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Research Article

Seven-Day Salt Loading Impairs Microvascular Endothelium-Dependent Vasodilation without Changes in Blood Pressure, Body Composition and Fluid Status in Healthy Young Humans

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High-salt diet · Microcirculation · Endothelium · Body composition · Renin-angiotensin system

Abstract
Objectives: We aimed to assess whether a 7-day high-salt (HS) diet affects endothelium-dependent and/or endothelium-independent microvascular function in the absence of changes in arterial blood pressure (BP), and to determine whether such microvascular changes are associated with changes in body composition and fluid status in healthy young humans. ***Materials and Methods:*** Fifty-three young healthy individuals (28 women and 25 men) were assigned to a 7-day low-salt diet (<3.5 g salt/day) followed by a 7-day HS diet (~14 g salt/day). Skin microvascular blood flow in response to iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) was assessed by laser Doppler flowmetry, and BP, heart rate (HR), plasma renin activity (PRA), serum aldosterone, serum and 24 h-urine sodium, potassium, urea and creatinine levels, together with body composition and fluid status measurement with a 4-terminal portable impedance analyzer were measured before and after diet protocols. ***Results:*** BP, HR, body composition and fluid status were unchanged, and PRA and serum aldosterone level were significantly suppressed after HS diet. ACh-induced dilation (AChID) was significantly impaired, while SNP-induced dilation was not affected by HS diet. Impaired AChID and increased salt intake, as well as impaired AChID and suppressed renin-angiotensin system were significantly positively correlated. Changes in

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body composition and fluid status parameters were not associated with impaired AChID. ***Conclusion:*** 7-day HS diet impairs microvascular reactivity by affecting its endothelium-dependent vasodilation in young healthy individuals. Changes are independent of BP, body composition changes or fluid retention, but are the consequences of the unique effect of HS on endothelial function.

Introduction

Excessive salt intake in most parts of the world presents an important issue because it not only acts as an essential risk factor for the development and progression of hypertension [1, 2], but also affects the vascular and endothelial functions even in the absence of changes in blood pressure (BP) [3]. Besides studies on experimental animals, a vast amount of this knowledge was brought by studies on large conductance arteries, such as the study of flow-mediated dilation of the brachial artery in healthy population [4–6]. Consistent with other studies [5, 6], we have reported that 7 days of high-salt (HS) intake impaired flow-mediated dilation of the brachial artery, independent of BP changes in healthy individuals [4]. Microcirculation is affected very early in various pathological conditions. Due to its easy accessibility, the skin presents an appropriate site for studying peripheral microcirculation [7]. We have previously reported that a 7-day HS diet impaired forearm skin microvascular reactivity in response to vascular occlusion (in vivo study) [8]. In another study we reported that, after a 7-day HS diet, the mechanisms of vasodilation in response to acetylcholine (ACh) and flow stimulation were switched to non-nitric oxide (NO)-dependent pathways in arterioles isolated from gluteal subcutaneous fat (in vitro study) in healthy women [4], both independent of BP changes.

It is well accepted that sodium is essential for controlling fluid balance in the body, and for maintaining blood volume and BP within a normal range. Hence, it is possible that impaired microcirculatory reactivity may be the consequence of body fluid retention. Contrasting data are published on the effects of HS intake (between 13 and 29 g of salt per day) on sodium balance and fluid retention [9, 10]. Either increases in serum sodium concentrations are accompanied by increased fluid retention [11, 12] or, in contrast, no further fluid retention was observed with increasing salt intake from an average normal intake (8.7 g/day) to a much higher level (above 17.4 g/day) in healthy men [9]. Altogether, impaired microvascular function can precede an increase in arterial BP. However, there have been no previous studies on the relation of body composition and fluid status to microvascular reactivity.

Thus, the present study was designed to determine in vivo whether a 7-day HS diet affects endothelium-dependent and/or endothelium-independent vasodilation of forearm skin microcirculation in young healthy adults. In addition, we sought to determine whether such HS intake changes body composition and fluid status of young healthy individuals of both sexes, and if potential changes in body composition/fluid status are associated with microvascular endothelium alterations.

Materials and Methods

Study Population

Fifty-three young healthy individuals (28 women and 25 men), aged 18–24 years, were recruited by advertisement at the Faculty of Medicine, University of Osijek to participate in

this study. Written informed consent was obtained from each subject. The study protocol and procedures conformed with the standards set by the latest revision of the Declaration of Helsinki and were approved by the Ethical Committee of the Faculty of Medicine, University of Osijek (Cl 602–04/15-08/08; No: 2158-61-07-15-68).

Study was performed in the Laboratory for Clinical and Sport Physiology, Department of Physiology and Immunology at the Faculty of Medicine, University of Osijek. All testing occurred in the morning after an overnight fasting. The focus of this study was on healthy adults; therefore, exclusion criteria included a history of hypertension, coronary artery disease, diabetes, hyperlipidemia, renal impairment, cerebrovascular, and peripheral artery disease. Participants who were obese (body mass index [BMI] >30 kg/m^2) or taking oral contraceptives, antihypertensive agents, anti-inflammatory non-steroidal drugs, steroids or other drugs that could affect the endothelium were also excluded from the study. Women entered the study protocol in the different phases of the menstrual cycle (randomized), which eliminated the effect of sex hormones fluctuation during the menstrual cycle [4, 8].

Dietary Salt Perturbation

This study was a non-randomized controlled experiment in which all subjects were subjected to the same protocol with multiple repeated measurements. During the study all participants had 3 visits to the laboratory – the first visit when entering the study, the second visit after 7 days of low-salt (LS) diet protocol, and the third visit after 7 days of HS diet protocol. All subjects were instructed to first maintain a LS diet, with an intake of <3.5 g of salt per day (Dietary Approaches to Stop Hypertension [DASH] eating plan; US Department of Health and Human Services, 2006) for 7 days, which was considered a "wash-out" period. This was followed by 7 days of a HS diet, which implied intake of 3.5 g of salt per day according to the DASH eating plan and 11.7 g of salt per day supplemented in the form of a salt powder, which represents a total of about 14.7 g of salt per day. The amount of salt that represented a HS diet in the present study was set at 14.7 g of salt per day since such salt intake is not uncommon in everyday life of many individuals (e.g., the average salt intake for adults in Croatia is 11.6 ± 4.3 g/day: 13.3 ± 4.3 g/day for men and 10.2 ± 4.2 g/day for women) [13, 14], and when taking into account that similarly designed studies commonly provide 250–400 mmol of Na+ per day (14.6–23.4 g of salt per day), the HS diet used in the present study can be considered as a moderate increase in dietary salt intake. During the whole study protocol, participants had free access to water.

Twenty-Four-Hour Urine Analysis and BP Measurement

Urine was collected during the last 24-h period of the LS and HS regimes [8]. Twenty-four-hour urine aliquots were analyzed for sodium, potassium, urea, creatinine coefficient, albumin and protein levels at the Department of Clinical Laboratory Diagnostics, University Hospital Osijek. Daily salt intake based on 24-h urinary sodium excretion was calculated using the appropriate formula (1 g salt [NaCl] = 393.4 mg Na = 17.1 mmol Na).

BP and heart rate (HR) were measured at the beginning of each visit after a 15 min rest in a seated position using an automated oscillometric sphygmomanometer (OMRON M3, OMRON Healthcare Inc., Osaka, Japan). The final values of BP and HR were the mean of 3 repeated measurements. Salt resistance was defined as a ≤5 mm Hg change in mean arterial pressure (MAP) determined on LS versus HS diet. Participants were classified as salt-sensitive or salt-resistant after they completed the full protocol. Participants classified as salt-sensitive (3 women and 2 men) were excluded from analysis, because they could not be used to test the BP-independent effects of dietary salt (study hypothesis). Forty-eight participants were included in the final data analysis (25 women and 23 men).

Body Composition and Body Fluid Status Measurement

BMI and waist-to-hip ratio (WHR) were measured at each study visit. Also, body composition and fluid status were measured using a 4-terminal portable impedance analyzer (Maltron Bioscan 920-II, Maltron International Ltd., Rayleigh, Essex, UK). Empirically derived formulas (the original manufacturer's software) were used to calculate the estimated BMI, fat free mass%, Fat% (Fat Mass%), total body water% (TBW%), extracellular water% (ECW%), intracellular water% (ICW%), plasma fluid (PF), interstitial fluid (IF), and body density (kg/L).

Assessment of Microvascular Endothelium-Dependent and -Independent Vasodilation

A noninvasive laser Doppler flowmetry (LDF; MoorVMS-LDF, Axminster, UK) measurement of skin microvascular blood flow in response to the iontophoresis of ACh, which is considered endothelium-dependent vasodilation, and to the iontophoresis of sodium nitroprusside (SNP), which is considered endothelium-independent vasodilation, were performed in each subject according to established guidelines and previously described protocols of our laboratory [15]. The laser probe was attached to the skin of the volar forearm at the same place during each study visit using doubled-sided adhesive discs provided by the manufacturer. Because LDF recordings are very sensitive both to the movement of the examinee and to the movement of the probe or the wire attaching the probe to the LDF device, besides adhesive discs that stabilized the probe to the skin, stationary position of the subject was achieved by a cushion that comfortably stabilized the subject's arm. After baseline recording for 5 min, either the positively charged vasodilator ACh (1%), or negatively charged SNP (1%) was applied by previously described protocols [16]. Microcirculatory blood flow in this test was expressed in arbitrary perfusion units and described as area under the curve during baseline flow and during ACh or SNP administration. The final result was expressed as an increase in blood flow following ACh or SNP administration in relation to baseline flow.

Blood Analysis

A venous blood sample was taken after 30 min resting in a supine position at each visit. Blood samples were analyzed for complete blood count, plasma electrolytes (sodium, potassium, calcium), urea, creatinine, fasting lipid panels (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides), fasting blood glucose, and high sensitivity C-reactive protein, using standard laboratory methods. Serum osmolarity was calculated according to the following formula: calculated serum osmolarity (mOsm/kg) = 2 Na + Glucose + Urea (all in mmol/L). Plasma renin activity (PRA) and serum aldosterone were measured via commercially available ELISA kits (PRA, No. DB52011, IBL International, Germany; Aldosterone Elisa, No. KAPDB450, DIAsource ImmunoAssays, Belgium). All measurements were performed at the Department of Clinical Laboratory Diagnostics, University Hospital Osijek.

Statistical Analysis

All results are reported as mean ± SD. The sample size required to show a potentially significant effect was calculated based on preliminary data collected from 10 subjects. To detect differences in primary study endpoints reported in this study (e.g., LDF measurement) with a level of significance of 0.05 and a statistical power of 80% for paired t-test, the needed sample size is 15 subjects per group. All variables that were measured before and after HS diet protocol were compared using a paired *t* test. The normality of data distribution was assessed by using the Kolmogorov-Smirnov normality test. The Wilcoxon rank-sum test was used when variables were not normally distributed. The correlations between change of AChID and corresponding parameters (salt intake, PRA, serum aldosterone level) before and

Table 1. Baseline participants' characteristics

Baseline characteristic	Value
Demographic data	
Number (men/women)	48 (23/25)
Age, years	21±2
BMI, kg/m^2	23.2±3.4
WHR	0.78±0.05
BP and HR	
Systolic BP, mm Hg	120±12
Diastolic BP, mm Hg	73±9
MPA, mm Hg	90±8
HR, beats per min	78±12
Biochemical parameters	
Serum sodium, mmol/L	138±2
Serum potassium, mmol/L	4.1±0.3
Serum calcium, mmol/L	1.9±0.2
Serum urea, mmol/L	4.7±1.5
Serum creatinine, mmol/L	70±16
FBG, mmol/L	5.0±0.4
High sensitivity CRP, mmol/L	0.96±1.08
Cholesterol, mmol/L	4.48±0.81
HDL cholesterol, mmol/L	1.54±0.40
LDL cholesterol, mmol/L	2.64±0.61
Triglycerides, mmol/L	0.89±0.37
Hemoglobin, g/L	143±18
Hematocrit, %	42.0±4.8
Leukocytes, ×10^9/L	6.7±2.1

Values are mean ± SD.

BMI, body mass index; WHR, waist-to-hip ratio; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BP, blood pressure; HR, heart rate; MPA, mean arterial pressure; FBG, fasting blood glucose.

after HS diet were determined by Pearson's or Spearman's correlation tests when appropriate. Multiple linear regression was used to examine the association between changes in AChID and body composition/fluid status parameters following HS diet. $p < 0.05$ was considered statistically significant. SigmaPlot, version 11.2 (Systat Software, Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Participants' clinical characteristics are presented in Table 1. All participants were lean, normotensive, with normal renal function, complete blood count, fasting blood glucose and fasting lipid panels within normal limits. All participants completed a 2-week dietary salt perturbation; the first week of LS diet which was a "wash-out" period and the second week of HS diet.

Effects of Dietary Salt Perturbation on Hemodynamic and Biochemical Parameters

Serum sodium increased, but calculated serum osmolarity did not change following a 7-day HS diet (Table 2). There was no significant difference in other serum electrolytes (potassium, calcium), hemoglobin, hematocrit or in urea and creatinine levels measured before and after HS diet protocol (Table 2). As expected, 24-h urinary sodium excretion as

Table 2. Hemodynamic and biochemical responses to dietary salt perturbation

	LS	HS
Biochemical parameters		
Serum sodium, mmol/L	137±2	138±3*
Serum potassium, mmol/L	4.1±0.3	4.1±0.3
Serum calcium, mmol/L	2.4±0.1	2.5±0.3
Serum urea, mmol/L	4.4±1.3	4.5±1.2
Serum creatinine, mmol/L	74±16	72±15
Hemoglobin, g/L	141±19	140±20
Hematocrit	0.418±0.052	0.413±0.052
Calculated serum osmolality, mOsm/kg	283.8±5.3	285.6±5.6
PRA, ng/mL/h	4.65±3.27	2.20±1.29*
Serum aldosterone, pg/mL	170±116	72±63*
24 h urine volume, mL	1,321±549	1,468±563
24 h urine sodium, mmol/dU	107±45	250±95*
24 h urine potassium, mmol/dU	45±21	53±26
24 h urine urea, mmol/dU	296±138	327±185
24 h urine creatinine coefficient, μmol/24 h/kg	177±49	179±63
24 h urine albumin, mg/dU	9.1±8.3	9.0±10.0
24 h urine proteins, mg/dU	104±64	113±60
Calculated daily salt intake, g/day	6.3±2.6	14.6±5.5*
BP and HR		
Systolic BP, mm Hg	116±12	117±12
Diastolic BP, mm Hg	73±7	72±7
MAP, mm Hg	87±8	87±8
HR, beats per min	75±11	72±9

Values are mean ± SD.

* $p < 0.05$ before HS vs. after HS.

LS, low salt; HS, high salt; PRA, plasma renin activity; BP, blood pressure; HR, heart rate; MAP, mean arterial pressure.

Table 3. Body composition and fluid status changes to dietary salt perturbation

	LS	HS
BMI, kg/m^2	23.26±3.27	23.58±3.38
WHR	0.77±0.06	0.77±0.06
FFM, %	82.1±7.2	80.3±8.0
Fat, %	17.9±7.2	19.7±8.0
TBW, %	62.4±7.9	58.8±11.8
ECW, %	43.5±4.2	43.2±2.9
ICW, %	56.5±4.2	56.8±2.9
PF, L	3.81±0.90	3.76±0.94
IF, L	13.32±3.14	13.16±3.30
Body density, kg/L	1.058±0.016	1.054±0.019

Values are mean ± SD.

LS, low salt; HS, high salt; BMI, body mass index; WHR, waist-to-hip ratio; FFM, fat free mass; TBW, TBW, total body water; ECW, extracellular water; ICW, intracellular water; PF, plasma fluid; IF, interstitial fluid.

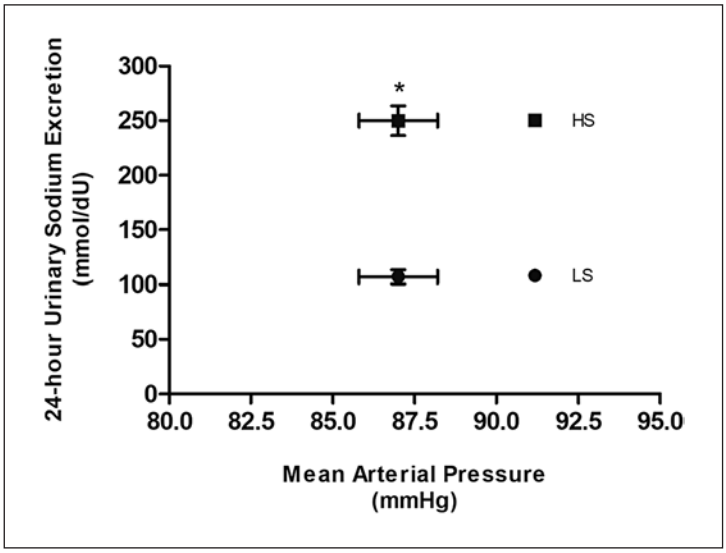

Fig. 1. 24-h urinary sodium excretion and MAP. Despite a significant increase in 24-h urinary sodium excretion during a 7-day HS diet, MAP did not change, confirming that participants were salt-resistant. * $p < 0.05$ LS versus HS. LS, low-salt; HS, high-salt; MAP, mean arterial pressure.

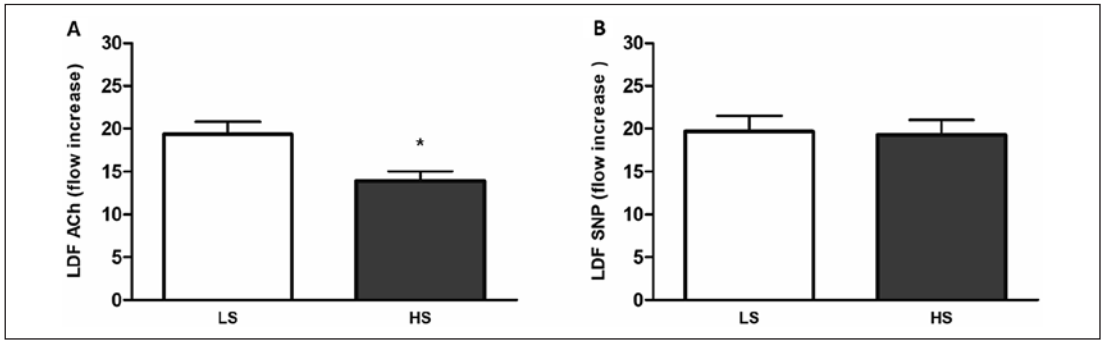

Fig. 2. Endothelium-dependent (**A**) and endothelium-independent vasodilation (**B**) of forearm skin microcirculation during HS diet. AChID (endothelium-dependent) was significantly impaired after a 7-day HS diet compared to corresponding values obtained after a LS diet. SNPID (endothelium-independent) did not differ between LS and HS diets. * $p < 0.05$ LS versus HS. LDF, laser Doppler flowmetry; ACh, acetylcholine; SNP, sodium nitroprusside; LS, low salt; HS, high salt.

well as calculated daily salt intake significantly increased (Table 2), while PRA and serum aldosterone level were significantly suppressed during the HS diet (Table 2). HS diet did not significantly change the 24-h urinary total volume, creatinine coefficient, potassium, urea, albumin, and protein excretion (Table 2). Values of systolic BP, diastolic BP and MAP during LS diet did not differ compared to the HS diet values (Table 2). Based on MAP, all subjects included in data analysis were characterized as salt-resistant (see Method section), as indicated by a large increase in 24-h urinary sodium excretion (and calculated daily salt intake) during the HS diet without a concomitant change in MAP (Fig. 1).

Effects of Dietary Salt Perturbation on Body Composition and Body Fluid Status

Body composition and fluid status responses to dietary salt perturbation are presented in Table 3. Seven-day HS diet did not induce any significant change in BMI, WHR, fat free mass%, Fat%, TBW%, ECW%, ICW%, PF, IF or body density in young healthy population (Table 3).

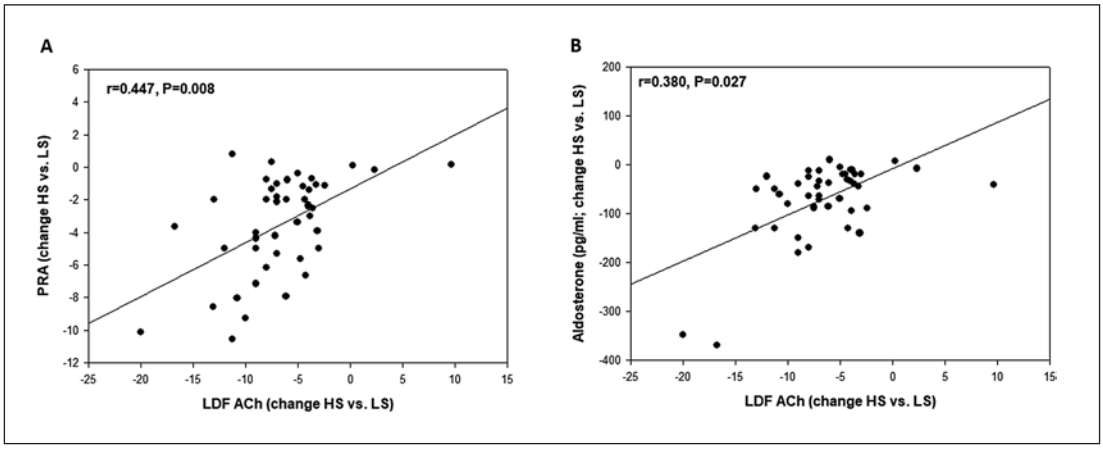

Fig. 3. Correlation between impaired AChID and suppression of RAS during HS diet. There was significant moderate correlation between impaired AChID and PRA suppression (**A**), and weak but significant correlation between impaired AChID and serum aldosterone level suppression (**B**) following a HS diet in the study population. LDF, laser Doppler flowmetry; ACh, acetylcholine; LS, low salt; HS, high salt; PRA, plasma renin activity.

Effects of Dietary Salt Perturbation on Body Skin Microvascular Endothelium-Dependent and Endothelium-Independent Vasodilation

Although LDF does not provide an absolute measurement of blood flow and cannot be used to compare absolute values of blood flow between individuals or within an individual at different times, baseline forearm skin microvascular flow values did not differ before and after HS diet protocol (baseline 1 min area under the curve before HS 548 ± 245 vs. after HS 589 ± 215; $p > 0.05$), which indicates consistency and reproducibility of our experimental design. Seven-day HS diet decreased ACh-induced dilation (AChID) of forearm skin microcirculation by approximately 32% (LDF ACh flow increase LS 21 ± 15 vs. HS 14 ± 6, $p < 0.05$; Fig. 2A). In contrast to AChID, SNP-induced dilation (SNPID) did not differ between LS and HS diet measurements (LDF SNP flow increase LS 20 ± 8 vs. HS 19 ± 7, $p > 0.05$; Fig. 2B).

Correlation between Impaired AChID and Salt Intake/Renin-Angiotensin System Suppression/Body Composition and Fluid Status Changes following a HS Diet

A significant moderate correlation was observed between AChID and increased salt intake in young healthy participants ($r = –0.507$, $p = 0.003$). There was also a significant moderate correlation between impaired AChID and PRA suppression ($r = 0.447$, $p = 0.008$; Fig. 3A), and a weak but significant correlation between impaired AChID and serum aldosterone level suppression ($r = 0.380$, $p = 0.027$) following HS diet in the study population (Fig. 3B). A multiple linear regression model to predict changes in AChID from measured body composition and fluid status parameters (body mass, WHR, Fat%, TBW%; with other variables eliminated due to multicollinearity) showed that measured changes in body composition and fluid status parameters were not significantly associated with impaired AChID following HS diet in the study population ($r^2 = 0.493$, $p > 0.05$).

Discussion

The salient finding of the present study is that 7 days of a HS diet significantly impaired endothelium-dependent vasodilation (AChID), but not endothelium-independent vasodilation (SNPID) of forearm skin microcirculation in young healthy participants (Fig. 2), without

affecting MAP (Fig. 1). Furthermore, suppressed PRA and serum aldosterone level significantly correlated with impaired AChID of forearm skin microcirculation (Fig. 3a, b), indicating that suppression of the renin-angiotensin system (RAS; and consequently ANG II levels) may be causal in the development of microvascular dysfunction, independent of the arterial BP levels. We did not observe any significant change in body composition and fluid status following the 7-day HS diet in healthy participants (Table 3).

Endothelium dysfunction is considered to be a precursor and the earliest detectable outcome of cardiovascular diseases [17], the leading cause of mortality and a major cause of morbidity in the world, with 3.9 million deaths in Europe and over 1.8 million deaths in the European Union [18]. As a result of large-scale intervention trials (INTERSALT, TOPH, DASH Sodium Trial) that provided robust evidence about the effect of salt restriction on reduction of BP and cardiovascular event rate [19–21], dietary salt restriction continues to be an important component in lowering BP and treating hypertension. However, in the last 15 years, a body of evidence in animal model studies demonstrated that increased dietary salt intake has an impact on vascular and endothelial function, independent of BP changes [22–24]. Some of the mechanisms have been elucidated, such as a profound reduction in nitric oxide bioavailability occurring with HS dietary intake [22–24], or shifts in the metabolisms of arachidonic acid [24]. However, even though a number of previous clinical trials aimed to examine the effect of dietary salt perturbation on endothelial function (besides its effect on BP), these studies have not been able to separate the effects of HS diet and BP on endothelial function, since they were predominantly performed on hypertensive patients. Thus, it was necessary to start investigating the vascular responses to sodium loading (and/or restriction) in healthy, normotensive individuals as well.

Besides the importance of conductance vessels damage in the etiopathogenesis of cardiovascular diseases [4–6, 25, 26], the impairment of microvascular function can also lead to deleterious end-organ damage and cardiovascular system failure (such as in untreated malignant hypertension, stroke, and sepsis) [7]. Our previous results indicated that vasoconstrictor metabolites of cyclooxygenase (COX) enzymes, especially COX-1, have a significant role in the development of impaired microvascular reactivity in response to vascular occlusion in healthy individuals on a HS diet [8]. Furthermore, we have also reported that vasodilation of arterioles isolated from gluteal subcutaneous fat in response to ACh and flow-induced dilation (in vitro) following a 7-day HS diet were no longer NO-dependent, but that COX and cytochrome P450 vasodilator mediators took part in this vasodilation [4]. However, in the present study we demonstrated, for the first time, that peripheral microvascular endothelial-dependent vasodilation, but not endothelial-independent vasodilation, declines in response to a 7-day HS diet in young healthy salt-resistant individuals of both sexes. To date, there is only one more study demonstrating that a 7-day HS intake reduced peripheral microvascular function (measured as reduced red blood cell flux during local heating-induced vasodilation using LDF) independent of BP in normotensive adults [27], which is in concordance with our results.

A growing body of evidence indicates that chronically suppressed RAS and low ANG II levels in rats fed a HS diet have deleterious effects that are almost identical to those occurring at pathologically elevated ANG II levels [23, 28, 29]. These include impaired vascular relaxation in different vascular beds in response to a number of endothelium-dependent and -independent vasodilator stimuli, reduced NO bioavailability, elevated levels of oxidative stress, and decreased vascular antioxidative capacity [23, 30]. The importance of physiological ANG II levels for maintaining normal vascular function was supported by a number of studies showing that vascular relaxation in response to various vasodilator stimuli following a HS diet is restored by continuous IV infusions of suppressor dose of ANG II [31, 32]. However, the potential link between RAS inhibition and disturbed vascular/endothelial function in

healthy humans is not sufficiently investigated. Accordingly, we have earlier demonstrated that 7 days of ANG II receptors 1 (AT1) blockade in young healthy women on a LS diet significantly increased plasma levels of the stable TXA2 metabolite, suggesting that crosstalk between RAS and production of arachidonic acid vasoconstrictor metabolites may be important in regulating vascular function during HS loading [33]. In the present study, we have found a significant correlation between impaired AChID and suppressed PRA and serum aldosterone levels following a HS diet in young healthy persons (Fig. 3a, b). Thus, it is possible that RAS suppression as a result of a HS diet has an important role in reducing AChID directly and/or via increased oxidative stress level due to disrupted antioxidant defense (or some other mechanism). The latter possibility remains to be investigated in future human studies.

The correlation between daily salt consumption and changes in body composition and/or fluid status is still an area with limited evidence. In healthy normotensive individuals, an increase in total body weight was observed following a HS diet, without effects on cardiac function (in particular, diastolic function) or cardiac output, suggesting that the increase in total body weight may predominantly reflect an expansion of extracellular volume rather than an increase in intra-vascular volume [34]. To our knowledge, the present study using a 4-terminal portable impedance analyzer is the first controlled interventional experimental study that investigated the effect of a 7-day HS diet (following a LS "wash-out" period) on body composition and fluid status in healthy individuals. We did not observe any significant change either in total body weight or fat mass, or in total body water or particular water compartments (ICW, ECW, PF or IF) following a HS diet in healthy lean participants. This is in concordance with other studies [9, 10], for example, increase in daily salt intake from the average normal (2.8 mmol NaCl/kg body mass per day) to a much higher level (7.7 mmol NaCl/kg body mass per day) showed that sodium was retained but without fluid retention, suggesting that the majority of sodium was stored in an osmotically inactive form [9]. There is some experimental evidence that tissues, in particular skin and muscle, can store sodium. However, those observations were made in severe diseases with significantly impaired sodium handling capacity (e.g., primary and secondary hyperaldosteronism, end-stage renal disease, refractory hypertension etc.) [35]. Additionally, there is currently no experimental evidence that skin sodium accumulation has any relationship to endothelial function [35–38]. Excessive sodium plasma concentrations may cause alterations in the endothelial glycocalyx (negatively charged surface rich in water and anionic glycosaminoglycans) [39, 40], as investigated by the Oberleithner group. Furthermore, it has been demonstrated that damaged endothelial glycocalyx could be related to altered endothelial function due to reduction in NO release [39, 40]. However, the utilized model of salt accumulation in endothelial cells is based on aldosterone effects on sodium retention and is opposite to the effects of dietary salt intake on aldosterone, where aldosterone is suppressed with a HS diet [41]. Taken together, the underlying regulation of such alternative sodium and fluid retention following HS dietary intake remains to be elucidated, particularly since an inability to store osmotically inactive sodium may underlie the pathophysiology of particular diseases related to the salt overconsumption in the Western world.

In conclusion, the results of this study indicate that microvascular changes induced by a 7-day HS diet are independent of body composition changes or fluid retention (just as they are BP-independent), but are a consequence of the unique effect of HS intake (and consequently suppressed RAS) on endothelial function.

Perspectives

One week of HS diet reduced endothelium-dependent vasodilation (AChID), but not endothelium-independent vasodilation (SNPID) of forearm skin microcirculation in young healthy participants. Observed microvascular reactivity impairment was independent of BP,

body composition or fluid status changes, but associated with increased salt intake and suppressed RAS. The present findings provide the foundation for further investigations into the exact mechanisms (e.g., RAS inhibition, increased oxidative stress level, changes in sympathetic activity, and/or endothelial-leukocyte activation) mediating microvascular endothelial alterations during HS loading in healthy individuals.

Statement of Ethics

Subjects have given their written informed consent.

The study protocol has been approved by the Ethical Committee of the Faculty of Medicine, University of Osijek (Cl: 602-04/15-08/08; No.: 2158-61-07-15-68).

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

L.B., I.D., and A.S.: were involved in formulating the research question(s), designing and conducting the study, gathering, analysis and interpretation of data, drafting the article and revising it critically. A.M., M.S., L.K., and Z.M.: were involved in carrying out the study, assembly, analysis and interpretation of data, drafting the article and revising it critically. H.L. and V.S.: were involved in designing the study, analysis and interpretation of data, drafting the article and revising it critically.

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