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Short term high salt intake reduces brachial artery and microvascular function in the absence of changes in blood pressure

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Abstract

Objectives—The aims of this study were to 1) test the hypothesis that short-term high salt (HS) intake reduces macro and micro vascular endothelial function in the absence of changes in blood pressure and 2) to determine if acute exercise restores endothelial function after HS in women.

Materials and Methods—12 women were administered high salt (HS; 11 g of sodium chloride for 7 days) and then underwent a weightlifting (WL) session. Brachial artery flow-mediated dilation (FMD) and nitroglycerin dilations (NTG) were measured with ultrasound at baseline, after HS, and after WL. Subcutaneous fat tissue biopsies were obtained at baseline and after HS and after WL. Resistance arteries (RA) from biopsies were cannulated for vascular reactivity measurements in response to flow (FID) and acetylcholine (ACh).

Results—Blood pressure was similar before and after HS diet. Brachial FMD was reduced after HS diet but was not affected by acute WL. Brachial NTG dilations were similar before and after HS. FID and ACh induced dilation (AChID) of RA were similar pre and post HS diet. FID and AChID was not altered by WL after HS diet. However, L-NAME significantly reduced FID at baseline and after exercise but had no effect dilator reactivity after HS diet alone.

Conclusion—These data suggest that 1) HS intake reduces brachial artery endothelial function and switches the mediator of vasodilation in the microcirculation to a non-nitric oxide dependent mechanism in healthy adults and 2) acute exercise may switch the dilator mechanism back to NO during HS diet.

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Keywords

high salt diet; endothelium; resistance exercise; flow mediated dilation; microcirculation

Introduction

During the past two decades, it has become more widely accepted that high dietary salt intake represents a risk factor for the development of cardiovascular (CV) disease that is independent [1, 2] of its association to increased arterial blood pressure [3–6]. Recent studies in normotensive animals [7–9] and in humans [10, 11] have revealed that a key-feature of this pressure-independent effect of high dietary salt intake is its adverse impact on endothelial function. Endothelial dysfunction is thought to be a precursor to CV disease, and is one of the earliest detectable outcomes in the pathophysiology of cardiovascular diseases, such as atherosclerosis and hypertension [3, 5]. Studies in animal models have demonstrated that high dietary salt intake significantly alters vascular reactivity in conduit vessels and the microcirculation and the likely mechanism is reduced NO bioavailability [7, 8, 12]. However, studies of high dietary sodium intake on vascular function in humans have shown more conflicting results. While some studies demonstrate that acute salt loading leads to reduced vascular NO bioavailability [10, 11], others have failed to find a link between dietary salt intake and NO-dependent vascular function [13–15]. The mechanisms by which HS intake impacts the endothelium and the time required for such changes to occur are unknown.

On the other hand, it is well recognized that EX protects against the development of cardiovascular diseases [16] and may improve endothelial function [17]. While aerobic EX clearly improves cardiovascular function, the benefits of acute resistance EX are less certain. Participants in resistance training programs are known to suffer fewer post-exertion cardiac events [18], and resistance EX is commonly recommended for cardiovascular health and disease prevention [19, 20]. However, weight lifting can induce large, transient increases in arterial pressure [21] and a pro-inflammatory response that may impair endothelial function [22]. The mechanisms by which acute EX restores endothelial function in conductance and resistance arteries after dietary salt manipulation are unknown.

Some evidence suggests that the classical risk factors for cardiovascular diseases may not apply to women as they do in the men, particularly in regard to coronary calcification, diastolic heart failure and microvascular disease [23, 24]. Moreover, recent studies reported that salt loading has divergent effects on vascular function between men and women [25]. Thus, the primary goals of the present study were to 1) test the hypothesis that short-term HS intake impairs brachial artery endothelial function and microvascular FID and AChID in the absence of changes in blood pressure or vascular stiffness and 2) to determine whether acute EX reverses endothelial function after short term HS loading in women.

Materials and Methods

Study participants

Twelve healthy inactive women without known cardiovascular disease risk factors were enrolled in this study. Women were recruited in an effort to isolate the vascular changes associated with HS intake in women. Exclusion criteria included men, diabetes, hypertension, pregnancy, cardiovascular disease or events, thyroid disease, pituitary tumor, a genetic disease causing disability, gout, illicit drug use, and body mass index $\geq 30 \text{ kg/m}^2$. Written informed consent was obtained from each participant. The study conformed to the standards set by the latest revision of the *Declaration of Helsinki* and was approved by the Institutional Review Board of the University of Illinois at Chicago. The study protocol was performed at the Clinical Interface Core at the University of Illinois at Chicago, Chicago, IL, USA. Experiments on isolated resistance arteries were performed at Vascular Biology Laboratory at the Department of Physical Therapy, College of Applied Health Sciences, University of Illinois at Chicago, Chicago, IL, USA.

Study protocol

Average daily salt intake in US is approximately 5 g of NaCl/day [26]. All participants were subjected to HS diet of 11 g NaCl by administration of 6 g salt supplement. A venous blood sample was taken before and after 7 days of HS via an intravenous cannula after 30 min in the supine position after an overnight fast. Fasting lipid panels (total cholesterol, lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), insulin, and renin and aldosterone levels were measured using spectrophotometric assay. Glucose concentration was measured using the glucose oxidase procedure (Beckman Coulter Inc; Fullerton, CA). A urine sample was collected before and after HS diet to test urine sodium and potassium levels. Manual blood pressure and heart rate (HR) were measured in a seated position and were recorded as the mean of three repeated measurements. For non-invasive blood pressure measurement oscillometric blood pressure monitor (WelchAllyn, Skaneateles Falls, New York, USA) was used, and between different stages of exercise protocol aneroid sphygmomanometer was used. Gluteal fat pad biopsies were obtained before and after HS diet or acute EX, as previously described [27].

Total body resistivity was measured with a four-terminal portable impedance analyzer (RJL Systems, Detroit, MI). Measurements were made while the subjects lay comfortably in supine. Empirically derived formulas provided by the manufacturer of the instrument were used to calculate estimated Fat Mass, Fat-Free Mass (FFM), Lean-Dry Mass (LDM), Total Body Water (TBW), Intra-Cellular Water (ICW) and Extra-Cellular Water (ECW) [28, 29].

Measurement of Flow Mediated Dilation (FMD)

All imaging studies were performed in the morning after an overnight fast. Similar to previous studies [29, 30], ultrasound imaging of the brachial artery was performed (Miromaxx; SonoSite, Seattle, Washington). Brachial FMD may vary during the menstrual cycle[31] and therefore, we recruited women during the early follicular phase of menses. Imaging of the brachial artery was performed in a longitudinal plane, at approximately 5 cm proximal to the antecubital fossa of the right arm, abducted approximately 80° from the

body, with the forearm supinated. The ultrasound probe (11 MHz) was positioned at a 60° insonation angle to visualize the anterior and posterior lumen-intima interfaces, to measure diameter or central flow velocity (pulsed Doppler). After baseline, Doppler readings of flow velocity were performed. A BP cuff was placed on the forearm, distal to the antecubital fossa of the imaged arm, and inflated to 60 mmHg above baseline systolic BP for 5 min. The brachial artery was then imaged continuously after BP cuff release. The response to NTG were used for the determination of endothelium-independent vasodilation. As previously described in adults by our group and others[30, 32], a sublingual NTG tablet (0.4 mg) was administered after obtaining a baseline brachial diameter, and brachial artery images and measurements were repeated for 5 minutes.

Images were digitally recorded using Brachial Imagery (Medical Imaging, Iowa City, Iowa) and analyzed as previously described [30]. Four hundred fifty frames were captured, digitized, and analyzed from M-line (the border between the intima and media of the brachial artery) of the same location of blood vessel using visible landmarks with edge detection software. Approximately 75 frames (7.5 frames/s for 10 s) were analyzed for each baseline and time point measurement through a mean of brachial artery diameters over the entire RR interval. FMD and the response to NTG were calculated using the averaged with the largest mean values obtained after release of the forearm occlusion or administration of NTG.

Measurement of carotid-radial PWV and the calculated central blood pressure

The PWV was determined using the peripheral tonometry (Sphygmocor; ATCOR Medical, Sydney, Australia) as previously described [33]. In brief, the common carotid artery and radial artery pressure waveforms were recorded non-invasively by using a pressure-sensitive transducer. The two pressure waveforms were then stored in a memory buffer. A preprocessing system automatically analyzed the gain in each waveform and adjusted it for equality of the two signals. When the operator observed a pulse waveform of sufficient quality on the computer screen, digitization was then suspended and calculation of the time delay between the 2 pressure upstrokes was initiated. The distance traveled by the pulse wave was measured manually with a tapeline as the distance between the two recording sites (D), while the pulse transit time (t), which was measured between the feet of the pressure waveforms that were recorded at these different points (the foot-to-foot method), was automatically determined. The PWV was automatically calculated as the $PWV=D/t$. Measurements were taken over 20 cardiac cycles and when the difference of consecutive mean data during the repeated measurements was less than 0.5 m/sec. The mean data was then used for the final analysis.

The calculated central blood pressure was obtained non-invasively by performing radial artery pulse wave analysis. The main principle of this method is based on deriving the central aortic pressure waveform from the peripheral pressure waveform by using generalized transfer functions. Analysis of the derived aortic pressure waveforms was performed with the Sphygmocor System (ATCOR Medical, Sydney, Australia) [33].

Exercise protocol

A free-weight leg press machine was used for this study as previously described [34]. Subjects sat in the machine with legs superior at an $\sim 30^\circ$ angle. Weight was added using standard steel plates. Each subjects started exercise (EX) with minimum resistance (~ 90 pounds) and weight was added as tolerated. The weight lifted was progressively increased with each set (up to 6 sets of 10 repetitions) until systolic blood pressure reached >170 mmHg during the final repetition.

Gluteal adipose biopsy and resistance artery function

Microvessels in subcutaneous fat are easily accessible and have been used to investigate microvascular function in humans [27, 35]. Approximately 2 ml of fat tissue was removed and transferred to (4°C) HEPES solution. Resistance arteries (approximately $150\ \mu\text{m}$) were isolated, prepared and cannulated in an organ chamber with glass micropipettes filled with KREBS solution ($\text{pH} = 7.40$) as previously described [27, 36]. Preparations were visualized with video cameras and monitors (model VIA-100, Boeckeler, Tucson, Arizona). The internal diameter of each vessel was initially measured at baseline (~ 60 mmHg intraluminal pressure) and after administration of endothelin-1, followed by incremental flow. Flow was produced by simultaneously changing the heights of the reservoirs in equal and opposite directions to generate a pressure gradient of 10, 20, 40, 60, 100 cmH₂O. In separate experiments, dilation to acetylcholine (ACh, 10^{-9} to 10^{-4} M) was determined. Arteriolar FID and AChID of arterioles were measured in absence and presence of: a) the NO synthase (NOS) inhibitor N ω -nitro-L-arginine methyl ester (L-NAME; 10^{-4} M), b) cyclooxygenase inhibitor indomethacin (INDO; 10^{-5} M), and c) the selective cytochrome P450 pathway inhibitor 17-octadecynoic acid (17-ODYA; 10^{-5} M). Inhibitors were added to the external bathing solution of the organ chamber 30 minutes. Maximal diameter of every vessel was determined in the presence of papaverine (10^{-4} M) at the end of each experiment. The diameter in response to flow at a gradient of 100 cmH₂O in both directions was measured in the presence of papaverine to confirm equal pipette diameter [36].

Materials

Chemicals were obtained from Sigma-Aldrich. Final molar concentrations of agents in the organ chambers were reported. None of pharmacological antagonists or inhibitors produced significant changes in baseline microvessel diameter and resulted in less than a 1% change in total volume.

Statistical Analysis

All results are presented as mean $\pm$ SD. Normality was tested using the Shapiro-Wilk test. Clinical characteristics before and after specific diet protocols were compared by paired t-test. Wilcoxon rank-sum test was used when variables were not normally distributed. A Student t-test was used to compare parameters between experimental protocols. When variables were not normally distributed, the Mann-Whitney Rank Sum Test was used. In separate analysis, the mixed effects model was performed to examine the effects of HS and exercise on FMD while controlling for baseline brachial diameter. Dilation due to FID or AChID is expressed as a percentage, with 100% representing the change from constricted

diameter to the maximal diameter at 60 cmH₂O intraluminal pressure (usually in the presence of papaverine). Responses to flow and ACh were analyzed with a 2 factor, repeated measures ANOVA to determine the effect of the treatment on the response. To compare maximum dilation to papaverine between experimental protocols Student t-test was used. Due to the pilot nature of the study, we did not conduct a formal power analysis and included only a small cohort of convenient sample (N=12). Regardless, our sample size of 12 has enabled us to detect a large effect size (ES>0.90) for primary study endpoints reported in this study (i.e., urine sodium, PRA, and %FMD). The results of our data could provide a more precise power estimate for future studies. Statistical significance was set at P<0.05. SigmaPlot (version 11.2, Systat Software, Inc, Chicago, USA) program was used for statistical analysis.

Results

Clinical characteristics

12 women (mean age 28±7 years) participated in the study. Anthropometric (BMI, WHR, and body fat) measurements are shown in Table 1. Urine sodium and potassium levels, and plasma renin activity and serum aldosterone levels were measured to confirm elevations in sodium intake. Urine sodium levels were significantly increased after HS diet protocol (Table 2). Urine potassium level was not significantly changed after HS diet. As expected, from HS diet plasma PRA was significantly decreased while aldosterone levels were similar (Table 2).

Blood pressure response to HS diet and exercise

Blood pressure measurements confirmed that the participants were normotensive before the diet period and BP did not change significantly after the HS diet period (Table 3). HR was similar before and after HS diet (HR/min baseline 67±13 vs. HS diet 71±13, P=0.288). All subjects showed an increase in SBP, DBP and MAP during heavy weight lifting compared to baseline blood pressure values (Table 3).

Brachial artery endothelium-dependent and endothelium-independent dilation

Blood vessel characteristics before and after HS diet and acute EX session are shown in Table 4. Baseline and peak brachial diameters were similar among the groups. Unadjusted brachial artery FMD was significantly reduced after 7 days of HS compared to baseline (%FMD baseline: 10.3±3.1 vs. post HS diet: 7.0±3.0, P=0.006). When baseline brachial artery diameter was tested as a predictor, a significant difference before and after HS was still observed (see data supplement table: P<0.0047). Brachial artery FMD was similar after acute EX (%FMD post HS diet 7.0±3.0 vs. post EX: 6.6±3.2, P=0.416) (Figure 1), adjustment for baseline diameter did not change the effect of exercise on FMD. Brachial NTG-mediated dilation was not significantly different before and after HS diet (%NTG baseline: 29.7±6 vs. post HS diet 32.1±7.8, P=0.517). Acute EX had no effect on NTG dilation (% NTG post HS diet 32.1±7.8 vs. post EX 29.4±6.3, P=0.526) (data supplement). While urinary sodium was increased on the HS diet there was no correlation found between the urinary sodium levels and %FMD (r= -0.143; P=0.542).

Carotid-radial PWV and the calculated central blood pressure

Carotid-radial PWV was not significantly changed after 7 days HS diet (PWV m/s before HS diet: 7.12 ± 1.31 vs. after HS diet: 6.63 ± 1.28 , $P=0.315$). Similarly, brachial artery SBP, DBP and MAP, and calculated central blood pressures were similar before and after HS diet (Table 3).

Microvascular resistance artery endothelial-dependent and endothelium-independent dilation

There was no significant reduction in FID and AChID of resistance arteries isolated from gluteal subcutaneous fat after 7 days of HS diet (FID % of maximal dilation before HS diet: 100 cmH₂O 79.2 ± 9.9 vs. post HS diet: 100 cmH₂O 66.5 ± 7 ; AChID % of maximal dilation before HS diet: 10^{-4} M ACh 75.5 ± 9.8 vs. post HS diet: 10^{-4} M ACh 62.7 ± 12.6). Furthermore, FID and AChID of resistance arteries were not altered following acute EX (FID % of maximal dilation post EX: 100 cmH₂O 76.8 ± 9.6 ; AChID % of maximal dilation post EX: 10^{-4} M ACh 71.8 ± 14.7). However, L-NAME reduced FID in resistance arteries at baseline and after EX, but had no effect immediately following HS (Figure 2A). In addition, indomethacin significantly reduced FID at baseline, but not after HS or EX (Figure 2B). The cytochrome P450 inhibitor, 17-ODYA had virtually no effect on FID except at higher pressure gradients at baseline (> 40 cmH₂O gradient; Figure 2C).

Dilation of resistance arteries to ACh was reduced by L-NAME, INDO, and 17-ODYA at baseline (Figure 3A). While the magnitude of inhibition was greatest after L-NAME (Figure 3A), there was no effect of L-NAME on arteriolar AChID after HS diet (Figure 3B). Following EX the effect of L-NAME on reducing AChID was restored (Figure 3C). Treatment of vessels following HS with INDO resulted in paradoxical vasoconstriction of arterioles in response to ACh (Figure 3B). However, indomethacin administration did not alter FID after EX (Figure 3C). In contrast to FID, 17-ODYA significantly reduced AChID after HS diet and EX (Figure 3B and 3C).

Discussion

There are several important findings of this study. First, elevated dietary salt intake for 7 days reduced brachial artery FMD in the absence of changes in blood pressure or vascular stiffness in healthy inactive women. The effect of HS on brachial FMD was not reversed by acute EX. Second, the mechanism of arteriolar dilations to ACh and flow following HS diet were no longer NO dependent. Third, NOS inhibition (but not cyclooxygenase inhibition) of AChID and FID was restored following acute EX after HS intake. These data suggest that elevation of daily salt intake for one week reduces endothelial function in conduit arteries. Interestingly, even though it seems that acute EX did not alter brachial artery endothelial function or microvascular FID and AChID following HS diet, it appears that acute exercise may have an important role in restoration of the NO-dependent vasodilation.

Excessive salt intake is a major problem in almost all parts of the world (e.g., Americans on average, consume more than 5g of salt daily) [26]. Moreover, HS intake contributes to the estimated one in three adults who will develop high blood pressure [26]. Sodium intake

during HS loading in this study was approximately 11 g of salt daily and the range of sodium intake in our study is not uncommon in westernized diets [2]. Elevated urinary sodium levels in subjects on HS diet confirm exposure to HS. In addition, PRA was suppressed after HS diet, providing additional confirmation of adherence to the HS diet (Table 2).

Many epidemiological and interventional studies have demonstrated a clear relationship between salt intake and hypertension [37, 38]. However, previous clinical studies have not been able to separate the effects of dietary salt intake and blood pressure on endothelial function [13]. Recent studies indicate that excess dietary salt consumption, even in the absence of elevations in blood pressure, may be one of the contributing factor to the development of endothelial dysfunction [10, 11]. Similarly, in our study, there was no effect of one week of HS on brachial SBP and DBP. Blood pressure sensitivity to changes in sodium intake in young healthy individuals and the duration of HS consumption needed to increase blood pressure in salt-sensitive ones is still not defined. Several recent studies have shown that HS intake reduced vascular reactivity in humans without elevations in blood pressure [10, 39, 40]. One study, demonstrated that 14 days of HS diet provoked minimal changes in brachial BP. However, there was a strong association between dietary salt and carotid BP [41]. In our study, there was no significant change in brachial artery SBP and DBP, as well as in calculated central SBP and DBP and MAP, after one week of HS diet. In none of our participants SBP values were not changed by more than 5 mmHg after HS diet, so we can conclude that they are salt resistant, at least for the salt effect on blood pressure values.

The results of present study show reduced brachial artery FMD after 7 days of elevated dietary salt intake in young healthy adults. These results are consistent with those of several other groups. For example, Lenon-Edwards et al. have shown that 7 days HS diet reduced FMD in both men and women [25]. DuPont et al. have demonstrated that high dietary sodium intake impairs FMD of brachial artery in healthy salt-resistant humans [40]. Tzemos et al. demonstrated reduced vascular function after 7 days of HS intake, as evaluated by measuring forearm blood flow by plethysmography [10]. Liu et al. observed lower FMD of brachial artery in normotensive salt sensitive compared to non-salt sensitive adults [14]. In contrast, Stein et al. found no effect of salt intake on methylcholine-induced vasodilation in the forearm in a group of healthy adults [11]. There was no effect of HS on dilations to NTG (supplement) suggesting that the effects on FMD are related to an endothelium dependent mechanism and not due to vascular sensitivity to NO. While the dose (0.4 mg) used in this study is higher than some reports is a potential limitation[42], we observed no effect of NTG on BP in our population.

Since microvascular dysfunction is an early sign of and precursor to end organ damage and may contribute to the early development of hypertension and CV events [43], it is important to evaluate changes in microvascular function in this population. To our knowledge, this study is the first to focus on the impact of HS diet on endothelial function in isolated arterioles in humans. Recently, the human subcutaneous microcirculation has emerged as an accessible and important assessment of human microvascular function [27, 35, 44]. Other studies by Greaney et al. who have demonstrated that HS intake reduced microvascular

function (measured as reduced red blood cell flux during local heating-induced vasodilatation) independent of changes in blood pressure [39]. On the other hand, this study is the first to focus on the impact of HS diet on microvascular endothelial function in healthy normotensive individuals using resistance arterioles. We found that HS diet did not significantly reduce FID and AChID of resistance arteries isolated from subcutaneous fat. Our results have demonstrated that NOS inhibition with L-NAME significantly reduced FID and AChID of resistance arteries isolated from gluteal subcutaneous fat at baseline but had no effect after HS diet, suggesting that HS diet altered the NO component of dilation to flow and ACh in resistance arteries (Figure 2 and 3). Furthermore, indomethacin significantly reduced FID (Figure 2B) before, but not after HS diet, suggesting that cyclooxygenase metabolites contribute to vasodilator responses to flow at rest but not after HS loading. This is consistent with other studies showing that non-steroidal anti-inflammatory drugs may reduce or impair endothelium-dependent vasodilation, increase arterial stiffness and possibly contribute to increases in blood pressure and cardiovascular risk [45, 46]. In contrast to flow, dilator responses to ACh were significantly reduced by indomethacin and 17-ODYA after HS diet suggesting a divergence induced by high salt in the mechanisms of endothelium-dependent dilation between these stimuli. Collectively, one of the possible mechanisms through which acute salt loading affects vascular function is altered production and/or bioavailability of NO.

While numerous studies reported the beneficial effect of exercise on cardiovascular function [16, 17], there is no evidence on whether acute EX improves vascular endothelial function in conductance and resistance arteries during dietary salt manipulation. The current study found that exercise did not alter brachial artery FMD after HS diet. In contrast, acute EX had no effect on FID and AChID of resistance arteries following HS diet (data supplement). However, since FID and AChID was impaired after HS diet and acute EX restored the NO component of dilator responses to flow and ACh (Figure 2A and 2B), it appears that acute resistance EX may improve the NO contribution of dilation to these stimuli in resistance arteries. The effects of acute exercise on FMD appear to depend on disease status and the mode of exercise administered [30, 34]. Faria et al. reported that acute resistance EX decreased reactivity of the tail artery to phenylephrine and increased endothelium-dependent relaxation [47]. The mechanism appears to involve NO and vasodilator prostaglandins, in spontaneously hypertensive rats [47]. Another study in rats reported that acute bout of endurance EX increased vascular responsiveness to ACh in aorta, carotid, iliac and femoral rat vessels [48]. Previous studies in our laboratory [29, 30], have shown that sedentary adults showed impaired brachial FMD following acute hypertension induced by a single weight lifting session. In contrast, other studies have shown reduced endothelial function in brachial arteries of hypertensive patients [49] and in radial arteries of healthy subjects [50] immediately following acute hand grip EX. Taken together, these results suggest that the dilator mechanism shifts away from an NO-dependent mechanism of dilation during HS diet in resistance arteries and that this mechanism appears restored after a single EX session.

In conclusion, elevated salt intake for 7 days reduced brachial artery endothelial function (in the absence of changes in blood pressure or arterial stiffness) but had no effect on resistance artery dilation to ACh and flow in healthy inactive women. Acute resistance EX did not restore brachial artery endothelial function after HS intake. In contrast, flow and ACh

dilation in resistance arteries demonstrate a larger NO-dependent component following acute exercise. Future studies are needed to clarify whether this type of EX may protect against altered vascular and hemodynamic responses to other physiological perturbations such as acute hypertension, systemic hypoxemia, and hyperglycemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Brachial artery flow mediated dilation (FMD) at baseline before high salt (HS) diet, after HS diet and after acute exercise. Values are means $\pm$ SD. * indicates significant difference from baseline ($P<0.05$).

A

Data were expressed as mean $\pm$ SD.
* P<0.05 vehicle vs. L-NAME

B

Data were expressed as mean $\pm$ SD.
* P<0.05 vehicle vs. Indomethacin

C

Figure 2.

Flow induced dilation (FID) of arteries isolated from gluteal subcutaneous fat in absence and presence of L-NAME (2A), indomethacin (INDO) (2B) and 17-octadecynoic acid (17-ODYA; 2C) before HS diet (baseline), after HS diet and following acute exercise (EX). There was a significant effect of L-NAME on FID in resistance arteries before HS and following EX compared. * indicates significant difference from vehicle control (P < 0.05).

A

Data were expressed as mean ± SD.

* $P < 0.05$ vehicle vs. L-NAME at all ACh concentrations

$P < 0.05$ vehicle vs. Indomethacin at all ACh concentrations

x $P < 0.05$ vehicle vs. 17-ODYA at all ACh concentrations

B

Data were expressed as mean ± SD.

$P < 0.05$ vehicle vs. Indomethacin at ACh concentration 10^{-7} - 10^{-4} M

x $P < 0.05$ vehicle vs. 17-ODYA at ACh concentration 10^{-6} and 10^{-5} M

C

Figure 3.

Acetylcholine induced dilation (AChID) of arteries isolated from gluteal subcutaneous fat in the absence and presence of L-NAME, indomethacin (INDO) and 17-octadecynoic acid (17-ODYA) in baseline (3A), after HS diet (3B) and after acute exercise (EX; 3C). There was a significant effect of L-NAME on FID in resistance arteries at baseline and following EX compared. * indicates significant difference from vehicle control (P<0.05).

Table 1.

Clinical characteristics

Number, n	12
Age, yr	28±7 (20–40)
White, %	50
Body Mass Index, kg/m ²	23.5±3.1 (19.6–29.4)
Waist-to-Hip Ratio	0.75±0.04 (0.67–0.83)
Body Fat, %	17±4 (12–23)
Fat-Free Mass (FFM), %	46±4 (39–52)
Lean Dry Mass (LDM), %	15±3 (9–19)
Total Body Water (TBW), %	32±4 (27–40)
Intra-Cellular Water (ICW), %	17±3 (15–23)
Extra-Cellular Water (ECW), %	15±2 (12–17)
Glucose, mg/dL	81.58±7.22 (72–95)
Insulin, uIU/mL	4.3±2.03 (1.6–6.9)
Tryglicerides, mg/dL	63.6±38 (33–170)
Cholesterol, mg/dL	151.3±26.1 (101–203)
HDL Cholesterol, mg/dL	59.1±11.9 (36–72)
nonHDL Cholesterol, mg/dL	92.3±22.2 (50–137)
LDL Cholesterol, mg/dL	79.5±18.7 (43–122)

Data are presented as mean±SD (MIN-MAX) or % except as indicated.

HDL - high-density lipoprotein; LDL - low-density lipoprotein

Table 2.

Biochemical parameters - indicators of confirmation to diet guidelines

Parameter	baseline (n=12)	after HS diet (n=12)	P value
urine sodium, mEq/L	76±69	119±75	0.035
urine potassium, mEq/L	41±34	46±27	0.672
plasma renin activity (PRA), ng/mL/h	1.34±1.20	0.75±0.43	0.032
plasma aldosterone, ng/dL	6.64±7.06	3.36±1.50	0.137

Data are presented as mean±SD.

Table 3.

Blood pressure response to HS diet and exercise

Parameter	Baseline	HS diet	HS diet+exercise (maximum BP)	P
brachial artery SBP, mmHg	113±8	110±8	163±33 *	<0.001
brachial artery DBP, mmHg	70±8	66±6	100±25 *	<0.001
brachial artery MAP, mmHg	83±8	81±5	121±25 *	<0.001
calculated central SBP, mmHg	100±11	99±10		0.609
calculated central DBP, mmHg	70±8	69±8		0.765
calculated central MAP, mmHg	83±9	82±9		0.595

Data are presented as mean±SD.

* P<0.001 HS diet+exercise vs. baseline and HS diet

Table 4.

Brachial artery characteristics

Variable	Baseline	Post HS diet	Post HS diet+exercise	P value
Baseline brachial FMD diameter, mm	29.7±3	29.7±3.1	30.7±3.3	0.766
Maximal brachial FMD diameter, mm	33±3.4	31.1±2.6	32.7±3.6	0.461
Baseline NTG- brachial diameter, mm	30.4±3.1	29.4±4.8	30.2±3.6	0.657
Maximal NTG- brachial diameter, mm	39±4.3	38.7±5.8	39.3±4.3	0.784

Data are presented as mean±SD.