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ORIGINAL ARTICLE

Dietary sodium intake does not alter renal potassium handling and blood pressure in healthy young males

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ABSTRACT

Background. The effects of sodium (Na^+) intakes on renal handling of potassium (K^+) are insufficiently studied.

Methods. We assessed the effect of Na^+ on renal K^+ handling in 16 healthy males assigned to three 7-day periods on low salt diet [LSD, 3 g sodium chloride (NaCl)/day], normal salt diet (NSD, 6 g NaCl/day) and high salt diet (HSD, 15 g NaCl/day), with constant K^+ intake. Contributions of distal NaCl co-transporter and epithelial Na^+ channel in the collecting system on K^+ and Na^+ handling were assessed at steady state by acute response to 100 mg oral hydrochlorothiazide and with addition of 10 mg of amiloride to hydrochlorothiazide, respectively.

Results. Diurnal blood pressure slightly increased from 119.30 ± 7.95 mmHg under LSD to 123.00 ± 7.50 mmHg (P = 0.02) under HSD, while estimated glomerular filtration rate increased from 133.20 ± 34.68 mL/min under LSD to 187.00 ± 49.10 under HSD (P = 0.005). The 24-h K^+ excretion remained stable on all Na^+ intakes (66.28 ± 19.12 mmol/24 h under LSD; 55.91 ± 21.17 mmol/24 h under NSD; and 66.81 ± 20.72 under HSD, P = 0.9). The hydrochlorothiazide-induced natriuresis was the highest under HSD (30.22 ± 12.53 mmol/h) and the lowest under LSD (15.38 ± 8.94 mmol/h, P = 0.02). Hydrochlorothiazide increased kaliuresis and amiloride decreased kaliuresis similarly on all three diets.

Conclusions. Neither spontaneous nor diuretic-induced K^+ excretion was influenced by Na^+ intake in healthy male subjects. However, the respective contribution of the distal convoluted tubule and the collecting duct to renal Na^+ handling was dependent on dietary Na^+ intake.

Keywords: hypertension, potassium, renal tubule, renin–angiotensin–aldosterone system, sodium intake

INTRODUCTION

A large body of evidence indicates that high sodium (Na^+) and low potassium (K^+) intakes are both involved in the pathogenesis of hypertension and cardiovascular diseases [1, 2]. Lifestyle modifications including dietary Na^+ restriction and increased K^+ intake efficiently can lower blood pressure (BP) and decrease cardiovascular risk [3, 4].

Renal Na^+ and K^+ handling are highly interdependent along the kidney tubule [5]. Filtered Na^+ and K^+ are mostly reabsorbed along the proximal tubule and then along the thick ascending limb of Henle. The fine tuning of Na^+ and K^+ balance occurs along the aldosterone-sensitive distal nephron. In this segment, which includes the distal convoluted tubule (DCT), the connecting tubule (CNT) and the collecting duct (CD), Na^+ is reabsorbed and K^+ is secreted. Na^+ reabsorption occurs preferentially in the DCT under low K^+ intake or in the CNT and CD on high K^+ diet [6].

The effects of dietary Na^+ intake on urinary K^+ have been scarcely studied [7]. Luft *et al.* reported that extremely high Na^+ intakes were associated with higher urinary K^+ excretion (U_KV) [8]. In a large international prospective cohort study, high urinary Na^+ excretion ($U_{Na}V$) was associated with higher levels of U_KV [2]. In mice, short-term high dietary Na^+ also induced higher K^+ urinary excretion [9]. Contrasting with these results, Burnier *et al.* found that in Caucasians urine K^+ excretion was unchanged after 6 days of moderate high Na^+ diet [10]. These observations call for a rigorous study of the effect of dietary Na^+ intake on K^+ excretion in human.

The first aim of our study was to characterize the effects of a 7-day low, normal and high Na^+ diets (LSD, NSD and HSD, respectively), with constant K^+ intake, on daily 24-h K^+ excretion in healthy human volunteers. Our second aim was to assess the effect of Na^+ diet on 24-h ambulatory BP (ABP) and estimated

KEY LEARNING POINTS

What is already known about this subject?

- sodium (Na^+) and potassium (K^+) handling are highly interdependent along the kidney tubule;
- high K^+ intake is associated with higher urinary Na^+ excretion ($U_{Na}V$); and
- the effects of dietary Na^+ intake on urinary K^+ have been poorly studied.

What this study adds?

- our study shows that urinary K^+ excretion is independent of dietary Na^+ intake of $U_{Na}V$ and of the activity of the renin–angiotensin–aldosterone system in healthy young males; and
- our results suggest that sodium chloride co-transporter is more active under high salt diet, while epithelial Na^+ channel is stimulated under low salt diet, when the renin–angiotensin system is activated.

What impact this may have on practice or policy?

- this study helps in understanding the relationships between Na^+ and K^+ renal handling; and
- these results may suggest that one should take into account whether patients adhere to a low- or high-Na^+ diet when choosing the appropriate diuretic in hypertensive patients.

glomerular filtration rate (eGFR), and our third aim was to determine the effect of dietary salt intake on the respective contributions of the DCT and the CNT/CD to renal Na^+ and K^+ handling.

MATERIALS AND METHODS

Subjects and inclusion criteria

We performed a prospective crossover controlled study on 16 healthy male volunteers.

Inclusion criteria were normotensive men aged ≥18–30 years, recruited among medical students of the Faculty of Medicine of Geneva, with K^+ plasma level ≤4.8 mmol/L, creatinine plasma level ≤100 µmol/L, and no medical history of cardiac, renal or endocrine disturbances. Exclusion criteria were hypertension defined as a mean office BP of three measurements after rest ≥140/90 mmHg, use of anti-inflammatory drugs, diuretics or corticoids, and a history of severe allergy. Participants received modest financial compensation for their participation. The protocol was approved by the University Hospital Ethical Committee (2016-01779), and written informed consent was obtained from each individual in accordance with the declaration of Helsinki. The study was conducted between 2016 and 2018 at the University Hospitals of Geneva, Switzerland.

Experimental procedure and key variables

All participants randomly received an LSD [3 g sodium chloride (NaCl)/day i.e. ∼52 mmol/24 h Na^+ and Cl^-], an NSD (6 g NaCl/day, i.e. ∼103 mmol/24 h Na^+ and Cl^-) and an HSD (15 g NaCl/day, i.e. ∼259 mmol/24 h Na^+ and Cl^-) for 1 week in a crossover design (Figure 1). Diet sequence was allocated by blocked randomization created by the computer, in order to prevent a sequence effect. All the meals were composed by a dietician, calibrated for constant K^+ (2.7 g K^+/day i.e. ∼70 mmol/24 h K^+) and Na^+ (3 g NaCl/day) intakes, and supplied by the university hospital kitchen. Subjects took away their daily prepared meals and ate them at home. They were not allowed to eat anything else, except for some collations defined in a precise list. To obtain the normal and high salt intakes, supplemental Na^+ was provided by addition of 500 mg NaCl tablets in order to get the precise intake desired. Salt tablets and diuretic packaging were prepared by the hospital pharmacy.

Volunteers were instructed to maintain a constant lifestyle during the study with prohibition of additional meals, sweet and salty drinks, alcohol and intensive physical activity. They were instructed to drink at least 1.5 L of water in a day.

Repeated 24-h urine samples were collected each day, during the three 7-day periods of different diets, and for two different 3-week session periods, to provide objective and quantitative assessment of Na^+ and K^+ intake (Figure 1). Concomitantly, urea and creatinine concentrations and excretion (mmol/24 h) were measured each day. Urine samples were collected in 2.5 L bottles under paraffin oil with addition of thymol to prevent bacterial proliferation.

At Day 7 of each diet period, individuals attended the clinical research centre early morning after a 12-h fasting period. Blood samples were collected to measure Na^+, K^+, urea, creatinine and cystatine C. Plasma renin activity, plasma and urinary aldosterone levels were measured at steady state by radioimmunoassay as published previously [11, 12]. The 24-h ABP was recorded at Day 6 of each diet period with a device validated by the British Society of Hypertension (Microlife, Watch BP, Baumann Medical, Wetzikon 8620, Switzerland A/A). Automated measurements were performed every 20 min from 07:00 to 22:00 h and every 30 min from 22:00 to 07:00 h.

In a first 3-week session, an acute functional assessment of NaCl co-transporter (NCC) was performed at the end of each

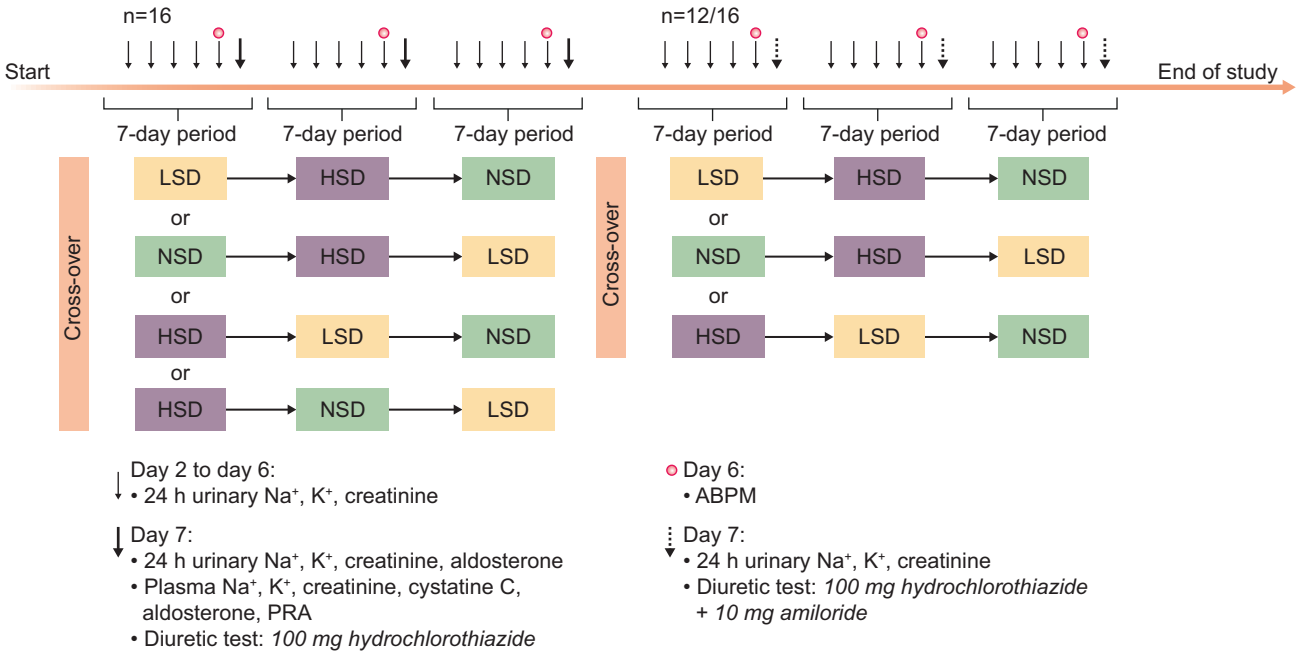

FIGURE 1: Design of the cross over study. This design was applied to two experimental sessions with three Na^+ diets: LSD, HSD and NSD. During the first 3-week sessions, the effect of hydrochlorothiazide was assessed, and during the second 3-week session, the effect of hydrochlorothiazide plus amiloride was studied. ABPM, ABP monitoring.

Table 1. Baseline characteristics of individuals

Parameters	Healthy volunteers ($n = 16$)
Age, years	22.00 ± 1.16
Weight, kg	76.55 ± 6.42
Height, cm	180.10 ± 5.92
Body mass index, kg/m^2	23.64 ± 2.23
Office SBP, mmHg	128.20 ± 10.35
Office DBP, mmHg	72.50 ± 9.56
HR, b.p.m.	62.63 ± 9.36
Plasma creatinine, µmol/L	83.31 ± 9.73
eGFR, mL/min/1.73 m^2	115.30 ± 12.30

Values are mean ± SD.

of the three 7-day period of specific diet, by an oral administration of 100 mg of hydrochlorothiazide. In a second 3-week session, an acute functional assessment of epithelial Na^+ channel (ENaC) was also performed in 12 of the 16 initial volunteers, by the administration of 100 mg of hydrochlorothiazide plus 10 mg of amiloride (Figure 1). There was no washout period in between those two periods of analysis.

The maximal single dose of diuretics usable in humans was chosen in order to generate a clear-cut renal response [13]. Because amiloride alone is not available in Switzerland, we have administered a combination of hydrochlorothiazide 100 mg plus amiloride 10 mg in the second separate 3-week session, and calculated the effects of blocking ENaC by removing the effect of hydrochlorothiazide (measured beforehand) from that of the combination of hydrochlorothiazide plus amiloride (Figure 1).

The subjects were preloaded with 1 L of water 2 h preceding the diuretic test. Plasma electrolytes were measured before diuretic administration. Urine volume, urine electrolytes concentrations and BP were measured before and 3 h after the administration of the diuretic. All tests were performed in the morning after first voiding.

Statistical analysis

As each participant served as his own control for the different Na^+ diets, and as meals were highly standardized, variability will be presumably low and statistical power sufficient to evaluate the endpoints. A sample size of 16 was calculated to identify a 1 standard deviation (SD) difference between groups with an 80% power ($\alpha = 5\%$). Quantitative and qualitative data are expressed as mean ± SD or as a percentage, respectively. Differences between continuous data when two groups were considered were analysed by a paired *t*-test when the distribution and variance met the conditions; otherwise, a Wilcoxon test was used. Differences between continuous data when more than two groups were considered were analysed by analysis of variance (ANOVA) for repeated measures when the distribution and variance met the conditions; otherwise, a Friedman test was used. When there was a global difference between groups, a multipair wise comparison between LSD, NSD and HSD was analysed. Correlations between urinary Na^+ and K^+ were analysed using a Spearman test. When two measures were available for one subject, the mean of the measures were calculated and taken into account.

A $P < 0.05$ was considered as statistically significant for Friedman and ANOVA global tests, for Wilcoxon and correlation tests. For multiple comparisons between the three groups (LSD, NSD and HSD), a $P < 0.02$ was considered as statistically

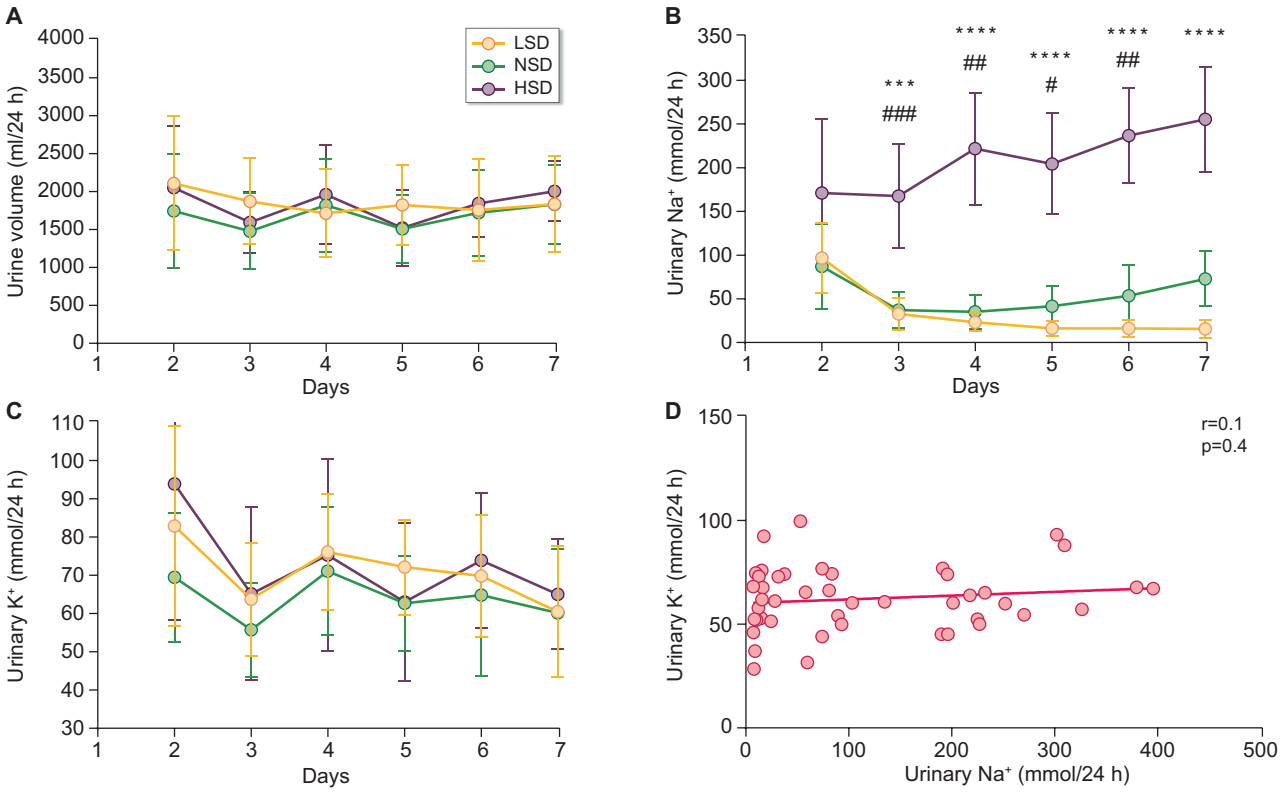

FIGURE 2: Urine volume, Na^+ and K^+ excretion on different salt intakes. (**A**) Time course of urine volume in mL/24 h. (**B**) Time course of urinary Na^+ in mmol/24 h. (**C**) Time course of urinary K^+ in mmol/24 h. (**D**) Correlations between urinary Na^+ and K^+ at Day 6. Here is shown the increase in urinary Na^+ excretion under HSD and its decrease under LSD, whereas urine volume and urinary K^+ were not modified by salt intake. Statistical differences between LSD, NSD and HSD were assessed by a Friedman test in (A–C) and correlations were assessed using a Spearman test in (D). In (B), multiple comparisons between HSD and LSD are shown as follows: ***$P < 0.001$, ****$P < 0.0001$; and differences between HSD and NSD are shown as follows: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$. $N = 13$–14 subjects in each subgroup.

Table 2. Physiological and biological parameters according to changes in Na^+ intake in healthy male volunteers

Parameters	LSD	NSD	HSD	Friedman global test, P-value
Weight, kg	78.50 ± 2.40	79.10 ± 2.50	80.00 ± 8.60	NS
Plasma creatinine, µmol/L	89.31 ± 12.86	86.94 ± 10.37	82.00 ± 11.55	0.02
Creatinine clearance, mL/min	133.20 ± 34.68	140.00 ± 26.11	187.00 ± 49.10$^{\#\#}$	0.005
Plasma urea, mmol/L	5.49 ± 0.73	4.81 ± 0.83	4.68 ± 0.64***	0.0002
Cystatin C, mg/L	0.83 ± 0.06	0.79 ± 0.07	0.78 ± 0.05**	0.004
Urine volume, mL/24 h	1836.00 ± 632.50	1831.00 ± 515.50	2004.00 ± 395.70	NS
U_{Urea}, mmol/24 h	495.40 ± 72.34	472.00 ± 150.00	512.80 ± 113.00	NS
$U_{Na}V$, mmol/24 h	18.78 ± 12.75	87.17 ± 39.85	266.00 ± 81.21****	<0.0001
U_KV, mmol/24 h	66.28 ± 19.12	55.91 ± 21.17	66.81 ± 20.72	NS
U_{Na}/U_K ratio	0.24 ± 0.14	1.27 ± 0.62	3.93 ± 1.02****	<0.0001

Values are mean ± SD.
**$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ versus LSD. $^{\#\#}P < 0.01$ versus NSD. NS, not significant.

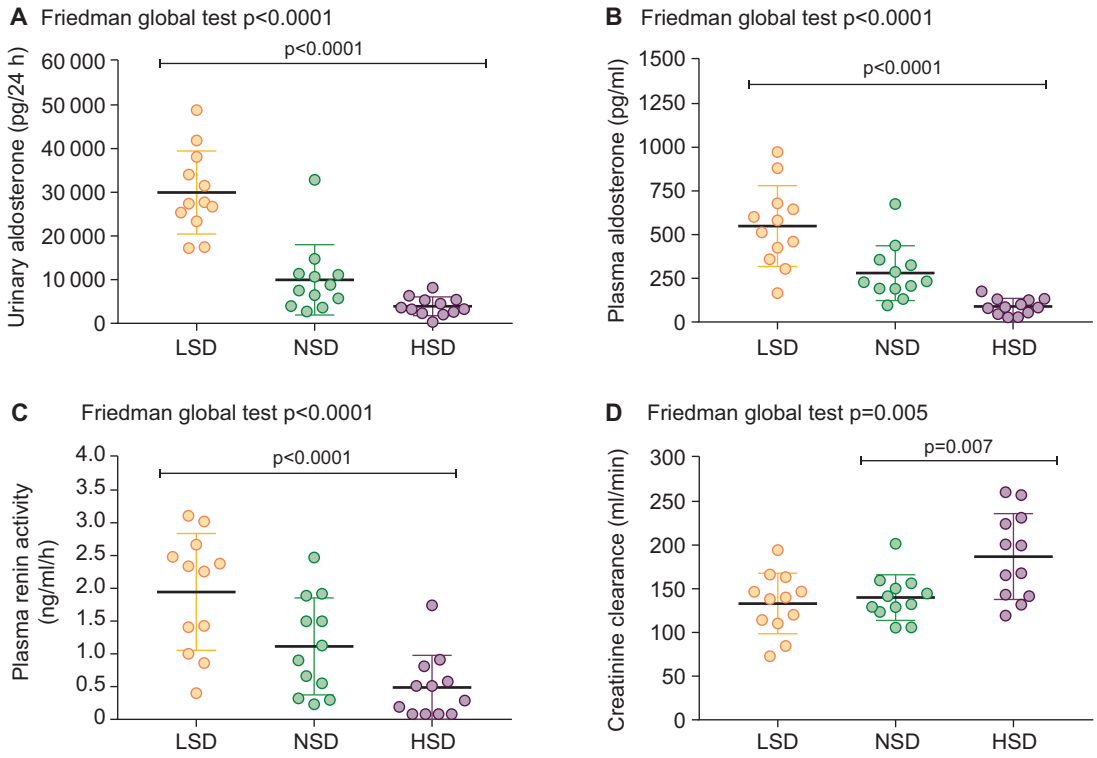

FIGURE 3: RAAS and GFR. (**A**) Urinary aldosterone levels in pg/24 h. (**B**) Plasma aldosterone levels in pg/mL. (**C**) Plasma renin activity in ng/mL/h. (**D**) Creatinine clearance in mL/min. As expected, (A–C) show the activation of the RAAS under LSD and its inhibition under HSD. Panel (D) shows the increase in eGFR estimated by creatinine clearance under HSD. Statistical differences between LSD, NSD and HSD were assessed by a Friedman test. $N = 12$ subjects in each subgroup.

significant following Bonferroni correction. GraphPad Prism® version 7.02 was used for analysis.

RESULTS

Effect of salt intake on urinary Na^+ and K^+ excretion and renin–aldosterone levels

Characteristics of the participants are shown in Table 1. Diuresis remained stable throughout the study period and did not change in response to the various Na^+ intakes (Figure 2A). Figure 2B shows that natriuresis was close to equilibrium after 3 days under each salt diet. The 24-h $U_{Na}Vs$ at Day 6 under various dietary salt intakes are shown in Table 2. Despite high intra-individual (within-person coefficients of variation varied from $39.51 \pm 19.02\%$ in LSD to $21.90 \pm 17.21\%$ in HSD, $P = 0.04$, Supplementary data, Figure S1A and S1B) and inter-individual (between-person coefficients of variation varied from $55.64 \pm 7.33\%$ in NSD versus $31.56 \pm 9.96\%$ in HSD, $P = 0.01$, Supplementary data, Figure S1A–C) variability of $U_{Na}V$, these results confirmed the good compliance of the volunteers to the assigned salt regimen.

Figure 2C shows that after a drop at Day 2, 24-h U_KV was almost constant from Days 3 to 6 and was not influenced by the dietary Na^+ intake. Furthermore, Figure 2D shows that at Day 6, 24-h urinary K^+ excretion was not correlated to 24-h urinary Na^+ excretion. Interestingly, the variability of K^+ excretion was less important than that of Na^+ excretion and did not differ between dietary conditions (within-person coefficients of variation varied from $19.17 \pm 7.86\%$ in LSD to $27.83 \pm 14.89\%$ in HSD, $P = 0.07$, Supplementary data, Figure S1D and E; and between-person coefficients of variation varied from 23.90 ± 5.32 in LSD to $30.75 \pm 6.28\%$ in HSD, $P = 0.4$, Supplementary data, Figure S1D and F). Under these conditions where urinary Na^+ excretion was altered in the absence of variation of urinary K^+ excretion, the urine Na^+/K^+ ratio calculated at Day 6 was 0.24 ± 0.14 under LSD, and increased to 1.27 ± 0.62 under NSD and to 3.93 ± 1.02 under HSD ($P < 0.0001$ for global test and for HSD versus LSD) (Table 2).

Plasma renin activity and aldosterone, and 24-h urinary aldosterone excretion were measured at Day 6. As expected, both plasma renin activity and aldosterone levels increased under LSD, and decreased under HSD (Figure 3A–C). Plasma Na^+ and K^+ concentrations measured at Day 6 were unchanged by either LSD or HSD as expected by physiological control (data not shown).

Effect of dietary salt intake on creatinine clearance and BP

Figure 3D shows that creatinine clearance increased progressively according to the level of dietary salt intake from LSD (133.20 ± 34.68) to HSD (187.00 ± 49.10 mL/min/1.73 m^2) ($P = 0.005$ for global test). Plasma cystatin C measurements also decreased from 0.83 ± 0.06 mg/L under LSD to 0.78 ± 0.05 mg/L under HSD ($P = 0.007$ for HSD versus LSD). These results indicated a positive relationship between dietary salt intake and filtered Na^+ load.

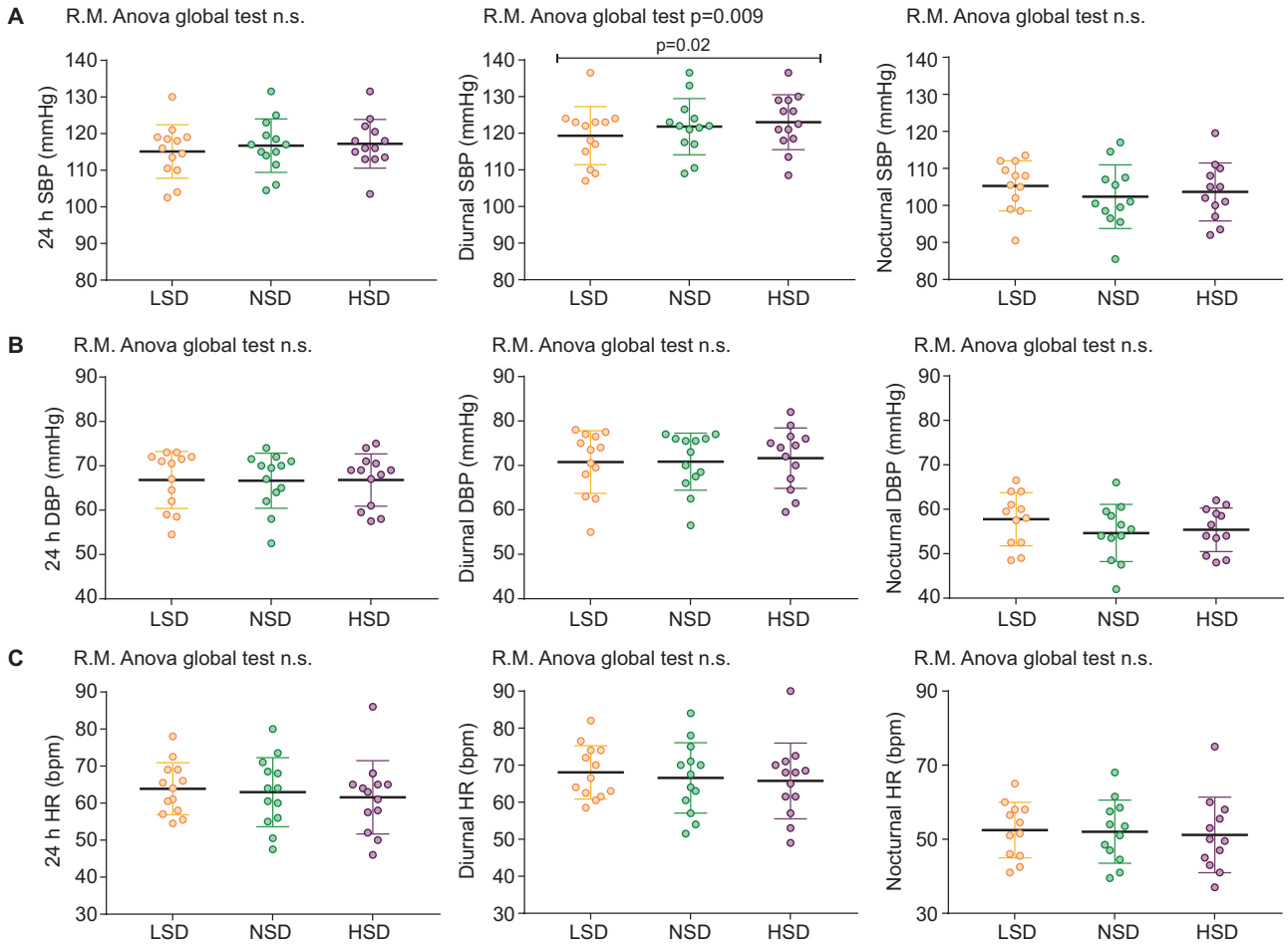

FIGURE 4: Effects of salt intake on ABP and HR. (**A**) Twenty-four-hour diurnal and nocturnal SBP in mmHg in each diet. (**B**) Twenty-four-hour diurnal and nocturnal DBP in mmHg in each diet. (**C**) Twenty-four-hour diurnal and nocturnal HR in beats per minute (b.p.m.) in each diet. Statistical differences between LSD, NSD and HSD were assessed using an ANOVA test for repeated measures. $N =$ 12 or 13 subjects in each subgroup. R.M., repeated measures; n.s., not significant.

Changes in dietary salt intake altered neither the global 24 h nor the nocturnal ABP in these healthy subjects (Figure 4). Interestingly, diurnal systolic BP (SBP) was slightly increased under HSD (123.00 ± 7.50 mmHg) compared with LSD (119.30 ± 7.95 mmHg) ($P = 0.009$ for global test and $P = 0.02$ for HSD versus LSD). Heart rate (HR) was unchanged in all dietary salt regimens, and dipping was conserved in all dietary salt regimens.

Effect of salt diet on NCC and ENaC activity

In response to acute NCC blockade along the DCT with 100 mg of hydrochlorothiazide, natriuresis increased over 3 h under all dietary salt regimens (Figure 5A–C). However, the difference between hydrochlorothiazide-induced natriuresis and baseline natriuresis was the highest under HSD (30.22 ± 12.53 mmol/24 h), intermediate under NSD (23.90 ± 8.26 mmol/24 h) and the lowest under LSD (15.38 ± 8.94 mmol/24 h) ($P = 0.02$ for global test and $P = 0.01$ for HSD versus LSD). K^+ excretion measured 3 h after hydrochlorothiazide administration increased to a similar extent under every salt diet (Figure 5D–F).

In the following 3-week session also including the same three Na^+ diets, we studied the effect of the combination of 100 mg hydrochlorothiazide plus 10 mg amiloride to inhibit both NCC along the DCT and ENaC along the CNT and the CD. Results depicted in Figure 5A–C showed that combination of both diuretics increased natriuresis over a 3-h period. The effect of amiloride was calculated by subtraction of the effect of hydrochlorothiazide plus amiloride measured during the second experimental session minus the effect of hydrochlorothiazide measured during the first experimental session. Interestingly, under LSD, a condition in which renin–angiotensin–aldosterone system (RAAS) is stimulated, the magnitude of hydrochlorothiazide- or amiloride-induced natriuresis was similar while under HSD and NSD, amiloride-induced natriuresis was significantly lower compared with that induced by hydrochlorothiazide (Figure 5A–C). In contrast, the effect of amiloride on kaliuresis was similar in all three groups (Figure 5D–F).

The diuretic-induced natriuresis occurred without change in office SBP under all three Na^+ diets while a slight decrease in diastolic BP (DBP) was observed 3 h after the administration of hydrochlorothiazide plus amiloride under LSD only (Figure 6).

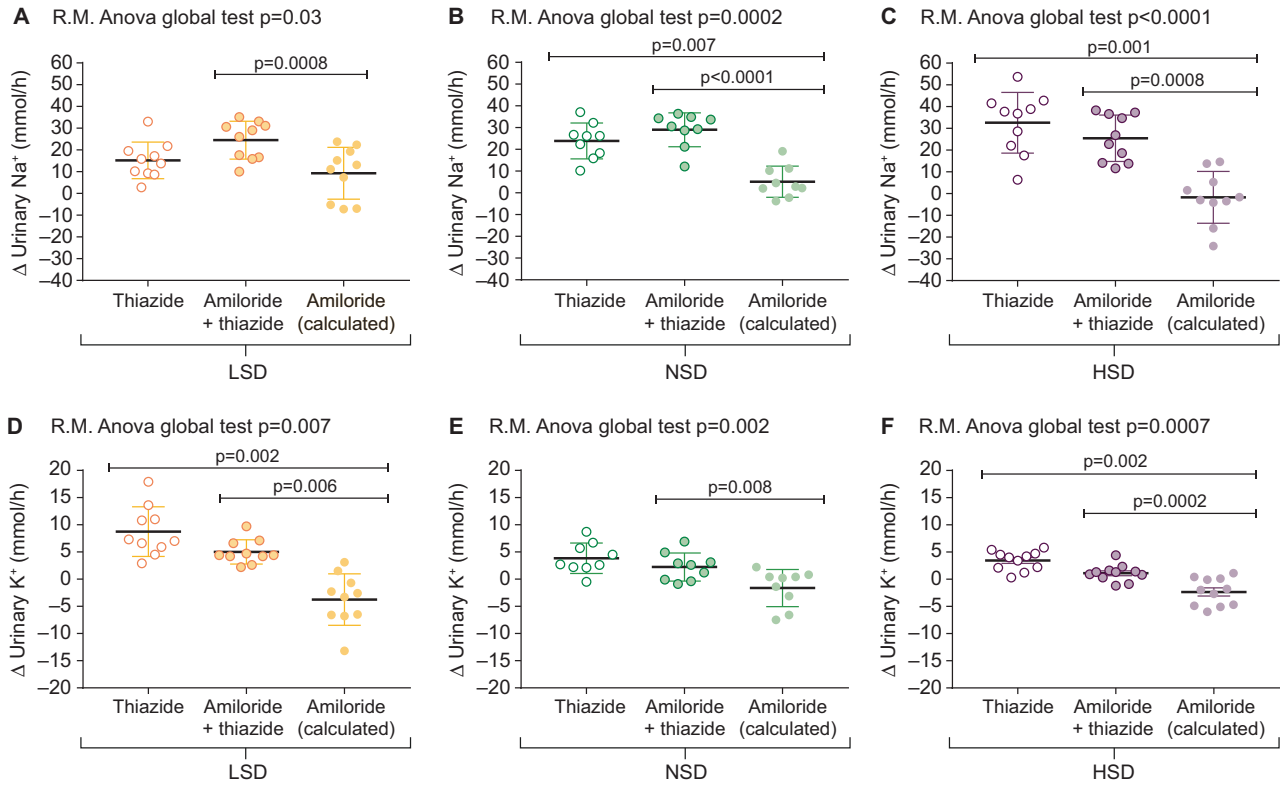

FIGURE 5: Assessment of DCT and CNT/CD function in each Na^+ diet. Difference between urinary Na^+ after acute NCC blockade by thiazide, after NCC plus ENaC blockade by thiazide plus amiloride, and after ENaC blockade alone, and baseline natriuresis, in LSD (**A**), NSD (**B**) and HSD (**C**). Difference between urinary K^+ after NCC blockade by thiazide, after NCC plus ENaC blockade by thiazide plus amiloride, and after ENaC blockade by amiloride and baseline kaliuresis, under LSD (**D**), NSD (**E**) and HSD (**F**). Urinary Na^+ and K^+ after amiloride administration were calculated by subtracting urinary Na^+ and K^+ obtained after thiazide from those obtained after thiazide plus amiloride administration. Statistical differences between each diuretic were assessed using an ANOVA test for repeated measures (R.M.). $N = 9$–11 subjects in each subgroup.

DISCUSSION

Whether dietary Na^+ intake directly influences renal K^+ excretion has been poorly studied and remains to be rigorously assessed in humans. We have previously shown in mice that a 7-day HSD increased U_KV despite blunted RAAS [9]. Here, we demonstrated that, at least on the short-term, large variations of dietary salt intake from 3 to 15 g/day do not alter 24-h U_KV measured daily for six consecutive days in healthy young human males with constant K^+ intake. Furthermore, kaliuresis was not correlated with natriuresis. In agreement with our results, Burnier *et al.* found that K^+ excretion measured at the end of a 6-day experimental period was unchanged by Na^+ intake [10]. In our study, LSD corresponds to a very low urinary Na^+/K^+ ratio (0.24 ± 0.14) close to that found in primitive cultures, and HSD to very high urinary Na^+/K^+ ratio (3.93 ± 1.02, $P < 0.0001$) observed in the upper range of industrialized populations. In 1979, Luft *et al.* reported that extremely high Na^+ intake (1200–1500 mmol/day) induced kaliuresis in both Black and Caucasian healthy males aged 18–40 years. Increased kaliuresis was not observed under Na^+ intakes between 10 and 800 mmol/day [8]. This observation can be explained by the massive intake of Na^+ leading to a rise in BP, increased tubular flow and Na^+ delivery to the CD leading to enhanced K^+ secretion. Results of time-course experiments in our study were strengthened by the absence of significant differences in hydrochlorothiazide-induced kaliuresis and amiloride-induced antikaliuresis under the three Na^+ diets. Therefore, the K^+ balance seems to be independent of variations of dietary salt intake within the physiological range and this despite the observed large variations of aldosterone secretion.

A large body of experimental evidence in rodents showed that increased aldosterone secretion observed under LSD increases K^+ secretion along the CNT/CD and thereby increases U_KV [5]. This effect was not observed, at least in the short-term, in our human volunteers suggesting that aldosterone-independent mechanisms preserved K^+ homoeostasis [7, 10]. On the other hand, animal studies have demonstrated that increasing tubular flow and/or Na^+ delivery to the CD promotes K^+ secretion via BK (apical calcium-activated K^+ channels) and ROMK, and thereby increases U_KV [14]. Our results showing that HSD leading to higher eGFR and thus higher distal Na^+ delivery does not increase U_KV suggests that in humans, flow-induced K^+ secretion is fully compensated by the inhibition of aldosterone secretion and the consecutive decrease in ENaC-dependent K^+ secretion via ROMK. This result is in line with the aldosterone paradox, which postulates that the kidney can handle both Na^+ and K^+ separately according to body homoeostasis requirements [15, 16].

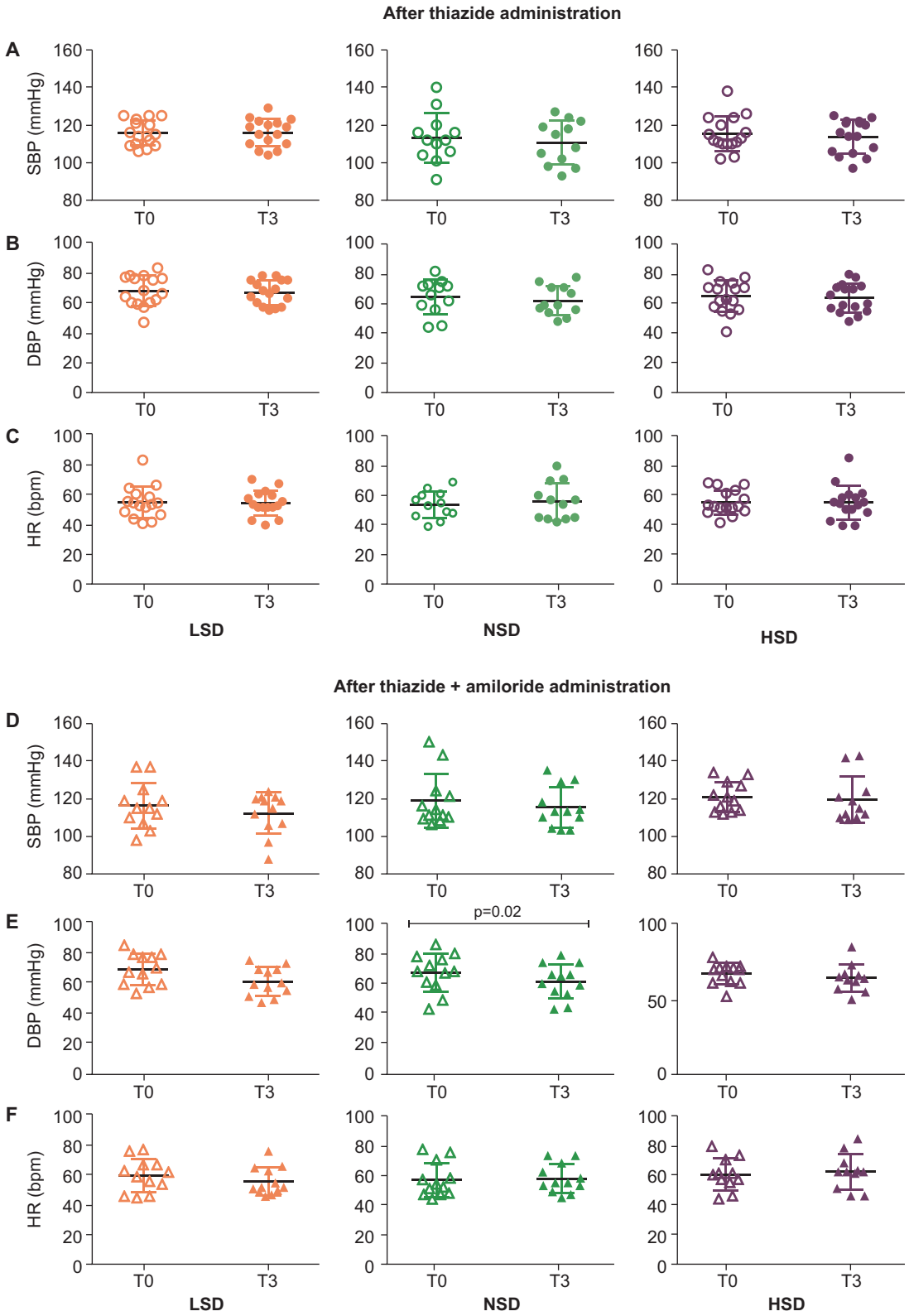

FIGURE 6: BP before and 3 h after diuretic administration. (**A**) SBP in mmHg after thiazide administration and (**D**) after thiazide plus amiloride administration under each diet. (**B**) DBP in mmHg after thiazide administration and (**E**) after thiazide plus amiloride administration under each Na^+ diet. (**C**) HR in b.p.m. after thiazide administration and (**F**) after thiazide plus amiloride administration under each diet. Statistical differences between baseline BP and 3 h after diuretic administration were assessed using a Wilcoxon test. $N = 11–16$ subjects.

The association between Na^+ intake and BP in young people is limited and equivocal [17]. In our study, ABP measurement revealed a very small increase in diurnal SBP under HSD, while nocturnal BP and HR were unchanged. This finding is consistent with the BP pattern observed in Caucasians with a normal renal function that is usually salt resistant [10].

Association studies have shown that eGFR increased under HSD under pathological conditions such as chronic kidney diseases, obesity and diabetes mellitus [18], or in salt-sensitive Black hypertensives, but not in salt-resistant White hypertensives [19]. However, our data show an increased creatinine clearance under HSD with no change in ABP after Na^+ loading [20]. This rise of eGFR increases the amount of filtered sodium and should trigger the tubulo-glomerular feedback. The increase in Na^+ concentration should be sensed by the macula densa in the distal tubule, then causing vasoconstriction of the afferent arteriole, a decrease in GFR and inhibition of the renin–angiotensin system, which promotes Na^+ retention and increased BP [21]. This effect might be associated with a 'pressure natriuresis' response to the slightly increased diurnal SBP leading to mostly unchanged 24-h ABP.

We showed that hydrochlorothiazide-induced natriuresis was the highest under HSD, suggesting a high Na^+ reabsorption rate in the DCT under HSD. Chiga's group showed in rodents that HSD decreases both NCC protein abundance and phosphorylation, which is commonly accepted as a surrogate for NCC activity [22]. In agreement with this result, we also previously showed in mice that a moderate HSD (1.25% Na wt/wt) was associated with decreased NCC protein abundance and phosphorylation, while the total amount of Na^+ reabsorbed via NCC in the DCT was higher under HSD than under NSD and LSD [9]. Indeed, one has to make a distinction between protein abundance, intrinsic activity of a transporter and the amount of transported ion that is also dependent on its concentration and delivery. In our study, eGFR was increased under HSD, therefore Na^+ delivery to the DCT as well as its concentration were most likely increased, leading to enhanced effective Na^+ transport in this segment and this despite reduced abundance of active NCC. Our results suggest that in human like in mouse, HSD increases the fractional Na^+ reabsorption by the DCT while abundance of active NCC is decreased to prevent Na^+ retention.

Under LSD, in which RAAS is stimulated, amiloride and hydrochlorothiazide-induced natriuresis were not different, whereas under NSD and HSD, thiazide-induced natriuresis largely exceeded that induced by amiloride. These results confirm that the effect of amiloride on natriuresis requires RAAS activation, which stimulates Na^+ reabsorption along the CNT/CD. These findings are consistent with animal studies showing that LSD for 5 days stimulates ENaC via aldosterone, and that blockade of the mineralocorticoid receptor with eplerenone reverses this activation [23]. In contrast to results of the present human study, the natriuretic response to amiloride in mice was maximal under HSD, intermediate under NSD and the lowest under LSD [9]. This species difference may rely on higher Na^+ reabsorption capacity of the human compared with the mouse DCT. One can speculate that mice must excrete in proportion much more K^+ than humans and always need some degree of ENaC-dependent Na^+ reabsorption along the CNT/CD to drive K^+ secretion.

Despite the controlled design of our study, the variability of 24-h $U_{Na}Vs$ was high, which is consistent with previous studies in young normotensive males and females [24, 25]. The cause of this variability was not any technical issue or lack of compliance, but could be 'endocrine-driven' [26]. This was highlighted in long-term balance studies that discovered an infradian rhythm period of about a month for Na^+ and K^+ urinary excretion [26]. In this study, Na^+ urinary excretion was also weakly correlated to K^+ urinary excretion [27]. However, BP varied with Na^+ intake [28], suggesting that we may have failed to detect a change in BP because of the short period of time, or that our younger subjects were more salt-resistant.

It should be mentioned that our study has other limitations. The first limitation was that amiloride alone is not available in Switzerland. This led us to use a combination of hydrochlorothiazide plus amiloride and to calculate the effects of blocking ENaC by subtracting the effect of hydrochlorothiazide (measured beforehand) from that of the combination of hydrochlorothiazide plus amiloride. Therefore, one can argue that this calculation does not truly reflect the basal Na^+ handling by the CNT/CD, because hydrochlorothiazide increased Na^+ delivery to this segment. However, one positive point is that increasing Na^+ delivery to the CD may sensitize the volunteers to the effect of amiloride. Secondly, we studied a high proportion of Caucasians, which limits the validity of the results to this specific population. For instance, Aviv *et al.* showed that Black people excrete less K^+ than Caucasians under similar Na^+ intake [29]. Thirdly, we studied only males. In a cohort of young nonhypertensive females, the BP response was also characterized by a salt-resistant pattern at any menstrual status. In this study as well, U_KV was unchanged under two different Na^+ diets, similar to ours, but uncontrolled K^+ intake was a clear limitation. At variance with this study, GFR was not increased under HSD [30]. Sexual dimorphism has been described in term of tubular transporters like NCC expression and function, in humans and mice [31, 32]; therefore, the response to diuretics described in the present work might differ in a controlled study involving females.

In conclusion, our human study shows that U_KV is independent of dietary Na^+ intake and $U_{Na}V$. We show that HSD increases eGFR and Na^+ reabsorption by NCC in the DCT while it blunts RAAS activity and subsequently ENaC-mediated Na^+ reabsorption by the CNT/CD. Finally, we confirm that ABP is salt-resistant in young healthy Caucasian males.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

A.P.-B. and E.F. designed and performed the study, K.U. and M.M. carried out experiments, A.P.-B., E.F. and V.O. analysed the data, V.O. made the figures and drafted the paper, and M.B., S.d.S, B.P. and P.-Y.M revised the paper; all authors approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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