $(d_1/d_2)$ was used to estimate the true (corrected) cross-sectional area $A_C = A_A\,(d_1/d_2)$, where $d_1$ = short axial diameter and $d_2$ = long axial diameter.

### Statistical analysis

All the data (haemodynamics, morphometric, ultrastructure, composite elastic modulus and compliance values) were analysed using two-way analysis of variance (ANOVA) followed by Bonferroni test and/or one-way ANOVA followed by Bonferroni test. The statistical analysis was carried out with the SigmaPlot statistical package (Systat Software, San Jose, California, USA). The data are presented as means ± s.e.m., and the sample size is the number of animals used in each experiment ($n = 5–8$). A value of $P$ less than 0.05 was considered significant.

## RESULTS

The bodyweight of Dahl salt-sensitive female rats was generally lower than that of the Dahl salt-sensitive male rats in both groups (high salt and regular diets). Within both groups (male and female), there was no significant difference between the body weights; male regular diet (MRD): 363.3 ± 7.7 g, male high salt diet (MHS): 373.9 ± 8.6 g, female regular diet (FRD): 241.3 ± 8.1 g and female high salt diet (FHS): 251.0 ± 4.7 g.

**TABLE 1. Hemodynamic measurements in Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks**

| Haemodynamic | MRD | MHS | FRD | FHS |
|---|---|---|---|---|
| HR (beats/min) | $373 \pm 2^{b}$ | $386 \pm 3^{a,c}$ | $356 \pm 5$ | $367 \pm 4^{b}$ |
| cSBP (mmHg) | $131 \pm 2$ | $161 \pm 3^{a,c}$ | $132 \pm 2$ | $147 \pm 2^{b}$ |
| cDBP (mmHg) | $96 \pm 1$ | $117 \pm 3^{a,c}$ | $99 \pm 2$ | $108 \pm 2^{b}$ |
| cPP (mmHg) | $37 \pm 1^{b}$ | $44 \pm 1^{a,c}$ | $40 \pm 1$ | $39 \pm 1$ |
| pSBP (mmHg) | $130 \pm 2$ | $158 \pm 3^{a,c}$ | $130 \pm 2$ | $144 \pm 2^{b}$ |
| pDBP (mmHg) | $92 \pm 1$ | $118 \pm 3^{a,c}$ | $91 \pm 1$ | $104 \pm 2^{b}$ |
| pPP (mmHg) | $38 \pm 1$ | $40 \pm 1$ | $39 \pm 1$ | $42 \pm 1^{b}$ |
| PWV (m/s) | $4.9 \pm 06$ | $6.4 \pm 0.8^{a,c}$ | $4.5 \pm 0.1$ | $5.5 \pm 0.1^{b}$ |

Each value is expressed as a mean ± s.e.m. ($n = 8$).
cDBP, central DBP; cPP, central pulse pressure; cSBP, central SBP; FHS, female high salt diet FRD, female regular diet; HR, heart rate; MHS, male high salt diet; MRD, male regular diet; pDBP, peripheral DBP; pPP, peripheral pulse pressure; pSBP, peripheral SBP; PWV, pulse wave velocity.
[a]Significantly different from MRD diet; $P < 0.05$.
[b]Significantly different from FRD diet; $P < 0.05$.
[c]Significantly different from FHS diet; $P < 0.05$.

There were significant differences between heart rate of males and females either within or between groups on regular compared with high salt diets. Consumption of high salt diet caused significant increases in heart rate in both male and females (Table 1). Moreover, consumption of high salt elevated central and peripheral, SBP and DBPs independent of sex. Central and peripheral, SBP and DBP of males on high salt diet were significantly higher than the corresponding values in females. However, there were no differences in the central and peripheral, SBP and DBPs of male and female on regular diet (Table 1). Central pulse pressure was elevated following high salt consumption in male but not female animals. However, PWV (an index of vascular stiffness) became significantly elevated in both male and female animals on high salt compared to those regular diet. PWV was also found to be significantly higher in males than in corresponding females on high salt diet (Table 1).

The ratio of the left ventricle and septum to right ventricle of MHS ($5.8 \pm 0.2$) was significantly greater than that of the MRD ($4.5 \pm 0.3$), and female groups FRD ($4.7 \pm 0.1$) and FHS ($4.7 \pm 0.2$). It is apparent that the significant left ventricular hypertrophy found in MHS group is likely due to greater increase in systemic arterial pressure as well as vascular stiffness.

**TABLE 2. Morphometric analysis from electron microscopy images of third-order mesenteric blood vessels fixed at 13.33 KPa (1 mmHg = 0.1333 KPa) obtained from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks**

| | MRD | MHS | FRD | FHS |
|---|---|---|---|---|
| Adventitia ($\mu m^2$) | $39 \pm 5$ | $34 \pm 4$ | $41 \pm 9$ | $41 \pm 8$ |
| Media ($\mu m^2$) | $89 \pm 18$ | $158 \pm 15^{a,b}$ | $80 \pm 18$ | $86 \pm 14$ |
| Intima ($\mu m^2$) | $18 \pm 5$ | $21 \pm 3$ | $21 \pm 6$ | $35 \pm 10$ |
| IEL ($\mu m^2$) | $2.4 \pm 0.7$ | $4.0 \pm 0.7$ | $3.6 \pm 1$ | $4.0 \pm 0.4$ |
| EEL ($\mu m^2$) | $0.2 \pm 0.05$ | $0.3 \pm 0.09$ | $0.27 \pm 0.1$ | $0.23 \pm 0.05$ |

Each value is expressed as a mean ± s.e.m. ($n = 5$).
EEL, external elastic lamina; FHS, female high salt diet; FRD, female regular diet; IEL, internal elastic lamina; MHS, male high salt diet; MRD, male regular diet.
[a]Significantly different from MRD diet; $P < 0.05$.
[b]Significantly different from FHS diet; $P < 0.05$.

## Morphometric analysis

There was a significant increase in the area of media in the third-order mesenteric blood vessels of males on high salt compared with those on a regular diet or the corresponding females. There were no other significant changes in areas of adventitia, intima, internal elastic lamina or external elastic lamina associated with either sex or diet (Table 2).

The internal and external elastic laminae demarcating the medial layer of the third order of the mesenteric blood vessel were prominent in both male and female groups (Fig. 1). However, the external elastic lamina was poorly developed in both sexes compared with the well developed internal lamina regardless of diet. Evidence indicated a distorted, fragmented and discontinuous endothelia cell layer in the arteries of males on a high salt diet. The medial layer of the vessel wall appeared to be thicker in the males on high salt diet with increased layers of smooth muscle cells compared with the other groups. The medial layer consists of mostly smooth muscle cell, with little elastin and collagen in the intercellular space consisting of fragmented, discontinuous layers of elastic lamina found in the media with collagen sparsely distributed in the media layer (Fig. 1).

There were significant increases in collage and smooth muscle cell areas in the third-order mesenteric blood arteries of males on high salt compared with either males on regular diet or corresponding females. There were no significant changes noted in elastin area for these blood vessels within or between any of the experimental groups (Fig. 2). Furthermore, no significant differences were observed in the ratio of the areas of collagen/elastin, vascular smooth muscle/collage or vascular smooth muscle/elastin within and between the various groups (Table 3).

## Vascular mechanics

The composite elastic Young's modulus, a measure of vascular stiffness independent of geometry, was found not to be significantly altered in the third-order mesenteric blood vessels among the experimental groups (Fig. 3a). However, in the presence of a vasoconstrictor, phenylephrine (0.3 μmol/l), the composite Young's modulus was significantly higher in blood vessels from males on high salt compared with males on regular diets and the corresponding females (Fig. 3b). The presence of the vasodilator and nitric oxide donor, sodium nitroprusside (0.3 μmol/l), resulted in a significant reduction in the composite Young's modulus in blood vessels of males on regular and high salt diets compared with the corresponding values in females (Fig. 3c).

The evidence from pressure-volume curves in the third-order mesenteric arteries revealed significant elevation in the volume at the two highest pressures in males on high salt compared with males on regular diets and the corresponding values in females (Fig. 4a). There were no significant differences between pressure-volume curves for females due to diet. In the presence of the vasoconstrictor, phenylephrine (0.3 μmol/l), a significantly higher volume was observed with females on a high salt diet compared with those on regular diets, at the two higher pressures

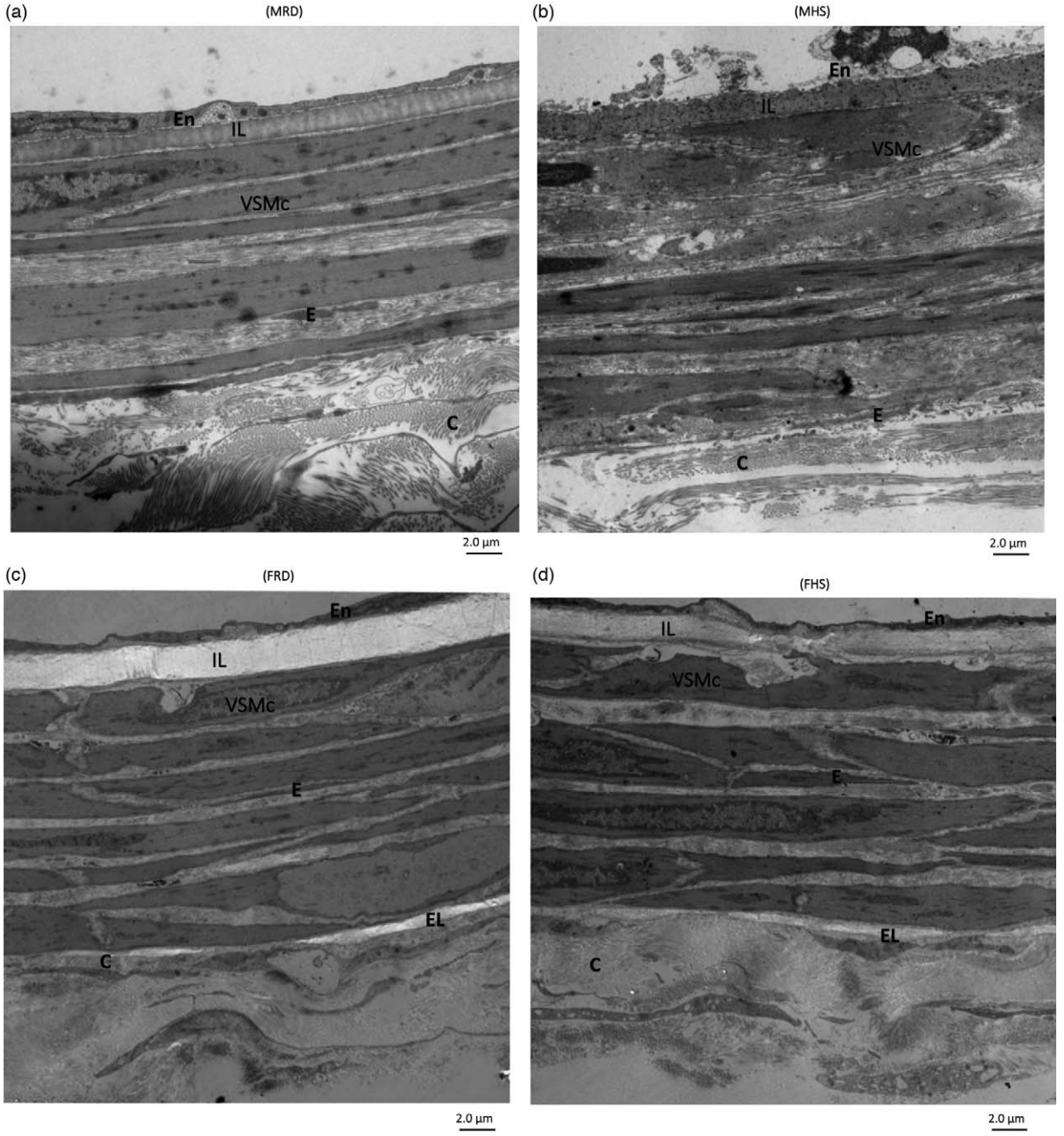


**FIGURE 1** Electron micrograph cross-sectional areas (direct mag: x1100) of the third-order mesenteric arteries from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks. C, collagen; E, elastin; EL, external elastic lamina; En, Endothelium; FHS, female high salt diet; FRD, female regular diet; IL, internal elastic lamina; MHS, male high salt diet; MRD, male regular diet.

(Fig. 4b). Also, a significantly higher volume was observed at the highest pressure in males on HSD with PE treatment (Fig. 4b). The presence of vasodilator, sodium nitroprusside (0.3 μmol/l), caused significant increases of volume in response to the two highest pressures in males compared with corresponding females on regular diet (Fig. 4c). It was evident that at the two highest pressures, significant increases in volume were observed in blood vessels from male compared with the corresponding females on high salt diet (Fig. 4c).

The calculated slope from the pressure-volume curves revealed significantly greater distended vascular compliance in males on high salt compared with males on regular diets, and the corresponding females on high salt diet (Fig. 5a). The presence of phenylephrine (0.3 μmol/l) did not significantly affect vascular compliance in males on regular compared with high salt diets (Fig. 5b). In contrast, phenylephrine significantly increased compliance in blood vessels of females on high salt compared with regular diets (Fig. 5b). The presence of sodium nitroprusside (0.3 μmol/l) significantly reduced vascular compliance in males on high salt compared with regular diets (Fig. 5c). The latter vasodilator also significantly increased vascular compliance in males on regular diet compared with the corresponding values in

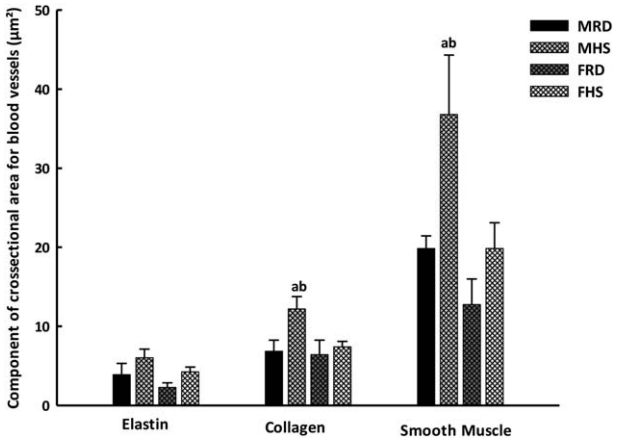


**FIGURE 2** Morphometric determination from electron microscopy images of content for elastin, collagen, vascular smooth muscle cell from third-order mesenteric blood vessels fixed at 13.33 KPa [1 mmHg = 0.1333 KPa) obtained from Dahl salt-sensitive] male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks. Each value is expressed as a mean ± s.e.m. ($n = 5$). FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet. [a]Significantly different from MRD diet; $P < 0.05$; [b]Significantly different from FRD; $P < 0.05$; [c]Significantly different from FHS; $P < 0.05$.

females also supporting the differential nature of the pressure-volume relationships in different sexes (Fig. 5c).

## DISCUSSION

Arterial stiffening results from complex interactions that involve structural and cellular elements, and whose changes are responsible for maintaining the mechanical properties of the arterial wall. The consequence of the alteration in the properties of load-bearing components of the arterial wall is the modifications of its mechanical characteristics, which are influenced by both intrinsic and extrinsic factors, such as sex differences and nutritional content [33–37]. Arterial stiffness and the resultant hemodynamic changes are now considered to be predictors of increase in morbidity and mortality; vascular stiffness is positively associated with increased risk of cardiovascular disease, including hypertension, myocardial infarction, heart failure, stroke [38–42]. Moreover, differential characteristics of the development of arterial stiffness between men and women appear to involve sex-specific mechanisms [43–47].

**TABLE 3. Morphometric determination from electron microscopy images of content for ratios for elastin, collagen and vascular smooth muscle cell (VSMC) from third-order mesenteric blood vessels fixed at 13.33 KPa (1 mmHg = 0.1333 KPa) obtained from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks**

| | MRD | MHS | FRD | FHS |
|---|---|---|---|---|
| Collagen/elastin | 2.68 ± 1.0 | 2.42 ± 0.6 | 3.14 ± 0.7 | 1.86 ± 0.2 |
| VSMC/collagen | 3.23 ± 0.7 | 2.75 ± 0.3 | 2.27 ± 0.5 | 2.75 ± 0.3 |
| VSMC/elastin | 6.96 ± 1.7 | 5.99 ± 0.9 | 6.18 ± 1.0 | 5.05 ± 0.8 |

Each value is expressed as a mean ± s.e.m. ($n = 5$).
FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet.

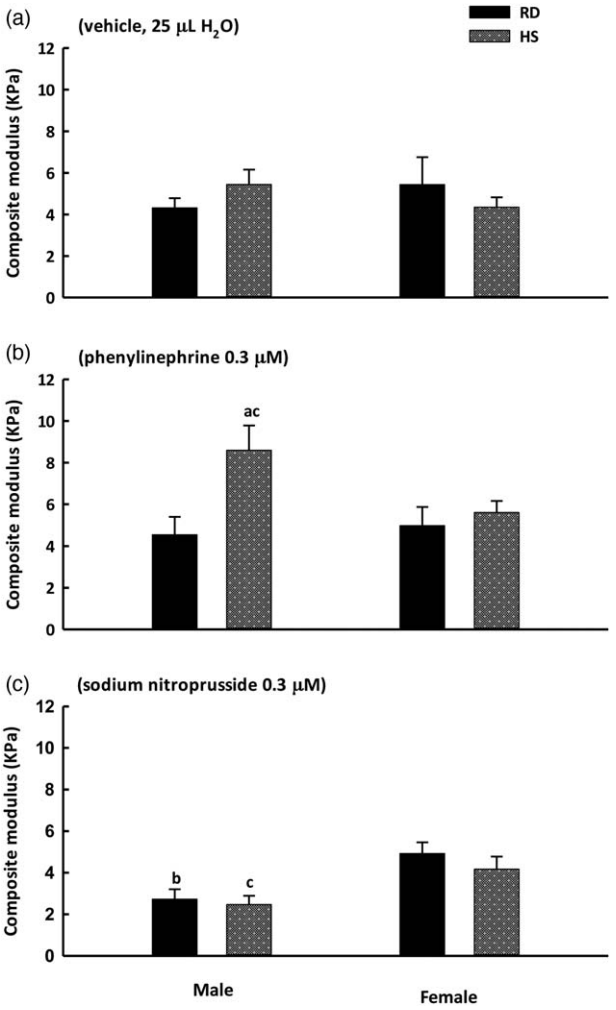


**FIGURE 3** Calculated values of composite Young's modulus (Kpa) using stress-strain plots in isolated third-order mesenteric arteries at various intravascular pressures from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks. Each value is expressed as a mean ± s.e.m. ($n = 8$). FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet. [a]Significantly different from MRD; $P < 0.05$; [b]Significantly different from FRD; $P < 0.05$; [c]Significantly different from FHS; $P < 0.05$.

Our investigation in Dahl salt-sensitive rats show that an increase in salt consumption in the diet induced a significant increase in both central and peripheral (systolic and diastolic) blood pressures, and heart rate of both males and females. However, the central and peripheral SBP and DBPs of the males were significantly higher than the corresponding female groups on high salt diet. This difference was absent in the male and female rats on a regular diet. Furthermore, the central pulse pressure, a surrogate of arterial stiffness, was elevated with increased salt consumption in males but not female animals. Sex-specific patterns have been reported in the association between high salt intake and arterial stiffness measured by PWV [43,48,49]. We found the PWV was significantly elevated in both male and females on high salt compared with regular diet. However, PWV was also significantly greater in males than the corresponding females on high slat diet. Surprisingly, on the basis of significant increase in the ratio of left ventricle plus septum to right ventricle in male on

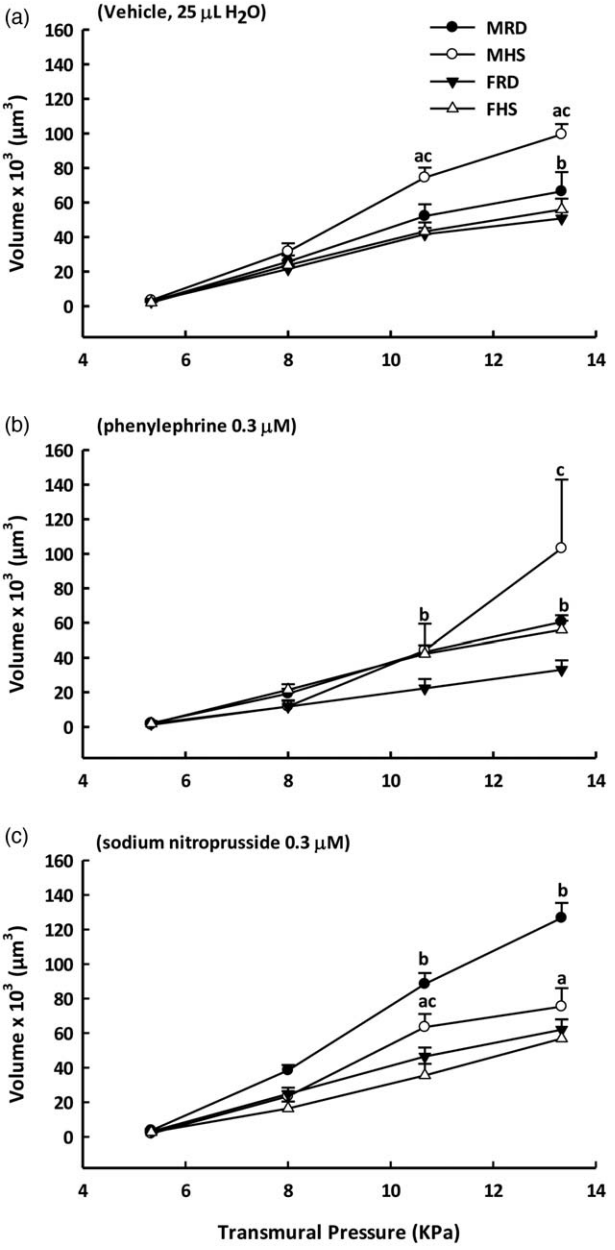


**FIGURE 4** Pressure-volume plots in isolated pressurised third-order mesenteric arteries from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks. Each value is expressed as a mean ± s.e.m. ($n = 8$). FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet. [a]Significantly different from MRD; $P < 0.05$; [b]Significantly different from FRD; $P < 0.05$; [c]Significantly different from FHS; $P < 0.05$.

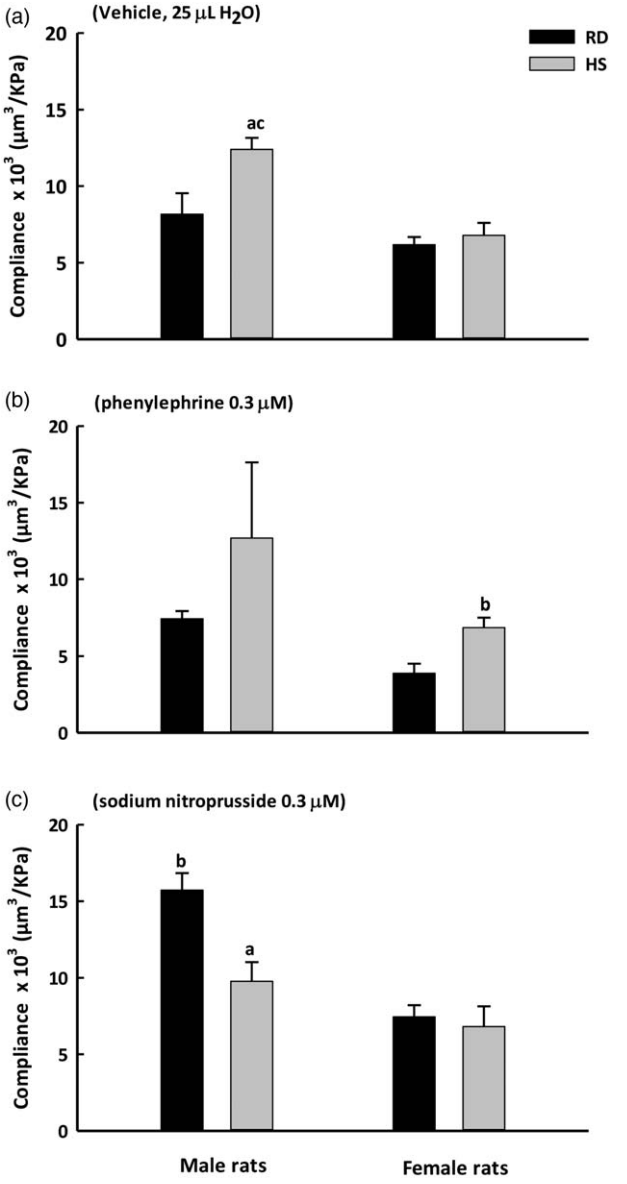


**FIGURE 5** Calculated values of compliance from pressure-volume plots in isolated third-order mesenteric arteries from Dahl salt-sensitive male and female rats on regular or high salt (4% NaCl) diets for 6–7 weeks. Each value is expressed as a mean ± s.e.m. ($n = 8$). FHS, female high salt diet; FRD, female regular diet; MHS, male high salt diet; MRD, male regular diet. [a]Significantly different from MRD; $P < 0.05$; [b]Significantly different from FRD; $P < 0.05$; [c]Significantly different from FHS; $P < 0.05$.

high-salt compared to all other groups, ventricular hypertrophy seems to only present in hypertensive males [50–52]. The results suggest the significant increase in systemic arterial blood pressure and PWV but not pulse pressure may lead to the existence of accommodating circumstance in the circulatory system of females on high salt diet that circumvents ventricular hypertrophy.

Evidence from several studies indicate a direct relationship between sodium intake and changes in systemic arterial blood pressure [34,53,54]. Accordingly, chronic consumption of high salt has been found to result in a significant increase in systemic arterial blood pressure linked to the onset of hypertension and increase morbidity and mortality [55–57]. In contrast, a decrease in salt consumption has been shown to decrease blood pressure, lower incidence of cardiovascular complications and better health outcomes [53,58,59]. Further, blood pressure responses to salt intake have also been reported to vary with sex and age [60–62].

Vascular changes from the large elastic arteries to microcirculation are known to occur as a result of elevated blood pressure [63–65]. Several studies have reported thickening of the walls of elastic and muscular arteries, remodelling of small muscular resistance arteries causing an increased wall to lumen ratio, as well as a reduction in the number of vessels in the microcirculation associated with elevated

systemic arterial blood pressure [66–68]. Our current findings show that there is structural remodelling in the third-order mesenteric vascular bed of the male group, that is an increase in collagen and smooth muscle component of the vascular wall. An effect that was absent in the parallel female group. Under normal physiological conditions, VSMCs are embedded in an elastin-rich ECM localized primarily in the media of the vascular wall. In hypertension, there is an increase in collagen synthesis and subsequent VSMC proliferation and migration that has been reported in the mesenteric and other vascular beds of several rat strains in response to increased stress on the vessel wall [69–71].

In contrast to the increased deposition of collagen due to the elevation of arterial blood pressure, collagen fibres are recruited over time at higher intravascular pressures to support passive tension resulting in increased vessel wall stiffening. Evidence in the literature also suggest that during elevated blood pressure, VSMCs undergo hyperplasia and hypertrophy, which is crucial for vascular remodelling and subsequent increase in the total peripheral resistance in response to stress from higher blood pressure [72]. In our present investigation, the increase in media thickness, cross-sectional area and increased media/lumen ratios suggest the possible development of hypertrophy and eutrophic remodelling associated with elevated blood pressure due to the consumption of high salt diet. The consequence of such modifications is an alteration of the stress/strain characteristics of the vessel wall, compliance and elastic modulus, which may predispose the circulatory tree to abnormal behaviour and hence, risks related to cardiovascular morbidity and mortality.

Morphological and physiological (functional) changes in the vasculature have been reported to occur during hypertension [73,74]. Salt-induced changes in vascular function is a consequential and/or contributing factor to vascular remodelling of the arterial wall that underlies elevated blood pressure. Our results from the pressure-volume curves reveal significantly greater distended vascular compliance in hypertensive (high salt diet) than normotensive (regular diet) males and the corresponding females on high salt diet. Our finding in these small resistance arteries is in contrast to other studies in large conduit vessels that suggest a decrease in compliance with hypertension due to the intake of high salt diet [75–77]. Sympathetic neural control is involved in the modulation of large artery function and vasomotor control of small resistance arteries. The influence of sympathetic nervous system as well as those of the local vasoactive mediators on vascular function are crucial elements in the development hypertension and related cardiovascular events.

In our current investigation, vasoconstriction due to the action of phenylephrine did not significantly affect vascular compliance over the pressure range in males on a regular compared with high salt diets. In contrast, phenylephrine significantly increased compliance in vessels of females on high salt compared with regular diets, likely due to the initial smaller lumen size diameter in males. Vasodilation using a nitric oxide donor, sodium nitroprusside, significantly reduced vascular compliance in males on high salt compared with regular diets. Furthermore, vasodilation significantly increased vascular compliance in males compared with females on regular diet. However, there was a significant decrease in vascular compliance in male compared with female on salt diet in the presence vasodilator, sodium nitroprusside, supporting the view that initial lumen diameter is likely responsible for the observed differences in vascular compliance over the pressure range in our investigation. The observed results may be due to differences in salt sensitivity and nitric oxide regulation between sexes as a result of the impact of hormonal regulation of endothelial function [78].

The composite elastic Young's modulus, a measure of vascular stiffness independent of geometry, was not significantly altered in the third-order mesenteric blood vessels among the experimental groups under baseline conditions. This is not perhaps surprising, as we noted that the ratios of the various components (i.e. collagen/elastin, VSM/collagen, VSMC/elastin) of vascular wall were found not to significantly differ in animals fed regular vs. high salt diets. However, in the presence of a vasoconstrictor (phenylephrine), the composite Young's modulus was significantly higher in blood vessels from males on high salt than males on regular diets and the corresponding females. This can likely be attributed to the combination of functional (i.e. pharmacomechanical coupling) and morphological changes in blood vessels of male animals on high salt diet [79–81].

In our studies, the presence of the exogenous nitric oxide donor, sodium nitroprusside, resulted in a significant reduction in the composite Young's modulus in blood vessels of males on regular and high salt diets compared with the corresponding values in females. Accordingly, such a response would be expected to mask the role of the active component of the vascular wall in modulating vascular function. The outcome may support the view that vascular tone is elevated *in vivo* due to reduction in endothelial cell function with increased salt consumption. The latter alteration in function may accentuate vascular stiffness due to the presence of vasoconstrictors even though under baseline condition composite Young's modulus was noted to be unchanged in animals fed a high salt diet. Moreover, this is an effect that seems to be suppressed in females perhaps due to the presence of other vasculoprotective factors such as. sex steroid hormones.

In summary, our findings suggest the link that exits between high salt diet and elevated blood pressure is sex specific. It likely involves sex-dependent changes in the ultrastructure of the blood vessel which ultimately could alter the biomechanics. Moreover, it is likely that both high salt intake and pressure play a role in the vascular remodelling, and the combination seem to produce a greater effect in male compared with female animals in our study. It is also possible that the haemodynamic functions of both micro-circulation and macro-circulation may lead to additive detrimental outcomes, and this is a novel concept that requires systematic clinical investigation.

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## Conflicts of interest

None.

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