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Original Article

High-salt intake affects sublingual microcirculation and is linked to body weight change in healthy volunteers: a randomized cross-over trial

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Background: The pathophysiology of salt-sensitive hypertension remains uncertain, but may involve microvascular alterations. High-salt intake decreases microvascular density in hypertensive patients, but due to lack of studies in normotensive patients the causal pathway remains unclear. We studied whether high-salt intake decreases sublingual microvascular density in normotensive individuals and assessed the influence of body weight on changes in microvascular density.

Methods: In an open label randomized cross-over trial 18 healthy men were included to study the effect of a 2-week high-salt (>12 g/day) and low-salt (<3 g/day) diet on microvascular (diameter <20 μm) density with sublingual sidestream darkfield imaging. We used sublingual nitroglycerin (NTG) to recruit microvessels.

Results: There was no significant difference in microvascular density between diets (0.96 ± 3.88 mm/mm^2; $P = 0.31$, following NTG; and -0.03 ± 1.64 mm/mm^2; $P = 0.95$, without NTG). Increased salt intake was correlated with a decrease in microvascular density following NTG ($r = -0.47$; $P = 0.047$), but not without NTG ($r = 0.06$; $P = 0.800$). The decrease in microvascular density following high-salt intake was significantly larger for those with a large change in body weight as compared with those with a small changer in body weight (-0.79 ± 1.35 and 0.84 ± 1.56 mm/mm^2 respectively, $P = 0.031$).

Conclusion: We demonstrate in healthy volunteers that higher salt intake is correlated with decreased sublingual microvascular density following administration of NTG and; larger changes in body weight following high-salt intake coincide with a larger decrease in microvascular density. Changes in microvascular density occurred without blood pressure effects, indicating that high-salt load as such contributes to microvascular changes, and may precede hypertension development.

Keywords: blood pressure, hypertension, microcirculation, salt sensitivity, sodium

Abbreviations: AVA, automated vessel analysis; BP, blood pressure; *CO*, cardiac output; ECFV, extracellular fluid volume; HSD, high-salt diet (>12 g/day); IDF, incident dark field imaging; LSD, low-salt diet (<3 g/day); NO, nitric oxide; NTG, nitroglycerin; PPV, proportion of perfused vessels; PVD_{small}, perfused vessel density diameter <20 μm; SDF, sidestream dark-field; SVR, systemic vascular resistance; TVD_{small}, total vessel density diameter <20 μm

INTRODUCTION

Daily dietary salt intake exceeds recommended maximum levels, [1] and has been linked to hypertension and increased cardiovascular risk [2]. However, not everyone responds to high-salt intake with a blood pressure (BP) increase [3]. The trait characterized by a BP increase following high-salt intake is known as salt-sensitivity, as opposed to salt-resistance [4]. The pathophysiology of salt-sensitivity remains to be elucidated. Until recently it was thought that in salt-sensitive individuals hypertension is caused via renal salt retention, leading to an increase in extracellular fluid volume (ECFV) and cardiac output (*CO*). Contrarily, recent studies have demonstrated that an increase in *CO* is also seen in salt-resistant individuals, but that compensatory reduction in systemic vascular resistance (SVR) is impaired in salt-sensitive individuals [5,6]. Nitric oxide (NO)-mediated pathways might explain this differential response in SVR, as activity of this endogenous vasodilator is generally decreased after high-salt intake, but is more extensively reduced in salt-sensitive individuals [7,8]. Theoretically this NO response could lead to a decrease in microvascular density that is demonstrated both in hypertensive patients [9–12] and following high-salt intake in both hypertensive and normotensive individuals [13–16]. This decrease in microvascular density can either

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be structural (anatomical absence of vessels) or functional (vessels are anatomically present yet not perfused), or both [9]. As reduction of microvascular density increases SVR [17], this phenomenon could be the missing link in understanding the mechanism of salt-sensitivity. It is yet unknown whether high-salt intake causes reduction of microvascular density in normotensive patients, and therefore the causal relationship with hypertension remains unclear. An alternative explanation for the differential response in SVR could involve recent findings of sodium compartmentalization. Sodium can be stored in various tissues without commensurate water retention and subsequent expansion of the ECFV [18]. Whereas salt-resistant individuals have this capacity for nonosmotic sodium storage, this seems perturbed in those who are salt-sensitive [6], resulting in weight gain and high SVR following salt loading via mechanisms not well understood [6]. So far, it has not been studied whether ECFV changes and microvascular alterations are related.

Improvements of imaging modalities grant the opportunity to have a closer look into the relationship between high-salt intake and microcirculatory changes. The aim of this study was to determine whether high-salt intake causes reduction of sublingual microvascular density in normotensive individuals. Though not the primary aim of this study, in light of newly emerged evidence regarding nonosmotic sodium storage, we also assessed the effect of sodium-induced body weight changes on the relationship between salt intake and microvascular density.

METHODS

We studied healthy male volunteers between 18 and 40 years old in an open label randomized crossover trial. The study was performed at the Academic Medical Center Amsterdam between October 2016 and April 2017 according to the principles of the Declaration of Helsinki [19]. Eligibility and exclusion criteria are presented in the Supplemental material (S1), http://links.lww.com/HJH/B43. Volunteers were recruited via local advertisement and provided informed written consent. The protocol was approved by the local ethics committee and registered at the Netherlands trial registry (NTR4785; http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4785).

All participants were asked to subsequently adhere to a high-salt diet (HSD) (>12 g/day) and low-salt diet (LSD) (<3 g/day) for 14 days each in randomized order. Randomization was performed by the research coordinator via sealed, opaque envelopes in blocks of four after assessment of eligibility and signing informed consent. There was no washout period between diets. Dietary compliance was verified at days 7 and 11 with collection of 24-h urine. On day 15 after an overnight fast participants visited our research department for measurement of microvascular density, hemodynamic parameters and laboratory testing.

The primary aim of this study was to assess a difference in sublingual microvascular density after a HSD compared with a LSD, with participants serving as their own controls. Microvascular densities were measured sublingually with sidestream dark-field (SDF) videomicroscopy (Microscan; Microvision Medical B.V. Amsterdam, The Netherlands) that captures hemoglobin (Hb) in passing red blood cells with green light-emitting diodes (540 nm). Therefore, vessels filled with red blood cells are captured, but nonperfused vessels are not. To visualize and maximize the residual capacity of the sublingual microcirculation (structural vessel density), vasodilation was induced via one dose of 0.4-mg sublingual nitroglycerin (NTG) [20].

Videos were assessed for sufficient quality (Supplemental methods S3, http://links.lww.com/HJH/B43) [21]. Video-image analysis was performed with the operator blinded for the characteristics of the participants using a semiautomated analysis program [automated vessel analysis (AVA) 3.2 and in concordance with the 2007 consensus statement [22]. With AVA 3.2 densities are measured as vessel-length per surface (mm/mm^2) for vessels with diameters less than 20 μm (TVD_{small}) and all vessels with diameters less than 150 μm ($TVD_{all\ vessels}$). Microvessels with diameters less than 20 μm are mostly capillaries, therefore we used TVD_{small} to answer our hypothesis. $TVD_{all\ vessels}$ mostly consists of venules and is considered a quality check [22]. The video analyst ranked the flow of erythrocytes through the vessels from no flow to continuous flow. The proportion of vessels with flow is expressed as PPV_{small} (% of vessels with diameters <20 μm with flow) and $PPV_{all\ vessels}$ (% of all vessels with flow). This proportion is multiplied with TVD to assess density of vessels with flow: perfused vessel density $(PVD)_{small}$ (density of vessels with diameters <20 μm with flow) and PVD_{all} vessels (density of all vessels with flow). Finally microvascular flow index was measured for all vessels and for small vessels, in which flow is ranked per video quadrant. More detailed methods regarding SDF analysis are provided in the Supplement (S3), http://links.lww.com/HJH/B43.

Hemodynamic parameters were measured after both diets. Seated SBP, DBP and heart rate (HR) (mean of the last two measurements) were done after 5-min rest at the nondominant arm with an automated device (Omron M4 oscillometric device; OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands). On day 14 of both diets 24-h ambulatory SBP and DBP, mean arterial pressure and HR were recorded at 15-min daytime intervals and 30-min night-time intervals (Mobil-O-Graph 24 h PWA Monitor; I.E.M. GmbH, Stolberg, Germany). The cuff was secured at the nondominant arm. When arm circumference exceeded 32 cm a large cuff was used.

Laboratory testing included plasma sodium, potassium and creatinine, and analysis of 24-h urinary sodium, potassium and creatinine levels. All biochemical tests were performed on a COBAS C8000 Modular Analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Participants were stratified to groups with a large and small salt-induced increase in weight, to assess the effect of sodium-induced body weight changes on the relationship between salt intake and microvascular density.

Sample size calculations were based on results of a previously conducted pilot study, showing that 18 participants were needed to detect a mean difference of 8.4% (SD of 12.0) in microvascular density measured with SDF-videomicroscopy, with 80% power using a two-sided paired *t* test at the 0.05 significance level. Continuous variables are reported as mean and SD, or as median and interquartile

range if the data were not normally distributed. We checked for period and carry-over effects (Supplemental methods S4, http://links.lww.com/HJH/B43) [23].

To compare the outcomes between diets, paired *t* tests or Wilcoxon signed ranks tests were used. Pearson correlation was used to test the correlation between the change in sodium excretion and the change in microvascular density between diets. Participants were stratified by median-split for amount of weight change. One-sample *t* tests or Wilcoxon rank sum tests were used to compare salt-induced changes in microvascular density between groups. As SDF imaging captures Hb, we suspected that salt-induced differences in hematocrit levels could influence our results. Therefore, we repeated these tests with a correction for hematocrit levels in a linear mixed model. A two-tailed *P* less than 0.05 was considered statistically significant. Statistical analyses were done in Rstudio (Version 1.0.136; RStudio, Inc., Boston, Massachusetts, USA).

RESULTS

Between October 2016 and April 2017 we included 18 individuals; 10 of whom were randomized to start with the HSD, and eight to start with the LSD. The mean (SD) age was 29 (5) years, and baseline BP was 118 (8)/73 (5) mmHg. Baseline characteristics are shown in Table 1.

Mean 24-h urinary sodium and creatinine excretion indicated overall compliance to both diets and complete urine sampling. Salt excretion was 15.2 g/day following high-salt intake and 2.3 g/day after low-salt intake (Table 2). An unintended effect of salt intake reduction was decreased 24-h potassium excretion ($P = 0.02$; Table 2). The difference in weight between diets was 1.8 (0.9) kg ($P < 0.0001$).

No differences in BP were observed (Table 2), irrespective of diet sequence. We found no differences in microvascular density between diets (Supplemental file S5, http://links.lww.com/HJH/B43). Mean microvascular density following NTG was slightly higher following the LSD, but the difference between diets was NS ($P = 0.31$, S5, http://links.lww.com/HJH/B43). No period or carry-over effects were detected. Adjustment for plasma hematocrit did not affect these results (data not shown).

There was no correlation between the increase in 24 h urine sodium excretion (i.e. salt exposure) and the change in microvascular density before administration of NTG (Fig. 1a and b), but there was a significant correlation between the increase of 24-h urine sodium excretion and the decrease in microvascular density following NTG administration (Fig. 1c and d).

When participants with a small change in weight were compared with participants with a larger change in weight, the change in microvascular density (TVD_{small}) before administration of NTG differed significantly between groups (Δ1.63 (1.43), $P = 0.031$, Fig. 2a and b). Whereas we found an increase in microvascular density following the HSD in the group with a small salt-induced change in

TABLE 1. Baseline characteristics

Healthy male participants, $n = 18$	
Age (years)	29.3 (4.5)
Weight (kg)	80.6 (8.5)
BMI (kg/m^2)	24.4 (2.6)
Waist-to-hip ratio	0.92 (0.04)[a]
Ethnicity	16 European descent, 2 Arabic descent
Plasma	
Sodium (mmol/l)	140 (3)[a]
Creatinine (μmol/l)	84 (11)[a]
eGFR (ml/min per 1.73 m^2)	106 (12)
Osmolality (mmol/l)	293 (2)
Glucose (mmol/l)	5.0 (1.0)
24-h Urine	
Volume (ml/24 h)	2262 (1269)[a]
Creatinine (μmol/24 h)	17.0 (4.6)
Sodium (mmol/24 h)	150 (77)[a]
Potassium (mmol/24 h)	83 (49)[a]
Creatinine clearance (ml/min)	141 (42)
Office BP	
SBP (mmHg)	118 (8)
DBP (mmHg)	73 (5)
Mean arterial pressure (mmHg)	88 (5)
Heart rate (bpm)	65 (8)

All values are expressed as mean (SD) unless otherwise marked. BP, blood pressure; eGFR, estimated glomerular filtration rate.
[a]Values are presented as median (interquartile range).

TABLE 2. Outcome measurements after high-salt and low-salt diet

	High-salt diet, $n = 18$	Low-salt diet, $n = 18$	*P* value
Weight (kg)	80.8 (8.1)	78.1 (8.4)	$P < 0.0001$
Plasma			
Sodium (mmol/l)	141 (1)	140 (2)	$P = 0.13$
Potassium (mmol/l)	3.9 (0.2)[a]	4.0 (0.4)[a]	$P = 0.092$
Osmolality (mOsm/kg)	293 (3)	291 (3)	$P = 0.020$
Hematocrit (l/l)	0.44 (0.03)	0.44 (0.02)	$P = 0.71$
24-h Urine			
Volume (ml/24 h)	2111 (1051)[a]	1973 (555)[a]	$P = 0.40$
Creatinine (μmol/24 h)	17.3 (2.8)	16.9 (3.3)	$P = 0.80$
Sodium (mmol/24 h)	264 (65)[a]	40 (19)[a]	$P < 0.0001$
Potassium (mmol/24 h)	114 (21)[a]	91 (29)[a]	$P = 0.022$
Creatinine clearance (ml/min)	157 (23)	128 (18)	$P < 0.0001$
Office BP			
SBP (mmHg)	114 (10)	110 (8)	$P = 0.022$
DBP (mmHg)	70 (7)	71 (5)	$P = 0.71$
Mean arterial pressure (mmHg)	85 (7)	84 (5)	$P = 0.50$
Heart rate (bpm)	60 (8)	62 (10)	$P = 0.35$
Ambulatory BP measurement (24-h)			
Daytime	($n = 17$)[b]	($n = 17$)[b]	
SBP (mmHg)	124 (6)	123 (6)	$P = 0.47$
DBP (mmHg)	74 (7)	73 (5)	$P = 0.66$
Mean arterial pressure (mmHg)	95 (5)[a]	96 (7)[a]	$P = 0.82$
Heart rate (bmp)	65 (6)	64 (6)	$P = 0.098$
Night-time	($n = 16$)[c]	($n = 15$)[d]	
SBP (mmHg)	110 (10)	108 (8)	$P = 0.91$
DBP (mmHg)	61 (7)	60 (5)	$P = 0.72$
Mean arterial pressure (mmHg)	83 (7)	82 (6)	$P = 0.64$
Heart rate (bmp)	55 (6)	53 (8)	$P = 0.22$
Nightly dipping systolic (%)	12.4 (5.5)	11.7 (4.9)	$P = 0.29$
Nightly dipping diastolic (%)	16.4 (7.5)	17.7 (5.5)	$P = 0.67$

All values are expressed as mean (SD) unless otherwise marked. BP, blood pressure.
[a]Values are presented as median (interquartile range).
[b]One participant had no daytime measurement after both high-salt and low-salt diet due to device malfunction.
[c]Two participants had no night-time measurements after high-salt diet due to device malfunction.
[d]Three participants had no night-time measurements after low-salt diet due to device malfunction.

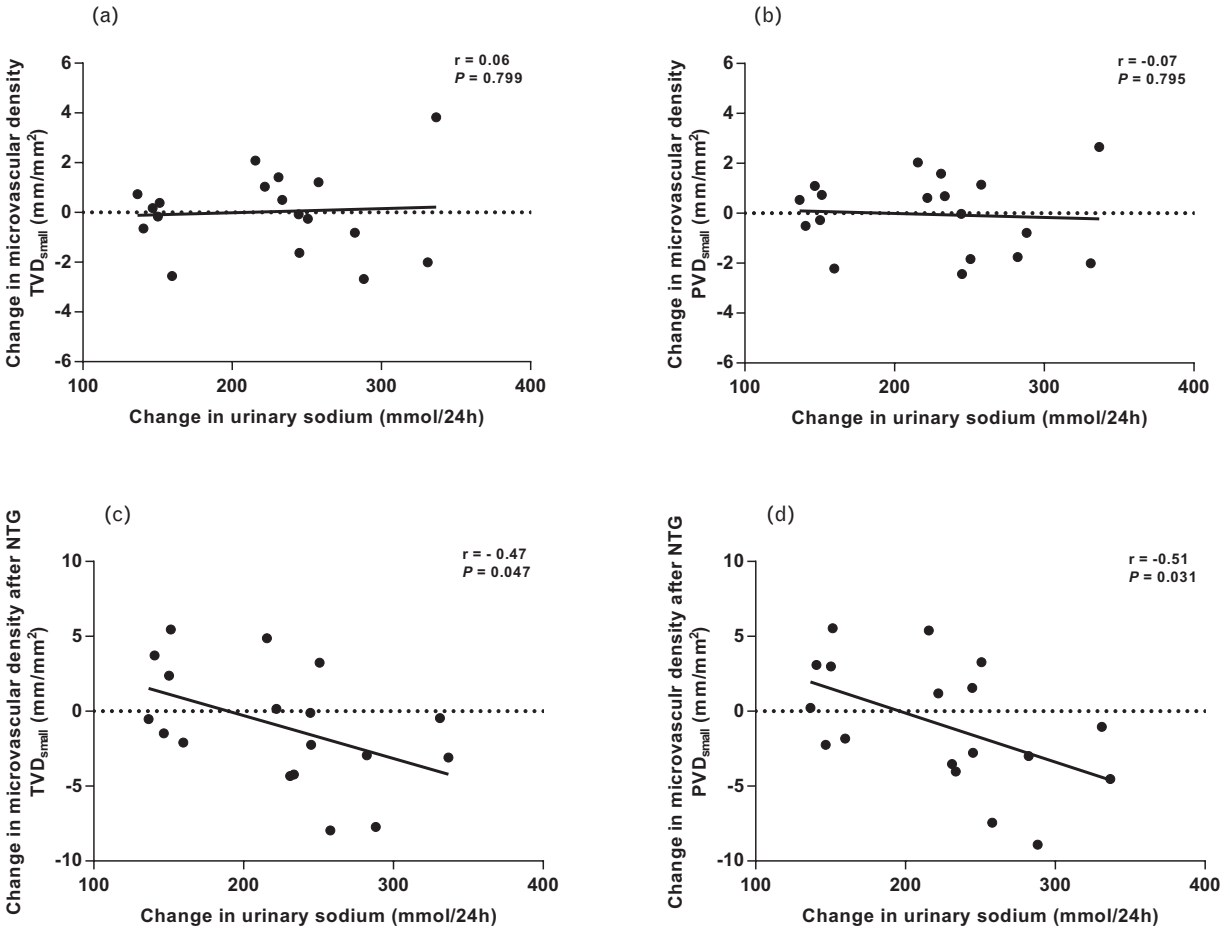

FIGURE 1 Scatterplots of the correlation between the change in 24 h urinary sodium excretion and the change in microvascular density. (a and b) There was no significant correlation between change in 24-h urinary sodium (i.e. salt exposure) and the change in microvascular density for total vessel density: diameter less than 20 μm or perfused vessel density diameter less than 20 μm. (c and d) There was a significant correlation between the increase in 24-h urinary sodium and decrease of microvascular density following nitroglycerin administration, for both total vessel density diameter less than 20 μm and perfused vessel density diameter less than 20 μm. NTG, nitroglycerin; PVD_{small}, perfused vessel density: diameter less than 20 μm; TVD_{small}, total vessel density: diameter less than 20 μm.

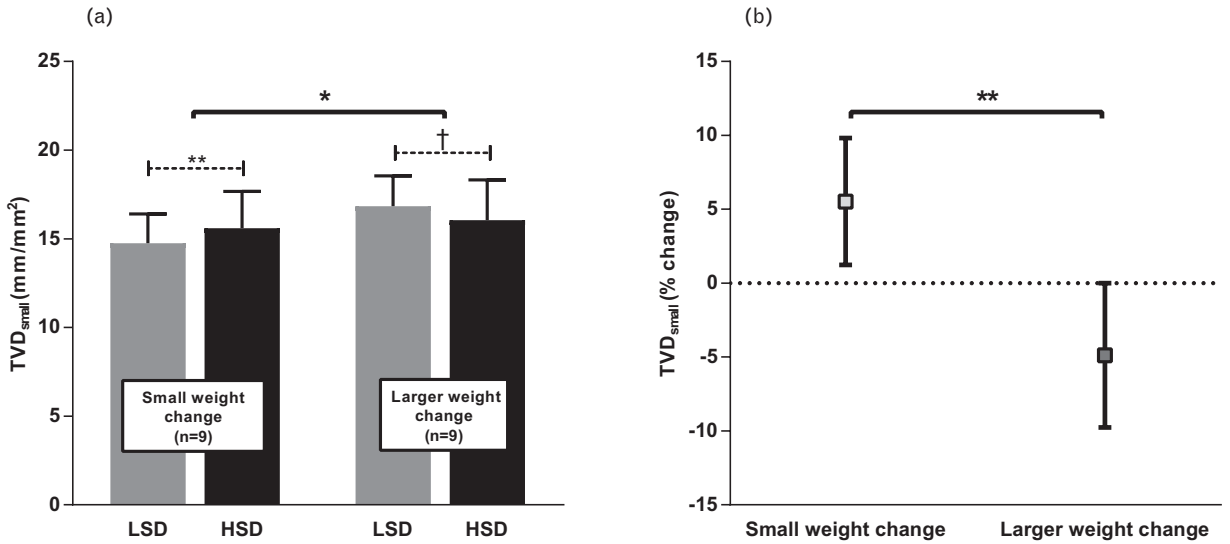

FIGURE 2 Results of the stratified analysis. Groups were defined via median-split for salt-induced change in weight. (a) Salt-induced change in microvascular density. Microvascular density after high-salt diet increases in the group with a small salt-induced change in weight and decreases in the group with a larger salt-induced change in weight. The change in microvascular (total vessel density: diameter <20 μm) differed significantly between groups. Bars and whiskers signify mean and SD. (b) Percentual change of salt-induced change in microvascular density. The change in microvascular density (total vessel density: diameter <20 μm) differed significantly between groups. Data are represented as means and SD. HSD, high-salt diet (>12 g/day); LSD, low-salt diet (<3 g/day); PVD_{small}, perfused vessel density: diameter less than 20 μm; TVD_{small}, total vessel density: diameter less than 20 μm. $^{**}P < 0.01$, $^{*}P < 0.05$, $^{\dagger}P < 0.10$.

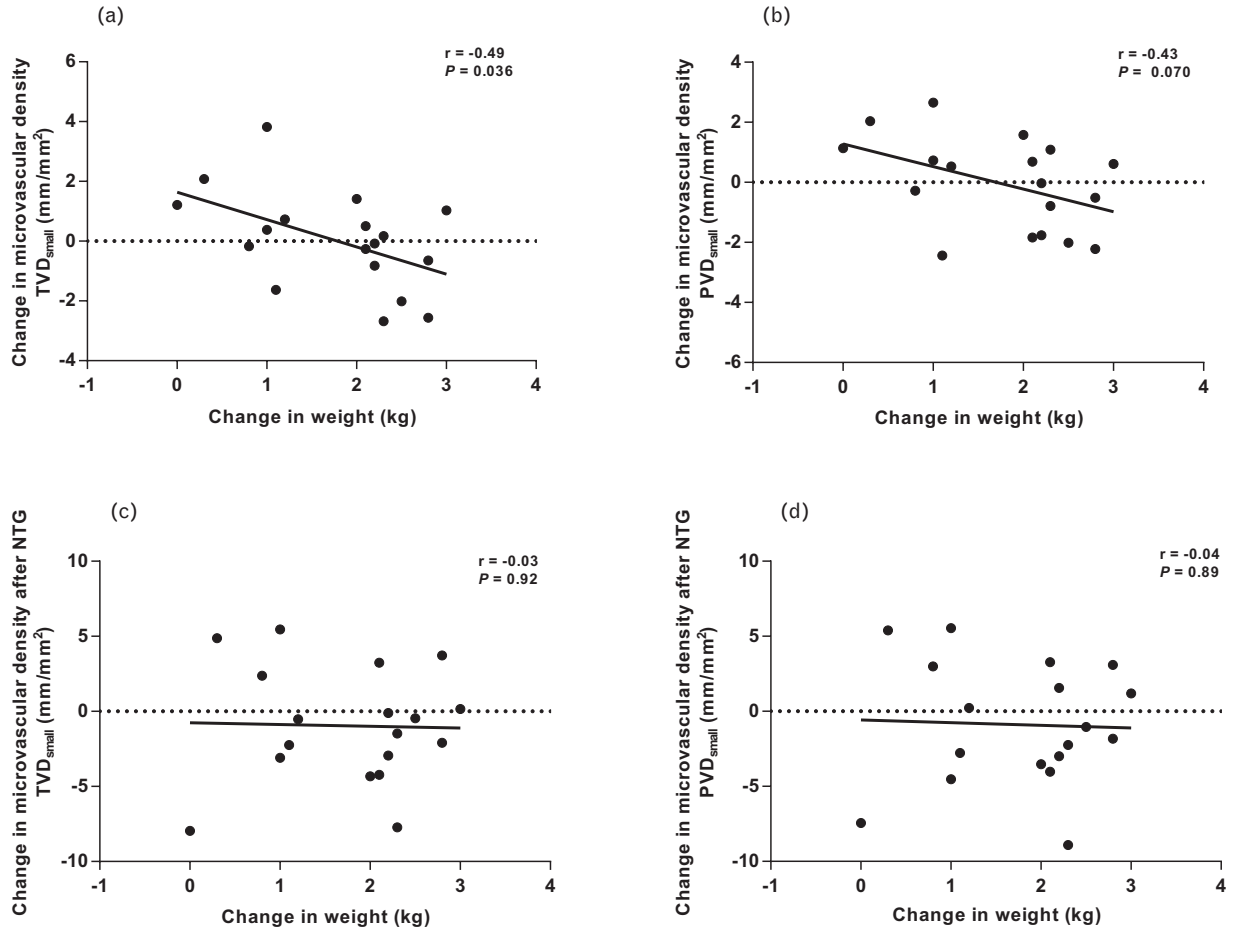

FIGURE 3 Scatterplots of the correlation between the change in weight and the change in microvascular density. (a) There was a significant correlation between the salt-induced change in weight and microvascular density for total vessel density diameter less than 20 μm. (b) There was no significant correlation between the change in weight and microvascular density (perfused vessel density diameter <20 μm). (c and d) There was no correlation between the change in weight and microvascular density after administering nitroglycerin spray for total vessel density diameter less than 20 μm and perfused vessel density diameter less than 20 μm. NTG, nitroglycerin; PVD_{small}, perfused vessel density: diameter less than 20 μm; TVD_{small}, total vessel density: diameter less than 20 μm.

weight ($P = 0.008$), a decrease in microvascular density was present in the group with a larger salt-induced change in weight ($P = 0.054$). In line with these results, the weight change of all participants demonstrated significant correlation with the change in microvascular density (TVD_{small}; Fig. 3). There was no correlation between weight change and the change in microvascular density following NTG (Fig. 3). Baseline characteristics, 24-h urine excretion and microvascular density following NTG did not differ significantly between participants with a small change in weight and those with larger change in weight (Table 3).

DISCUSSION

In this study, we aimed to obtain insight into the effect of salt intake on the sublingual microcirculation in healthy men. Overall no microvascular differences were found between the LSD and HSD. However, the increase in salt consumption from the LSD to the HSD significantly correlated with a lower recruitment rate of sublingual capillaries after administration of NTG, which indicates lower structural microvascular density. In individuals with larger body weight increase following high-salt intake, we observe significantly lower rates of perfused capillaries reflecting impaired functional microvascular density. As both phenomena occur independently of BP effects, our study indicates that high-salt load as such contributes to microvascular dysfunction, which is generally considered as an early feature of end-organ damage in various cardiovascular risk patients, including hypertensive patients.

To our knowledge this is the first study that has investigated the effect of salt on microvascular densities in normotensive patients using in-vivo sublingual imaging. With the use of other in-vivo techniques and in different microvascular tissues, previous studies have demonstrated reduction of capillary density in hypertensive [9,10,24], borderline hypertensive individuals [25,26] and offspring of hypertensive individuals [27,28], suggesting that reduction of microvascular density is an early feature of increased BP. However, these studies did not take sodium intake nor sodium-sensitivity into account as possible contributors to the BP associated microvascular changes. Other studies that did explore the combined effects of sodium, BP and the microcirculation, have shown that sodium-sensitive individuals with borderline hypertension or normal BP had significantly lower capillary density in the conjunctival

TABLE 3. Difference in outcome measurements between groups stratified for weight change

Δ High-salt diet – low-salt diet	Group with small change in weight, $n = 9$	Group with larger change in weight, $n = 9$	*P* value
Weight (kg)	1.1 (0.7)	2.5 (0.3)	NA
Plasma			
Sodium (mmol/l)	0 (2)	2 (3)	$P = 0.63$
Potassium (mmol/l)	−0.1 (0.4)	−0.3 (0.3)	$P = 0.53$
Osmolality (mmol/l)	1 (3)	2 (3)	$P = 0.59$
Hematocrit (l/l)	−0.01 (0.02)	0.00 (0.01)	$P = 1.0$
24-h Urine			
Volume (ml/24 h)	298 (977)	93 (983)	$P = 0.66$
Creatinine (μmol/24 h)	−0.4 (2.3)	0.8 (3.3)	$P = 0.40$
Sodium (mmol/24 h)	283 (100)	253 (120)	$P = 0.57$
Potassium (mmol/24 h)	21 (39)	22 (36)	$P = 0.93$
Creatinine clearance (ml/min)	30 (25)	29 (16)	$P = 0.96$
Office BP			
SBP (mmHg)	4 (6)	2 (6)	$P = 0.63$
DBP (mmHg)	−1 (6)	−1 (4)	$P = 0.99$
Mean arterial pressure (mmHg)	1 (5)	0 (4)	$P = 0.30$
Heart rate (bpm)	−3 (6)	0 (7)	$P = 0.82$
Ambulatory BP measurement (24-h, average of daytime and night-time measurements)			
SBP (mmHg)	−1 (7)	3 (5)	$P = 0.13$
DBP (mmHg)	0 (3)	−1 (4)	$P = 0.53$
Mean arterial pressure (mmHg)	0 (4)	1 (4)	$P = 0.84$
Microvascular density			
Total vessel density small (mm/mm^2)	0.84 (1.56)	−0.79 (1.35)	$P = 0.03$
Perfused vessel density small (mm/mm^2)	0.45 (1.71)	−0.55 (1.24)	$P = 0.17$
PPV small (%)	−2.49 (4.30)	−0.98 (3.58)	$P = 0.08$
MFI small	0.0 (0.0)	0.0 (0.0)	$P = 0.36$
Total vessel density all vessels (mm/mm^2)	0.35 (1.40)	−0.97 (1.23)	$P < 0.05$
Perfused vessel density all vessels (mm/mm^2)	−0.10 (1.97)	−0.68 (1.25)	$P = 0.46$
PPV all vessels (%)	−2.37 (5.03)	1.13 (4.28)	$P = 0.13$
MFI all vessels	0.0 (0.2)	0.0 (0.2)	$P = 0.39$
Microvascular density following sublingual nitroglycerin			
Total vessel density small (mm/mm^2)	−0.24 (4.56)	−1.10 (3.89)	$P = 0.45$
Perfused vessel density small (mm/mm^2)	−0.09 (4.68)	−0.81 (3.98)	$P = 0.43$
PPV small (%)	0.71 (4.08)	1.50 (5.29)	$P = 0.70$
MFI small	0.0 (0.1)	0.0 (0.1)	$P = 0.50$
Total vessel density all vessels (mm/mm^2)	−0.35 (4.00)	−0.88 (2.90)	$P = 0.49$
Perfused vessel density all vessels (mm/mm^2)	−0.28 (4.22)	−0.63 (3.03)	$P = 0.41$
PPV all vessels (%)	0.20 (5.13)	1.36 (5.99)	$P = 0.96$
MFI all vessels	0.0 (0.2)	0.0 (0.2)	$P = 0.55$

All values are presented as mean (SD). Vessel parameters marked as 'small' have diameters less than 20 μm, representing capillary density. Vessel parameters marked as 'all vessels' include vessels of all sizes and is considered a quality check. BP, blood pressure; MFI, microvascular flow index; PPV, proportion of perfused vessels.

microvasculature [14] and demonstrate an inverse association of nailfold capillary recruitment and the sodium-sensitive BP response among hypertensive and normotensive individuals [15]. As these studies had a cross-sectional design and did not report dietary sodium status at time of measurements, direct effects of sodium reduction and associated BP response on the microcirculation were not evaluated. So far, data on effects of sodium reduction on microvascular networks are therefore currently available in untreated hypertensive individuals, in either the conjunctival vascular bed [16] or skin capillaries [13]. In contrast to these studies we could not demonstrate salt-induced microvascular changes when comparing LSD vs. HSD in a crossover fashion. Yet, there was a significant correlation between the increase in salt intake and decrease in microvascular density following administration of NTG. This suggests that salt exposure has negative effects on the recruitment of sublingual microvasculature, as they move together in a linear fashion. Our results are in line with studies in the cremaster muscle of rats in which high-salt intake led to decrease in microvascular densities [29,30]. However, we were unable to detect differences in microvascular density between diets. The absence of BP effects might be explanatory for our observation that there was no difference in microvascular density when comparing the HSD and LSD. He *et al.* [13] have demonstrated an increase in both functional and structural microvascular density following sodium reduction among hypertensive patients. This may be related to the fact that He *et al.* reported an increase in microvascular density with a decrease in BP, while we did not find a change in BP.

The data of our stratified analysis are of interest in light of the recently rediscovered concept of nonosmotic sodium storage and the effect of salt on microvascular density. In the current study, we observed that the amount of weight change between diets differed substantially between participants that, considering the duration of the salt intervention periods, can be attributed to changes in ECFV. Although there was a difference in salt-induced fluid expansion between individuals, there was no difference in salt

excretion. This is in line with previous observations of Laffer *et al.* [6] who demonstrated that with the same amount of total body sodium, salt-sensitive individuals had an increase in body weight, whereas salt-resistant individuals did not. Our observations with a wide range of weight change for similar salt levels challenge traditional beliefs that salt retention induces iso-osmolar water retention. Via ^{23}Na MRI it was shown that high amounts of nonosmotic sodium storage in striated muscle were associated with hypertension [31]. This was further substantiated in studies in mice in which disruption of salt efflux from the nonosmotic storage compartment led to high BP [18]. One may hypothesize that saturation of the nonosmotic storage compartment leads to a subsequent smaller capacity for nonosmotic storage of added salt. The following increase in BP may be related to microcirculatory alterations.

We did not observe a difference in BP response and therefore we can only assume that those who showed a larger salt-induced fluid expansion might become more salt-sensitive in terms of BP response at an older age. Furthermore, after administration of NTG microvascular densities in the group with large salt-induced weight gain increased to similar levels of vessel densities measured in the group with small weight changes, dissolving the correlation between weight change and vessel densities. This suggests a relatively larger response to exogenous NO in the group with a larger salt-induced fluid expansion, indicating a decrease of NO activity in those individuals. These results are in line with studies among salt-sensitives. Schmidlin *et al.* [8] demonstrated an increase in asymmetric dimethylarginine (ADMA), a NO inhibitor, in salt-sensitives but not in those salt-resistant. Another study rendered similar results measuring plasma NOx (NO metabolites nitrate and nitrite) concentrations [7]. Our results add to their findings and show that NO activity might play a role in the interaction between microvascular changes and a smaller capacity for nonosmotic sodium storage.

Our study has some limitations. First, we only assessed sublingual microcirculatory parameters. It remains uncertain if our findings can be extrapolated to other microvascular beds. Also, though SDF imaging obtains high-resolution images, incident dark field imaging (IDF) is considered to provide images with higher resolution [32]. However, images were graded for sufficient quality, and discarded if necessary. Also, the fact that administering NTG led to significant increase in vessel density suggests that our image quality was sufficient for detecting differences between groups. Finally, our analyses generated similar results in comparison to a study using IDF imaging that also used NTG [20]. Another limitation might be that we did not precisely measure the ECFV, but used an indirect measurement (i.e. body weight). Given the short-time frame of our intervention, most of the differences in body weight are likely to be attributable to changes in body water, also because 24-h creatinine excretion levels, reflecting muscle mass, remained similar. Also, we studied healthy male individuals to exclude the influence of menstrual cycle related hormonal changes, and therefore one might question whether our results are applicable to other patient categories. Finally, we found that potassium excretion was also significantly different between both intervention periods. Considering the effects of potassium intake on BP, this may explain why no BP effects were observed. Yet, potassium excretion levels were above WHO recommendations of 90 mmol/day in both interventions, and no associations between potassium excretion and BP or sodium-to-potassium ratio and BP were seen.

Perspectives

We show that increment of dietary salt intake is associated with a reduction in sublingual microvascular density following administration of NTG among healthy men. Our results suggest that the ability of our healthy male volunteers to maintain sufficient microvascular density is vital in maintaining normal BP. We demonstrate that *in vivo* a decrease of sublingual microvascular density is present in individuals with a larger change in body weight which may reflect smaller capacity for nonosmotic sodium storage. Furthermore, when exogenous NO in the form of NTG was administered, no change in microvascular density was present, suggesting that impaired activity of NO may play a role in the link between nonosmotic sodium storage and microvascular alterations. This also may imply that healthy individuals may benefit from dietary sodium reduction before hypertension or microvascular end-organ damage becomes apparent. More research is needed to further assess underlying pathophysiological mechanisms and longitudinal consequences of the interaction between the microcirculation and salt intake.

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Conflicts of interest

There are no conflicts of interest.

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