Original Article

Short-term changes in dietary sodium intake influence sweat sodium concentration and muscle sodium content in healthy individuals

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Objective: There is increasing evidence that sodium can be stored in the skin and muscles without being osmotically active, yet whether acute changes in dietary sodium intake alter sweat and muscle sodium content has not been investigated previously.

Methods: In a cross-over design, we assessed muscle sodium content by ^{23}Na-MRI in 38 healthy normotensive volunteers (aged 33.5 ± 11.1 years, 76.3% women) after 5 days of high-sodium diet (6 g of salt added to their normal diet) and 5 days of a low-sodium diet. In a subgroup of 18 participants (72.2% women) we conducted quantitative pilocarpine iontophoretic sweat collections and measured the sodium concentration in sweat. Plasma aldosterone and plasma renin activity levels were measured in all participants.

Results: Under high-sodium diet conditions urinary sodium excretion, muscle sodium content and sweat sodium concentration all increased significantly. Muscle sodium content ($r_m = 0.47$, $P = 0.03$) and sodium sweat concentration ($r_m = 0.72$, $P < 0.001$) correlated positively with salt intake as estimated by 24-h urine sodium excretion. Age, sex or the phase of the menstrual cycle did not influence muscle or sweat sodium concentrations or their changes. In contrast, plasma aldosterone levels were negatively associated with both muscle sodium ($r_s = -0.42$, $P = 0.0001$) and sweat sodium content ($r_s = -0.52$, $P = 0.002$). Plasma renin activity correlated negatively with sweat sodium ($r_s = -0.43$, $P = 0.012$) and muscle sodium levels ($r_s = -0.42$, $P < 0.001$).

Conclusion: Muscle and sweat sodium concentrations are significantly higher on a high-salt intake in healthy male and female individuals, suggesting that muscle and sweat play a role in regulating sodium balance in humans.

Keywords: aldosterone, blood pressure, MRI, muscle, sex, sodium, sweat

Abbreviations: BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; ENaC, epithelial sodium channel; MR, magnetic resonance; RAS, renin–angiotensin system; TSE, turbo spin echo

INTRODUCTION

The kidney has long been placed at the very center of extracellular volume, sodium (Na^+) and blood pressure (BP) homeostasis. Recently, Titze *et al.* [1] suggested that the skin and muscle also contribute to the regulation of sodium balance in humans. In a long-term Mars flight simulation study, they reported that healthy male individuals accumulated Na^+ in considerable amounts without concomitant weight gain, in contrast to the generally accepted theory that changes in total body sodium are paralleled by changes in extracellular volume [2]. They stated that Na^+ can accumulate in the skin and muscles where it is stored without being osmotically active, bound to negatively charged glycosaminoglycans, and representing thus a third compartment of storage which is regulated by the immune system [3].

Sweat – the major product of the skin – may also be involved in the control of the human sodium balance, but this has received little attention so far. Of interest, sweat glands are able to secrete high amounts of water and salt, but can also reabsorb significant part of Na^+ ions in their duct via epithelial sodium channels (ENaCs) that are under the control of aldosterone [4]. These data suggest that sweat participates to Na^+ homeostasis but it is currently unknown whether dietary sodium intakes influence sodium excretion in sweat. In addition, it is unknown whether sex differences exist in the influence of dietary sodium intake on sweat and muscle sodium content, as most studies included mainly men. Sparse data suggest that estrogen promotes sweating, whereas progesterone has the opposite effect [5].

A technique of functional MRI called ^{23}Na-MRI allows to measure simultaneously sodium and water content in human tissues [6]. This technique has shown that sodium accumulates

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in the skin and muscles of patients with hypertension, primary hyperaldosteronism or on hemodialysis [6–8]. The same authors demonstrated that skin and muscle sodium content decrease acutely after a hemodialysis session and after adrenalectomy in primary hyperaldosteronism. However, it remains so far unknown whether changes in dietary sodium intake induce changes in muscle sodium content of healthy individuals, which would be another argument for a role of muscles in sodium homeostasis.

The aim of this study was therefore to assess whether short-term changes in sodium intake induce parallel changes in the sodium concentration of sweat and muscle sodium deposition as measured by ^{23}Na-MRI in men and women.

METHODS

The current study was approved by the local institutional review committee (Ethical Committee of the Canton de Vaud, Switzerland), and conducted according to the principles expressed in the Declaration of Helsinki. All study participants provided written informed consent.

Study population and design

Healthy volunteers were recruited by local advertisement. Inclusion criteria were minimum age of 18 years; office BP less than 135/85 mmHg without a history of hypertension; normal renal function [estimated glomerular filtration rate (eGFR) > 90 ml/min per 1.73 m^2], no albuminuria (<30 mg/day) and no history of kidney disease; negative pregnancy test in women and ability to understand the study protocol and to sign an informed consent form. Participants were excluded in case of concomitant diseases, drug treatment, pregnancy, history of drug abuse and in case of a contra-indication to MRI such as claustrophobia or implanted metallic device.

During the screening visit a complete physical examination was performed. Body weight and height were measured using calibrated electronic scales (Seca, Hamburg, Germany) and BMI was calculated as weight (kilograms) divided by squared height (square meters). BP was measured with an automated Omron 705IT oscillometric device (HEM-759-E, Omron Corporation, Kyoto, Japan) after 10 min of rest in the sitting position in each arm. Subsequently, five consecutive BP measurements were taken on the side with the highest BP [9]. Electrolytes, renal function, blood glucose, spot urine albuminuria were measured using standard clinical laboratory methods. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was used to calculate the eGFR [10].

This was a cross-over study. Each participant performed two study visits: once after 5 days of a high-salt diet and once after 5 days of a low-sodium diet. Participants followed a high-sodium diet by adding 6 g of NaCl to their regular diet during 5 days before the study visit. Low-salt diet was obtained by providing dietary advice and documents with menu proposals. A wash-out period of 30 days was respected between the two study visits. Participants were randomly assigned to start with a high-sodium diet or low-sodium diet. In women, each study visit was performed at the same phase of the menstrual cycle.

On the day before each study visit, all participants performed a 24-h urine collection following oral and standardized written instructions for the measurement of urinary volume, excretion of sodium, albumin, urea and creatinine. The 24-h urine collection was considered as incomplete, that is if urinary volume was less than 500 ml/24 h, participants reported not having collected all 24-h urine or urinary excretion of creatinine was equal to or below the 10th percentile in both sex categories [11,12]. The last condition was arbitrarily chosen to eliminate collections most likely to be incomplete. The 24-h collection was considered as overcollected if urinary excretion of creatinine was above the 90th percentile in both sex categories.

Each study visit included standardized BP measurement, blood sampling (including electrolytes, oestradiol, progesterone, aldosterone, renin and full blood count) and ^{23}Na-MRI. Quantitative pilocarpine iontophoretic sweat collection was performed in a subset of participants.

Plasma renin activity and aldosterone quantification

Plasma aldosterone concentrations were measured in supine condition at 30 min with the Aldo-Riact RIA kit (CISBio International, Yvette Cedex, France). Plasma renin activity was measured using a radioimmunoassay commercial kit for the quantitative determination of Angiotensin I in human plasma (REN-CT2; CISBio International).

Quantitative pilocarpine iontophoretic sweat collection

Sweat collections were performed using the validated Macroduct sweat collection system based on pilocarpine iontophoresis (Webster Sweat Inducer Model 3700; ELITechGroup, Puteaux, France) [13]. Macroduct is approved for sweat collection by the Cystic Fibrosis Foundation, and quantitative pilocarpine iontophoresis sweat chloride test is the gold standard for the diagnosis of cystic fibrosis [14]. The preferred site for sweat collection was the lower portion of the flexor aspect of the forearm. The selected site had to be free of observable abnormalities in the skin. Hereafter, local sweating was induced by iontophoresis of pilocarpine with a disposable Pilogel electrode using the Webster Sweat Inducer for 5 min, as published previously [13]. Sweat sodium and potassium levels were measured in the local laboratory by flame photometry (Instrumentation Laboratory IL 943 Flame Photometer; Lexington, Massachusetts, USA), and sweat chloride was measured with a chloride analyzer (Model 926S; Sherwood Scientific Ltd, Cambridge, UK).

^{23}Na-MRI

The magnetic resonance (MR) antenna (RAPID Biomedical, Rimpar, Germany) contained a hydrogen tuned butterfly coil for scout images and an excite and receive (Tx/Rx) sodium-tuned surface coil made of a single circular loop of 14 cm for sodium images [15]. This antenna is able to measure muscle sodium content, but not skin sodium content. In each scan, four reference phantoms containing respectively 10, 20, 30 and 40 mmol/l NaCl and 2% agar gel in which placed symmetrically under the coil by the help of a dedicated Plexiglas support. They served to transform the sodium signal intensity into a sodium concentration. Each volunteer was lying supine with the leg flat on the antenna and the feet entering first into the scanner. The acquisitions

were done at the level of the upper leg with a 3T-whole-body MR system (Magnetom Prisma; Siemens Medical Systems, Erlangen, Germany). The image reconstruction for the radial trajectory of the ultra-short echo time sequence was implemented on Matlab 8.6 R2015b (The MathWorks Inc., Natick, Massachusetts, USA) [16,17]. The data acquired along the radial trajectory were first interpolated on a three-dimensional Cartesian grid before applying the conventional inverse Fourier transform. A convolution-based interpolation was used with a Kaiser–Bessel convolution kernel. The density compensation was performed as previously reported [18]. For the quantification of the sodium concentration, the sodium signal was averaged in regions of interest drawn around the muscle mass on turbo spin echo (TSE) images. Regions drawn on the TSE images allowed to exclude voxels containing large vessels or fat infiltration. The mean sodium signal intensity was then converted into a sodium concentration by a piecewise linear interpolation of the reference phantoms signals.

Statistical analysis

Quantitative variables were expressed as mean ± SD or as median (25th–75th percentile range), as appropriate. Qualitative variables were expressed as number of patients and percentage. Shapiro–Wilk test was used to assess whether data were normally distributed. To compare distributions of continuous variables we used paired *t* tests or *t* tests as appropriate if the data were normally distributed and Wilcoxon signed-ranks tests for data that were not normally distributed. Sweat sodium concentration was log transformed because of nonnormal distribution. To assess the relationship between continuous variables, we used Spearman's correlation tests. We represented these relationships with lines and [95% confidence intervals (CIs)] resulting from linear regression analyses. To determine the common within-individual association for paired measures of two continuous variables assessed on two occasions (after low-sodium and high-sodium diets), we used the repeated measures correlation method implemented in the package *rmcorr* for use in R (R Core Team, 2017; R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) [19]. All other analyses were performed with Stata 14.1 (Stata Corp, College Station, Texas, USA). To assess the relationship between invariant baseline characteristics and paired measures of a continuous variable, we performed a repeated measures analysis of variance (ANOVA) with baseline characteristics as a time invariant covariate. The nominal level of statistical significance was set at *P* less than 0.05.

The power calculation for dietary-induced changes in sweat sodium concentration was based on an anticipated change of 20% in sweat sodium and mean concentrations as reported previously by Buono *et al.* [20]. Based on these assumptions, 15 persons had to be included for a power of 80%. As for muscle sodium concentration, we expected a dietary-induced change of 10%. Based on values reported by Kopp *et al.* [6], and an alpha of 0.05 at two-sided significance level, we calculated that a minimum of 10 participants had to be included to assure a power of 80%. To detect between-group differences of the same size, with the predefined intention to recruit at least twice more women than men, 25 women and 13 men had to be included to assess sex differences.

RESULTS

A total of 38 participants (76.3% women) were included. Their baseline characteristics are shown in Table 1. All urine collections fulfilled the predefined criteria of completeness. Of the 29 women, 19 participated during the follicular phase and 10 during the luteal phase. All the 38 participants underwent successfully ^{23}Na-MRI, whereas a subgroup of 18 volunteers (72.2% women) underwent quantitative pilocarpine iontophoretic sweat collection. Their baseline characteristics did not differ from the participants who underwent only ^{23}Na-MRI, apart from a higher age in men in the sweat assessment group.

Dietary-induced changes in participant characteristics

Serum sodium was significantly higher under high-sodium diet conditions, as expected like urinary sodium and chloride excretion (Table 2) [21]. Body weight and eGFR increased, but not significantly. Urinary potassium excretion was not influenced by the dietary regimen, whereas, circulating aldosterone levels and plasma renin activity were significantly lower under high-sodium diet. Both sweat sodium and chloride concentrations increased significantly under high-sodium diet, in contrast to sweat potassium concentration that was lower under high-sodium diet. Muscle sodium content was also significantly higher after high-sodium diet compared with low-sodium diet ($P < 0.001$).

TABLE 1. Baseline characteristics of participants[a]

	^{23}Na-MRI muscle assessment			Sweat assessment		
	Total	Women	Men	Total	Women	Men
n	38	29	9	18	13	5
Age (years)	33.5 ± 11.1	33.4 ± 11.0	33.7 ± 12.1	36.2 ± 13.2	35.2 ± 11.9	40.3 ± 20.5
Weight (kg)	66.3 ± 12.2	62.7 ± 6.8	77.5 ± 17.9	66.1 ± 11.9	61.9 ± 5.1	75.0 ± 17.6
Height (cm)	166.8 ± 7.9	164.2 ± 7.1	174.7 ± 4.6	170.1 ± 7.5	166.8 ± 5.4	177.5 ± 6.1
BMI (kg/m^2)	23.8 ± 3.7	23.3 ± 3.0	25.3 ± 5.3	22.7 ± 2.8	22.3 ± 2.1	23.6 ± 4.2
SBP (mmHg)	113.6 ± 11.6	110.1 ± 8.7	123.5 ± 13.8	114.5 ± 8.8	113.1 ± 6.7	119.5 ± 15.3
DBP (mmHg)	69.8 ± 8.6	68.9 ± 6.8	72.3 ± 12.6	68.1 ± 7.8	67.5 ± 6.6	70.5 ± 13.0
eGFR (ml/min per 1.73 m^2)	107.4 ± 13.6	105.8 ± 14.5	112.4 ± 9.8	105.7 ± 15.3	104.6 ± 16.0	110.6 ± 13.3

BP, blood pressure; eGFR, estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation.
[a]Mean ± SD.

TABLE 2. Participant characteristics after high and low-salt diet[a] ($n = 38$)

	High salt	Low salt	*P*
Weight (kg)	66.2 ± 12.0	65.2 ± 11.8	0.68
BMI (kg/m^2)	23.7 ± 3.7	23.4 ± 3.7	0.61
SBP (mmHg)	113.6 ± 11.5	112.9 ± 9.9	0.79
DBP (mmHg)	69.6 ± 8.6	69.4 ± 7.0	0.82
eGFR (CKD-EPI, ml/min per 1.73 m^2)	107.2 ± 13.6	102.9 ± 13.2	0.15
Serum sodium (mmol/l)	140.4 ± 1.5	139.1 ± 1.7	<0.001
24-h urine volume (ml)	1859.1 ± 766.2	1820.5 ± 749.3	0.93
24-h urinary sodium excretion (mmol/day)	227.0 ± 104.2	51.7 ± 60.1	<0.001
24-h urinary chloride excretion (mmol/day)	235.1 ± 80.2	39.0 ± 30.7	<0.001
24-h urinary potassium excretion (mmol/day)	63.6 ± 22.8	66.6 ± 23.6	0.49
24-h urinary salt excretion (g/day)	13.4 ± 6.1	3.0 ± 3.5	<0.001
Sweat sodium (mmol/l)[b]	43.6 ± 18.1	34.2 ± 20.2	0.01
Sweat chloride (mmol/l)[b]	25.2 ± 13.0	17.6 ± 12.9	0.02
Sweat potassium (mmol/l)[b]	8.1 ± 1.9	10.3 ± 3.1	0.01
Muscle sodium content (mmol/l)	10.7 ± 1.4	9.7 ± 1.0	<0.001
Plasma aldosterone (pg/ml)	56.7 ± 56.7	308.7 ± 195.2	<0.001
Plasma renin activity (ng/ml per h)	0.74 ± 1.43	1.90 ± 1.40	<0.001
PAC/PRA (h 10^2)	15.3 ± 17.2	22.5 ± 21.8	0.08

eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRA, plasma renin activity.
[a]Paired *t* tests or Wilcoxon signed ranks test as appropriate; expressed in mean ± SD.
[b]Analysis in subgroup of 18 participants.

Factors associated with sweat sodium concentration

We found a strong positive association between sweat sodium concentration and 24-h urinary sodium excretion [$r_m = 0.72$, 95% CI (0.39–0.89), $P < 0.001$], as graphically illustrated in Fig. 1. Sweat sodium concentration was similar in men and women after low-sodium diet (29.3 ± 10.5 vs. 36.5 ± 23.4 mmol/l, $P = 0.49$) or high-sodium diet (40.3 ± 16.9 vs. 45.0 ± 19.1 mmol/l, $P = 0.61$). During the follicular phase, women tended to have a higher sweat sodium concentration than women during the luteal phase (58.9 ± 26.9 vs. 24.0 ± 8.5 mmol/l, $P = 0.06$). However, there was no significant relationship between sweat sodium concentration and oestradiol (Spearman's $r_s = -0.33$, $P = 0.102$) or log transformed progesterone levels ($r_s = -0.46$, $P = 0.097$) in female participants. Sodium concentration in sweat was not associated with age, nor after high-sodium diet ($r_s = -0.09$, $P = 0.73$) nor low-sodium diet ($r_s = 0.11$, $P = 0.67$). A repeated measures ANOVA with age as a time invariant covariate also showed that age was not related to sweat sodium concentrations ($P = 0.069$). SBP or DBP were not correlated with sweat sodium concentration after low-sodium diet or high-sodium diet (Supplementary materials, http://links.lww.com/HJH/B143).

Factors associated with muscle sodium concentration

There was a positive correlation between muscle sodium content and 24-h urinary sodium excretion across individuals [$r_m = 0.47$, 95% CI (0.17–0.69), $P = 0.003$] as illustrated in Fig. 2. Muscle sodium concentration was similar in women

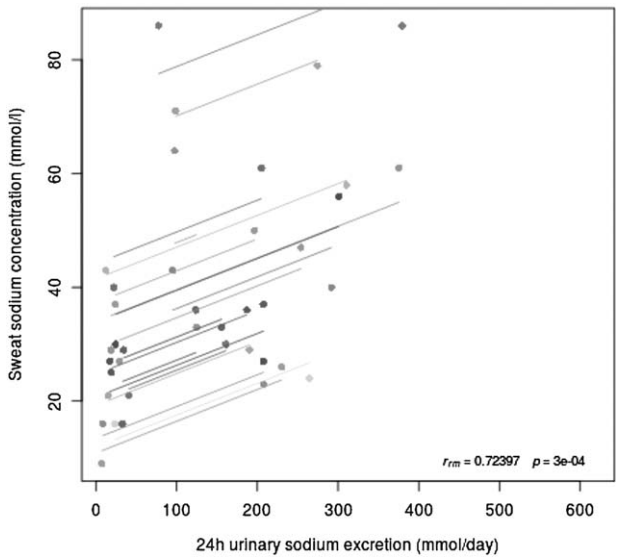

FIGURE 1 Repeated measures correlation between sweat sodium concentration and 24-h urinary sodium excretion. Each dot represents a single observation for a participant. Observations from the same participant at different times are given the same color, with corresponding lines to show the common linear fit for each participant.

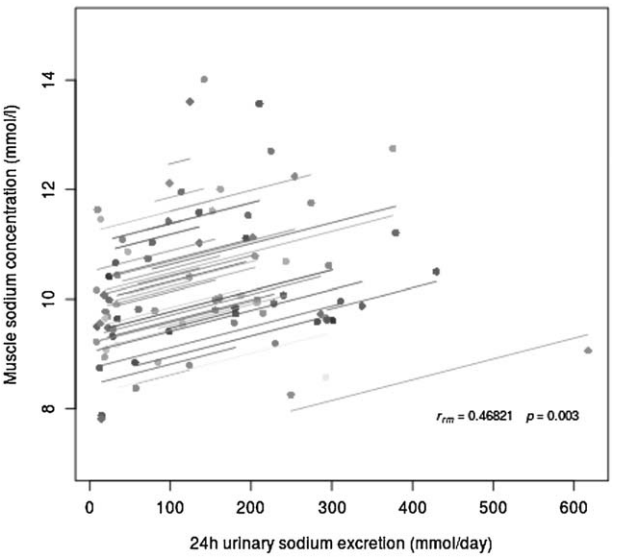

FIGURE 2 Repeated measures correlation between muscle sodium concentration and 24-h urinary sodium excretion. Each dot represents a single observation for a participant. Observations from the same participant at different times are given the same color, with corresponding lines to show the common linear fit for each participant.

and men after low-sodium diet (9.9 ± 1.0 vs. 9.3 ± 0.9 mmol/l, $P = 0.13$) and high-sodium diet (10.6 ± 1.3 vs. 10.9 ± 1.6 mmol/l, $P = 0.62$) as was the change in muscle sodium content from low-sodium diet to high-sodium diet (0.86 ± 1.5 mmol/l in women vs. 1.1 ± 1.2 mmol/l in men, $P = 0.63$). In female participants, muscle Na^+ content was not influenced by the follicular or luteal phase. In addition, there was no correlation between muscle sodium concentration and oestradiol concentration ($r_s = -0.136$, $P = 0.302$) or log transformed progesterone ($r_s = -0.33$, $P = 0.068$).

There was a positive correlation between BMI and 24-h urinary Na^+ excretion after high-sodium diet ($r_s = 0.41$, $P = 0.005$) but not low-sodium diet ($r_s = 0.18$, $P = 0.28$). Significantly, muscle sodium content was lower in overweight or obese participants (BMI ≥ 25 kg/m^2) ($n = 16$; 42.1%) compared with normal weight volunteers after low-sodium diet (8.9 ± 0.8 vs. 10.1 ± 0.9 mmol/l, $P = 0.0003$) and high-sodium diet (9.8 ± 0.8 vs. 11.1 ± 1.4 mmol/l, $P = 0.002$). Furthermore, there was a significant negative correlation between muscle sodium concentration and BMI after high-sodium diet ($r_s = -0.39$, $P = 0.008$) and low-sodium diet ($r_s = -0.34$, $P = 0.036$).

We found no significant correlation between muscle sodium content and age after low-sodium diet ($r_s = 0.18$, $P = 0.26$) or high-sodium diet ($r_s = 0.09$, $P = 0.55$). Age was not significantly related to muscle sodium concentration in a repeated measures ANOVA analysis ($P = 0.072$). In participants undergoing sweat assessment, muscle sodium concentration correlated positively with sweat sodium concentration ($r_s = 0.49$, $P = 0.005$) (Fig. 3). Finally, there was a significant negative correlation between DBP and muscle sodium content after low-sodium diet ($r_s = -0.37$, $P = 0.02$) (Supplementary materials, http://links.lww.com/HJH/B143).

Influences of plasma aldosterone concentration and plasma renin activity

As expected, plasma aldosterone was markedly lower on high-sodium diet than on low-sodium diet ($P < 0.001$) and correlated negatively with urinary sodium excretion ($r_s = -0.77$, $P < 0.001$) (Table 2). Plasma aldosterone was negatively associated with both sweat sodium ($r_s = -0.52$, $P = 0.002$) and muscle sodium content ($r_s = -0.42$, $P = 0.0001$) (Fig. 4a and b). In addition, plasma aldosterone correlated positively with urinary potassium excretion ($r_s = 0.22$, $P = 0.036$) and sweat potassium concentration ($r_s = 0.49$, $P = 0.004$) (Fig. 4c).

Plasma renin activity also correlated negatively with urinary sodium excretion ($r_s = -0.59$, $P < 0.001$), sweat

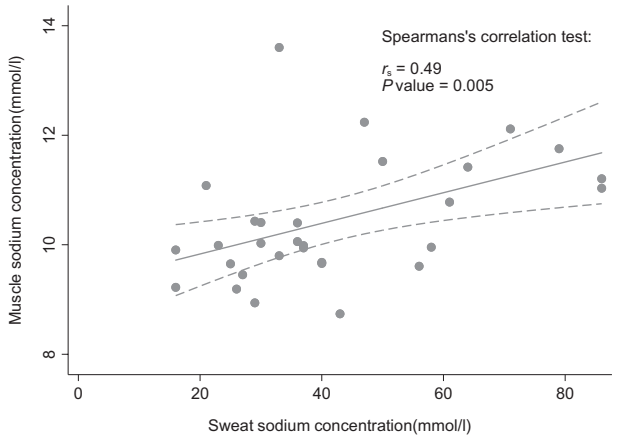

FIGURE 3 Correlation between muscle sodium and sweat sodium concentration. Data are Spearman's rank correlation coefficient (r_s) with associated P value. The line with a 95% confidence interval results from the prediction of a linear regression.

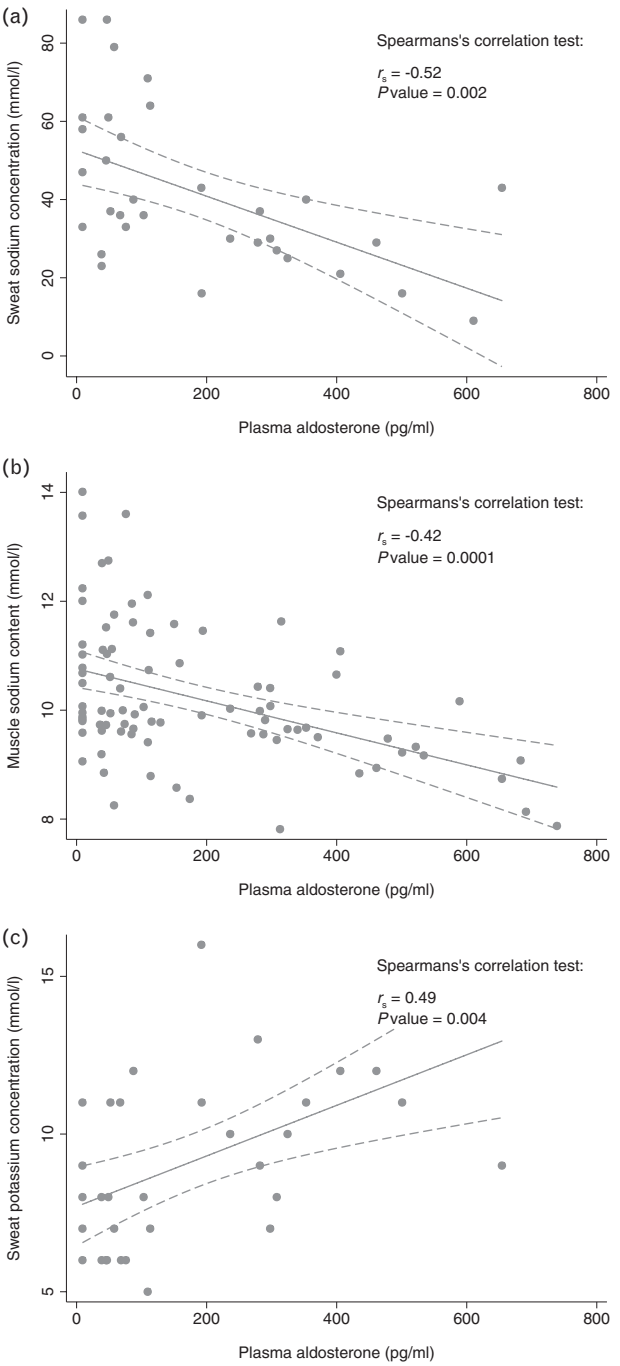

FIGURE 4 Correlation between sweat sodium, muscle sodium and sweat potassium concentration with plasma aldosterone. (a) Correlation between sweat sodium concentration and plasma aldosterone. (b) Correlation between muscle sodium concentration and plasma aldosterone. (c) Correlation between sweat potassium concentration and plasma aldosterone. Data are Spearman's rank correlation coefficient (r_s) with associated P value. The line with a 95% confidence interval results from the prediction of a linear regression.

TABLE 3. Univariate associations with delta sweat Na and delta muscle Na[a,b]

	Δ Sweat Na		Δ Muscle Na	
	r_s	*P*	r_s	*P*
Age	−0.24	0.36	−0.09	0.61
BMI	−0.18	0.45	−0.03	0.86
Δ Body weight	0.09	0.71	0.37	0.02
Δ SBP	−0.11	0.71	0.02	0.92
Δ DBP	0.05	0.86	−0.14	0.41
Δ Plasma aldosterone	−0.55	0.03	−0.21	0.21
Δ Plasma renin activity	−0.56	0.02	−0.10	0.53

[a]Data are Spearman's rank coefficient with associated *P* value.
[b]Delta (Δ) defined as difference between high and low-salt diet.

sodium content ($r_s = -0.43$, $P = 0.012$) and muscle sodium levels ($r_s = -0.42$, $P < 0.001$). BP levels were not related to plasma renin activity or aldosterone levels (Supplementary materials, http://links.lww.com/HJH/B143).

Factors associated with changes of sweat and muscle sodium content

Differences in weight between low-sodium diet and high-sodium diet were wide ranging from −1.3 to +5.0 kg, with 18.7% of participants having lost more than 0.5 kg weight after switching from high-sodium diet to low-sodium diet and 35.4% having lost less than 0.5 kg of weight. Changes in body weight did not correlate with the changes in sweat Na^+ concentration, but correlated positively with the change in muscle sodium content ($r_s = 0.37$, $P = 0.02$) (Table 3). Changes in SBP or DBP were not correlated with changes in sweat or muscle Na^+ content. Finally, both the change in plasma aldosterone and plasma renin activity correlated strongly and negatively with changes of sweat Na^+.

DISCUSSION

The main finding of the current study is that sweat and muscle sodium concentrations are significantly higher under high-salt diet as compared with low-salt diet in young healthy individuals supporting the hypothesis that sweat and muscle may play a role in the regulation of sodium balance in humans. Age, sex or the phase of the menstrual cycle in women do not influence sweat and muscle Na^+ concentrations or their changes in this study, whereas, circulating aldosterone levels and plasma renin activity are strongly associated with both sweat and muscle sodium contents in men and women. In addition, overweight or obese participants appear to have lower sweat and muscle sodium contents than individuals with a normal weight.

The sodium concentration of sweat increased significantly on a high-salt intake. The changes in sweat sodium content were rather small (∼25%), suggesting that its role in sodium homeostasis may be limited as compared with the kidneys, but the skin surface is large. Moreover, large differences were seen between individuals, with some participants showing sweat sodium concentrations up to 86 mmol/l. If we consider that the average daily sweat production is ∼1 l (and even much higher during intense exercise), salt losses by the skin can be considerable and almost equal to renal losses [4].

The activity of the renin–angiotensin system (RAS) is highly dependent on the salt intake. Our data suggest that salt-induced changes in RAS activity also play a role in the regulation of sweat sodium excretion. Indeed, plasma aldosterone and plasma renin activity correlated negatively with sweat sodium, and positively with sweat potassium levels, supporting an effect of aldosterone on the Na^+/K^+ exchange in cutaneous glands that was firstly described more than 40 years ago [22]. By acting on ENaCs aldosterone increases ductal reabsorption of Na^+ in sweat glands in exchange for potassium [23]. Of interest, mutations in ENaC subunit genes lead to pseudohypoaldosteronism type I, which is characterized by an excessive salt loss from sweat glands [24]. In theory, mineralocorticoid antagonists might also alter sweat sodium and potassium content, but to the best of our knowledge, this has not been studied yet in healthy individuals or patients treated with mineralocorticoid antagonists.

On the contrary, our ^{23}Na-MRI was not able to measure skin sodium content, and therefore, we could not verify whether sweat sodium correlates directly with skin sodium content. However, a recent skin biopsy study in 48 healthy participants maintained on low-salt diet (4 g/day) and subsequently randomized to placebo or high-sodium (200 mmol/day) tablets for 7 days revealed that skin sodium content (as measured with inductively coupled plasma optical emission spectrometry in the biopsies) clearly increased after dietary salt loading [25].

Our MRI findings suggest that changes in dietary sodium intake induce parallel changes in muscle Na^+ content. However, diet-induced changes in muscle sodium were much lower (∼1 mmol/l) than those observed in sweat or skin biopsies, and their impact is therefore probably smaller, though the global muscle mass represents about 30 kg in a 70 kg men. We found no significant differences in muscle or sweat Na^+ concentrations according to age, sex or menstrual phase. These results are in contrast with previous reports showing that tissue sodium content increases with age [7,26]. However, we included mainly young participants and a small number of individuals. These two factors may explain the lack of association with age.

Muscle sodium content was associated with circulating aldosterone levels: the higher aldosterone, the lower muscle sodium content. These results seem in contrast with previous data that described a high muscle sodium content in patients with primary hyperaldosteronism that was reversed with spironolactone [7]. We have no clear explanation for these discrepant findings. We hypothesize that sodium is essentially stored in the muscles when there is an excessive sodium intake (high-sodium diet) or sodium retention associated with elevated plasma aldosterone levels as in primary hyperaldosteronism. When sodium intake is low, there is no need to store sodium in the muscle or skin, and in this case maintenance of plasma sodium is probably the main goal of the regulatory processes. As aldosterone secretion is suppressed after a short-term high-salt diet and vice versa, our findings confirm that on a low-sodium intake, elevated aldosterone represent a compensatory mechanism, which is proportional to sodium depletion. The apparent contradiction of high sweat/muscle sodium levels in primary aldosteronism suggests that the relationship between sweat and

to a lesser extent muscle Na^+ levels with salt intake is similarly sodium excess driven, and that in physiological conditions such as sodium depletion, high aldosterone is only a normal physiological response.

Surprisingly, participants with a large dietary sodium-induced weight change had also a large change in muscle sodium content. This is in contrast with the concept developed by Titze *et al*. [7] stating that Na^+ storage in muscles is osmotically not active and prevent changes in body weight. As we did not assess simultaneous muscle water content by proton MRI we are unable to prove that muscle storage of sodium is exclusively osmotically inactive. In addition, as we performed a short-term study after an oral salt load, it is possible that nonosmotically active sodium storage in muscles takes more time than the 5 days in this study. Indeed, in response to a high-salt diet, enzymes involved in skin and muscle glycosaminoglycan synthesis are upregulated, yet this has been demonstrated after 8 weeks and not after a few days [27,28].

Significantly, sex or the phase of the menstrual cycle in women did not seem to influence sweat and muscle Na^+ concentrations. There were no significant differences in sodium muscle content or sweat sodium concentration between women tested during the follicular and women tested during the luteal phase. Female sex hormones are known to influence the systemic and renal hemodynamic response to salt but their effect on muscle sodium content is currently unknown [29]. Regarding sweat Na^+, data suggests that estrogen stimulates sweating, whereas progesterone has the opposite effect [5]. In our study women tended to have a higher sweat sodium concentration during the follicular phase than women during the luteal phase but this did not reach statistical significance. It has previously been shown that when controlled for age, skin Na^+ content is lower in women than in men [7]. Furthermore, sodium content appears to be higher in skin than in muscle for men, in contrast to women who tend to have higher muscle sodium than skin sodium [26]. It remains however unclear whether these observed sex differences are related to hormonal influences or rather structural factors such as skin thickness or muscle lipid content. Because of the absence of significant relationship between sweat or muscle sodium concentration and oestradiol/progesterone, our results would rather argue against an influence of female reproductive hormones, but the limited number of participants limits definite conclusions.

Finally, we found an inverse relation between muscle Na^+ deposits and BMI, meaning that overweight and obese participants had less muscle Na^+ stores than patients with normal BMI. This is surprising since participants with higher BMI tend to have higher salt intake which is indeed supported by our data as we found a positive correlation between BMI and 24-h urinary Na^+ excretion after high-sodium diet [30]. A possible explanation is a measurement artifact because of the increased distance between the antenna and the muscles in obese individuals. Another explanation may be that obesity is associated with a higher fat content in muscles fibers [31].

Our study has several strengths and limitations. One of the strengths is that we measured for the first time simultaneously sweat sodium content by quantitative pilocarpine iontophoresis and muscle sodium content with the ^{23}Na-MRI technique in healthy individuals. In addition, the measurement of sweat sodium concentration was standardized. The large difference in urinary sodium excretion between low-sodium diet and high-sodium diet suggests that participants adhered well to their prescribed diet.

Limitations are that sweat sodium was only measured in a local sample through stimulated sweating but not in whole-body washdown which is the current reference method for determining sweat Na^+ loss [32]. Nonetheless, studies have shown that local sweat Na^+ is highly and significantly correlated with whole-body washdown sweat Na^+ [32]. Another limitation was that we did not measure skin sodium content by ^{23}Na-MRI for technical reasons. Furthermore, we may have underestimated muscle sodium content due the inherent very low average signal-to-noise ratio of sodium images. However, since each volunteer was its own control, the systematic error is likely to be the same in both ^{23}Na-MRI (after high-salt and after low-salt diet). Our method should therefore still be sensitive to detect sodium concentration differences. Another limitation is that we used a single 24-h urine collection to assess Na^+ intake, whereas several collections would have increased precision. Indeed, a 24-h urine collection is unable to identify precisely the Na^+ intake of a single individual because of the biological variability of the urinary excretion of sodium. In fact, a 3 g difference in salt intake per day is detected correctly through a 24-h urine collection in only 50% [33]. Finally, the number of participants was rather small in this exploratory study; confirmation in larger studies is therefore needed. We estimated that 15 persons had to be included to assess the dietary-induced changes in sweat sodium for a power of 80%. Although we included a total of 18 participants, the observed SDs of the sweat sodium concentrations were larger in our study than in the study of Buono *et al*. [20] and a larger sample size would have strengthened our results. Furthermore, our study was underpowered to assess sex differences due to the low number of male participants, therefore definite conclusion concerning sex differences cannot be drawn.

In conclusion, we found that muscle and sweat sodium concentrations correlate positively with increases in salt intake in healthy individuals supporting a role of muscle and sweat in the regulation of human sodium balance upon changes in dietary sodium intake.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be conceived as a potential conflict of interest.

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