Dietary sodium intake does not alter renal potassium handling and blood pressure in healthy young males
Antoinette Pechère-Bertschi¹, Valérie Olivier¹,², Michel Burnier³, Khalil Udwan², Sophie de Seigneux¹,², Belén Ponte¹, Marc Maillard³, Pierre-Yves Martin¹,², Eric Feraille¹,²

¹Service of Nephrology and Hypertension, University Hospital Geneva, Switzerland
²Department of Cell Physiology and Metabolism, University of Geneva, Switzerland
³Service of Nephrology and Hypertension, CHUV, Lausanne, Switzerland

Correspondence to: Antoinette Pechère-Bertschi; E-mail: antoinette.pechere@hcuge.ch

Short title: Effect of Sodium Intake on Potassium Excretion
ABSTRACT

Background. The effects of sodium intakes on renal handling of potassium are insufficiently studied.

Methods. We assessed the effect of sodium on renal potassium handling in 16 healthy males assigned to three 7-day periods on low (LSD, 3g NaCl/d), normal (NSD, 6g NaCl/d) and high (HSD, 15g NaCl/d) sodium diet with constant potassium intake. Contributions of distal NaCl co-transporter and epithelial sodium channel in the collecting system on potassium and sodium 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). Twenty-four hours potassium excretion remained stable on all sodium intakes (66.28 ± 19.12 mmol/24h under LSD; 55.91 ± 21.17 mmol/24h 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 3 diets.

Conclusion. Neither spontaneous nor diuretic-induced potassium excretion were influenced by sodium intake in healthy male subjects. However, the respective contribution of the distal convoluted tubule and the collecting duct to renal sodium handling was dependent on dietary sodium intake.

Keywords: hypertension, potassium, renin angiotensin aldosterone system, renal tubule, sodium intake
KEY LEARNING POINTS

What is already known about this subject?

- Sodium and potassium handling are highly interdependent along the kidney tubule.
- High potassium intake is associated with higher urinary sodium excretion.
- The effects of dietary sodium intake on urinary potassium have been poorly studied.

What this study adds?

- Our study shows that urinary potassium excretion is independent of dietary sodium intake, of urinary sodium excretion and of the activity of the renin-angiotensin-aldosterone system in healthy young males.
- Our results suggest that NCC is more active under high salt diet while ENaC 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 understanding the relationships between sodium and potassium renal handling.
- These results may suggest that one should take into account whether patients adhere to a low or to high sodium diet when choosing the appropriate diuretic in hypertensive patients.
ABBREVIATIONS
ABP, ambulatory blood pressure; eGFR, estimated glomerular filtration rate; TGF, tubulo-glomerular feedback; RAAS, renin-angiotensin-aldosterone system; BP, blood pressure; NCC, sodium-chloride co-transporter; CNT, connecting tubule; CD, collecting duct; DCT, distal collecting tubule, ENaC, epithelial sodium channel; HSD, high salt diet; LSD, low salt diet; NSD, normal salt diet.

INTRODUCTION
A large body of evidence indicates that high sodium and low potassium intakes are both involved in the pathogenesis of hypertension and cardiovascular diseases (1,2). Lifestyle modifications including dietary sodium restriction and increased potassium intake efficiently lower blood pressure (BP) and decrease cardiovascular risk (3,4). Renal sodium and potassium handling are highly interdependent along the kidney tubule (5). Filtered sodium and potassium are mostly reabsorbed along the proximal tubule and then along the thick ascending limb of Henle. The fine tuning of sodium and potassium 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), sodium is reabsorbed and potassium is secreted. Sodium reabsorption occurs preferentially in the DCT under low potassium intake or in the CNT and CD on high potassium diet (6).

The effects of dietary sodium intake on urinary potassium have been scarcely studied (7). Luft et al reported that extremely high sodium intakes were associated with higher urinary potassium excretion (8). In a large international prospective cohort study, high urinary sodium excretion was associated with higher levels of urinary potassium excretion (2). In mice, short-term high dietary sodium also induced higher potassium urinary excretion (9). Contrasting with these results, Burnier et al. found that in Caucasians urine potassium excretion was unchanged after 6 days of moderate HSD (10). These observations call for a rigorous study of the effect of dietary sodium intake on potassium excretion in human. The first aim of our study was to characterize the effects of a 7-day low, normal and high sodium diets with constant potassium intake, on daily 24h potassium excretion in healthy human volunteers. Our second aim was to assess the effect of sodium diet on 24h
ambulatory BP and estimated 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 sodium and potassium 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 potassium 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 a 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 a low (3 g NaCl/day i.e. ~ 52 mmol/24h Na⁺ and Cl⁻, LSD), a normal (6 g NaCl/day, i.e. ~ 103 mmol/24h Na⁺ and Cl⁻, NSD) and a high (15 g NaCl/day, i.e. ~ 259 mmol/24h Na⁺ and Cl⁻, HSD) sodium diet for one week in a crossover design (Figure 1). Diet sequence was allocated by blocked randomization created by computer, in order to prevent a sequence effect. All the meals were composed by a dietician, calibrated for constant potassium (2.7 g K⁺/day i.e. ~70 mmol/24h K⁺) and sodium (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 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 diuretics 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 liters of water in a day. Repeated 24-hour urine samples were collected each day, during the three 7-day periods of different diets, and for 2 different 3 week’s session periods, to provide objective and quantitative assessment of Na\(^+\) and K\(^+\) intake (Figure 1). Concomitantly urea and creatinine concentrations and excretion (mmol/24h) were measured each day. Urines 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 center early morning after a 12-hour fasting period. Blood samples were collected to measure sodium, potassium, urea, creatinine, cystatin C. Plasma renin activity, plasma and urinary aldosterone levels were measured at steady state by radioimmunoassay as published previously (11,12). Twenty-four hours ambulatory blood pressure (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-minutes from 07:00 to 22:00 hours and every 30 minutes from 22:00 to 07:00 hours.

In a first 3 weeks session, an acute functional assessment of NCC was performed at the end of each of the three 7-day period of specific diet, by an oral administration of 100 mg of hydrochlorothiazide. In a second 3 weeks session, an acute functional assessment of 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 wash out period in between those two periods of analysis.

The maximal single dose of diuretics usable in humans were 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 weeks session, and calculated the effects of blocking ENaC by removing the effect of hydrochlorothiazide (measured beforehand) to that of the combination of hydrochlorothiazide plus amiloride (Figure 1). The subjects were preloaded with 1 L of water 2 hours preceding the diuretic test. Plasma electrolytes were measured before diuretic administration. Urine volume, urine
electrolytes concentrations and BP were measured before and 3 hours 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 sodium diets, and as meals were highly standardized, variability will be presumably low and statistical power sufficient to evaluate the end points. A sample size of 16 was calculated to identify a 1 SD difference between groups with a 80% power ($\alpha=5\%$). Quantitative and qualitative data are expressed as mean ± standard deviation or as a percentage, respectively. Differences between continuous data when two groups were considered, were analyzed 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 analyzed by 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 analyzed. Correlations between urinary sodium and potassium were analyzed using a Spearman test. When two measures were available for one subject, the mean of the measures were calculated and taken into account.

A $P$ value < 0.05 was considered as statistically significant for Friedman and ANOVA global tests, for Wilcoxon tests and for correlation tests. For multiple comparisons between the three groups (LSD, NSD and HSD), a $P$ value < 0.02 was considered as statistically significant following Bonferroni correction. GraphPad Prism® (version 7.02) was used for analysis.

**RESULTS**

**Effect of salt intake on urinary sodium and potassium 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 sodium intakes (Figure 2A). Figure 2B shows that natriuresis was close to equilibrium after 3 days under each salt diet. Twenty-four hours urinary sodium excretions at day 6 under various dietary salt intakes are shown in Table 2. Despite high intra- (within-person coefficients of
variation varied from 39.51 ± 19.02 % in LSD to 21.90 ± 17.21 % in HSD, P=0.04, Figure S1A-B) and inter-individual variability (between-person coefficients of variation varied from 55.64 ± 7.33 % in NSD vs 31.56 ± 9.96 % in HSD, P=0.01, Figure S1A-C) of urinary sodium excretion, these results confirmed the good compliance of the volunteers to the assigned salt regimen.

Figure 2C shows that after a drop at day 2, 24h urinary potassium excretion was almost constant from day 3 to day 6 and was not influenced by the dietary sodium intake. Furthermore, Figure 2D shows that at day 6, 24h urinary K⁺ excretion was not correlated to 24h 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 ± 7.86 % in LSD to 27.83 ± 14.89 % in HSD, P=0.07, Figure S1D and E; and between-person coefficients of variation varied from 23.90 ± 5.32 in LSD to 30.75 ± 6.28 % in HSD, P=0.4, 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 vs LSD) (Table 2).

Plasma renin activity and aldosterone, and 24h 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 Blood Pressure**

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/1.73m²), (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 vs LSD). These results indicated a positive relationship between dietary salt intake and filtered sodium load.

Changes in dietary salt intake did not alter the global 24h, nor the nocturnal ambulatory BP in these healthy subjects (Figure 4A-C). Interestingly, diurnal SBP was slightly
increased under HSD (123.00 ± 7.50 mmHg), compared to LSD (119.30 ± 7.95 mmHg) (P = 0.009 for global test and P = 0.02 for HSD vs LSD). Heart rate 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 3h under all dietary salt regimens (Figures 5A, B, C). However, the difference between hydrochlorothiazide-induced natriuresis and baseline natriuresis was the highest under HSD (30.22 ± 12.53 mmol/24h), intermediate under NSD (23.90 ± 8.26 mmol/24h) and the lowest under LSD (15.38 ± 8.94 mmol/24h) (P = 0.02 for global test and P = 0.01 for HSD vs LSD). Potassium excretion measured 3h after hydrochlorothiazide administration increased to a similar extent under every salt diet (Figure 5D, E, F).

In the following 3 weeks session including also the same 3 sodium 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 Figures 5A, B, C show that combination of both diuretics increased natriuresis over a 3h 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 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 to that induced by hydrochlorothiazide (Figure 5A, B, C). In contrast, the effect of amiloride on kaliuresis was similar in all 3 groups (Figure 5D, E, F).

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

Whether dietary sodium intake directly influences renal potassium excretion has been poorly studied and remained to be rigorously assessed in humans. We have previously shown in mice that a 7-days HSD increased urinary potassium excretion 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 per day do not alter 24h urinary potassium excretion measured daily for 6 consecutive days in healthy young human males with constant potassium intake. Furthermore, kaliuresis was not correlated with natriuresis. In agreement with our results, Burnier et al. found that potassium excretion measured at the end of a 6 day experimental period was unchanged by sodium 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 sodium intake (1200-1500 mmol/day) induced kaliuresis in both Black and Caucasian healthy males aged 18-40. Increased kaliuresis was not observed under sodium intakes between 10 to 800 mmol/day (8). This observation can be explained by the massive intake of sodium leading to a rise in blood pressure, increased tubular flow and sodium delivery to the collecting duct leading to enhanced potassium secretion. Results of time-course experiments in our study were strengthened by the absence of significant difference in hydrochlorothiazide-induced kaliuresis and amiloride-induced antikaliuresis under the 3 sodium diets. Therefore, the potassium 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 potassium secretion along the CNT/CD and thereby increases urinary potassium excretion (5). This effect was not observed at least on the short-term, in our human volunteers suggesting that aldosterone-independent mechanisms preserved potassium homeostasis (7,10). On the other hand, animal studies have demonstrated that increasing tubular flow and/or sodium delivery to the CD promotes potassium secretion via BK (apical calcium-activated potassium channels) and ROMK and thereby increases urinary potassium excretion (14). Our results showing that HSD leading to higher eGFR and thus higher distal sodium delivery does not increase
urinary potassium excretion suggests that in humans, flow-induced potassium secretion is fully compensated by the inhibition of aldosterone secretion and the consecutive decrease in ENaC-dependent potassium secretion via ROMK. This result is in line with the aldosterone paradox, which postulates that the kidney can handle both sodium and potassium separately according to body homeostasis requirement (15,16).

The association between sodium intake and BP in young people is limited and equivocal (17). In our study, ambulatory BP measurement revealed a very small increase in diurnal systolic BP under HSD while nocturnal BP and heart rate were unchanged. This finding is consistent with the BP pattern observed in white 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 hypertensive, but not in salt-resistant white hypertensive (19). However, our data show an increased creatinine clearance under HSD with no change in ambulatory BP after sodium loading (20). This rise of eGFR increases the amount of filtered sodium and should trigger the tubulo-glomerular feedback (TGF). 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 systolic BP leading to mostly unchanged 24h ambulatory BP.

We showed that hydrochlorothiazide-induced natriuresis was the highest under HSD, suggesting high sodium reabsorption rate in the DCT under HSD. Uchida’s group showed in rodents that HSD decreases both NCC protein abundance and phosphorylation, which is commonly accepted as 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 sodium 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 sodium delivery to the DCT as well as its concentration were most likely increased
leading to enhanced effective sodium 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 sodium reabsorption by the DCT while abundance of active NCC is decreased to prevent sodium 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 sodium 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 with 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 sodium reabsorption capacity of the human compared to the mouse DCT. One can speculate that mice must excrete in proportion much more potassium than humans and always need some degree of ENaC-dependent sodium reabsorption along the CNT/CD to drive potassium secretion.

Despite the controlled design of our study, the variability of 24h urinary sodium excretions 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 which discovered an infradian rhythm periods of about a month for sodium and potassium urinary excretion (26). In this study, sodium urinary excretion was also weakly correlated to potassium urinary excretion (27). However, blood pressure varied with sodium intake (28), suggesting that we may have failed to detect a change in blood pressure 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) to that of the combination of hydrochlorothiazide plus amiloride. Therefore, one can argue that this
calculation does not truly reflect the basal sodium handling by the CNT/CD, because hydrochlorothiazide increased sodium delivery to this segment. However, one positive point is that increasing sodium delivery to the CD may sensitize the volunteers to the effect of amiloride. Second, 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 potassium than Caucasians under similar sodium intake (29). Thirdly, we studied only males. In a cohort of young non hypertensive females, the blood pressure response was also characterized by a salt-resistant pattern at any menstrual status. In this study as well, urinary potassium excretion was unchanged under two different sodium diets, similar to ours, but uncontrolled potassium intake was a clear limitation. At variance with the current study, GFR was not increased under high sodium diet (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 urinary potassium excretion is independent of dietary sodium intake and urinary sodium excretion. We show that HSD increases eGFR and sodium reabsorption by NCC in the DCT while it blunts RAAS activity and subsequently ENaC-mediated sodium reabsorption by the CNT/CD. Finally, we confirm that ambulatory BP is salt-resistant in young healthy Caucasian males.

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CONFLICT OF INTEREST STATEMENT
None declared

AUTHORS’ CONTRIBUTIONS
A.P.B and E.F. designed and performed the study, K.U., M.M carried out experiments, A.P.B, E.F and V.O. analyzed the data, V.O. made the figures and drafter the paper, M.B.,
S.D.S, B.P., P.Y.M, revised the paper, all authors approved the final version of the manuscript.

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**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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### Table 1. Baseline Characteristics of Individuals

| Parameters                                      | Healthy volunteers (n= 16) |
|------------------------------------------------|--------------------------|
| Age, year                                       | 22.00 ± 1.16             |
| Weight, kg                                      | 76.55 ± 6.42             |
| Height, cm                                      | 180.10 ± 5.92            |
| Body mass index, kg/m²                          | 23.64 ± 2.23             |
| Office systolic blood pressure, mm Hg           | 128.20 ± 10.35           |
| Office diastolic blood pressure, mm Hg          | 72.50 ± 9.56             |
| Heart rate, beats/minute                        | 62.63 ± 9.36             |
| Plasma creatinine, μmol/l                       | 83.31 ± 9.73             |
| Estimated glomerular filtration rate (ml/min/1.73m²) | 115.30 ± 12.30           |

Values are mean ± SD
Table 2. Physiological and biological parameters according to changes in sodium intake in healthy male volunteers

| Parameters                | LSD          | NSD          | HSD          | Friedman global test p |
|---------------------------|--------------|--------------|--------------|------------------------|
| Weight, kg                | 78.50 ± 2.40 | 79.10 ± 2.50 | 80.00 ± 8.60 | n.s.                   |
| 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/24h      | 1836.00 ± 632.50 | 1831.00 ± 515.50 | 2004.00 ± 395.70 | n.s.                   |
| U_{Urea}, mmol/24h        | 495.40 ± 72.34 | 472.00 ± 150.00 | 512.80 ± 113.00 | n.s.                   |
| U_{NaV}, 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 | n.s.                   |
| 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 vs LSD. ## P < 0.01 vs NSD.

Abbreviations are: LS, low sodium; NS, normal sodium; HS, high sodium; U_{NaV}, urinary sodium excretion; U_{KV}, urinary potassium excretion.
Figure legends:

**FIGURE 1: Design of the cross over study**
This design was applied to 2 experimental sessions with 3 sodium diets: LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet. 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.

**FIGURE 2: Urine volume, Na\(^+\) and K\(^+\) excretion on different salt intakes.**
A. Time course of urine volume in ml/24h. B. Time course of urinary Na\(^+\) in mmol/24h. C. Time course of urinary K\(^+\) in mmol/24h. 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 panels A, B, and C and correlations were assessed using a Spearman test in panel D. In panel 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 to 14 subjects in each subgroup. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.

**FIGURE 3: Renin angiotensin aldosterone system and glomerular filtration rate.**
A. Urinary aldosterone levels in pg/24h. B. Plasma aldosterone levels in pg/ml. C. Plasma renin activity in ng/ml/h. D. Creatinine clearance in ml/min. As expected, panels A, B and 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. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.

**FIGURE 4: Effects of salt intake on ambulatory blood pressure and heart rate.**
A. Twenty-four hour, diurnal and nocturnal systolic blood pressure in mmHg in each diet. B. Twenty-four hour, diurnal and nocturnal diastolic blood pressure in mmHg in each diet.
diet. C. Twenty-four hour, diurnal and nocturnal heart rate (HR) in beats per minute (bpm) 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. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.

**FIGURE 5: Assessment of DCT and CNT/CD function in each sodium 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. N=9 to 11 subjects in each subgroup. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.

**FIGURE 6: Blood pressure before and 3h after diuretic administration**

A. Systolic blood pressure in mmHg after thiazide administration and D. after thiazide plus amiloride administration under each diet. B. Diastolic blood pressure in mmHg after thiazide administration and E. after thiazide plus amiloride administration under each sodium diet. C. Heart rate (HR) in beats per minute (bpm) after thiazide administration and F. after thiazide plus amiloride administration under each diet. Statistical differences between baseline blood pressure and 3h after diuretic administration were assessed using a Wilcoxon test. N=11 to 16 subjects. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.

**FIGURE S1: Variability of Na\(^+\) and K\(^+\) urinary excretion.**

A. 24h Urinary excretion of Na\(^+\) in ml/24h, in each diet. B. Intra-individual variability assessed by within-person coefficients of variation of 24h urinary Na\(^+\) from day 4 to day 7. C. Inter-individual variability assessed by between-person coefficients of variation of 24h urinary Na\(^+\). D. 24h Urinary excretion of K\(^+\) in ml/24h, in each diet. E. Intra-
individual variability assessed by within-person coefficients of variation of 24h urinary K+ from day 4 to day 7. F. Inter-individual variability assessed by between-person coefficients of variation of 24h urinary K+. Statistical differences between LSD, NSD and HSD were assessed by a Friedman test. N=6 to 14 subjects in each subgroup. LSD, low sodium diet; HSD, high sodium diet, NSD, normal sodium diet.
Day 2 to day 6:
- 24 h urinary Na⁺, K⁺, creatinine

Day 7:
- 24 h urinary Na⁺, K⁺, creatinine, aldosterone
- Plasma Na⁺, K⁺, creatinine, cystatin C, aldosterone, PRA
- Diuretic test: 100 mg hydrochlorothiazide

Day 6:
- ABPM

Day 7:
- 24 h urinary Na⁺, K⁺, creatinine
- Diuretic test: 100 mg hydrochlorothiazide + 10 mg amiloride

Legend:
- LSD
- HSD
- NSD

Crossover

n=16
7-day period 7-day period 7-day period
Start

n=12/16
7-day period 7-day period 7-day period
End of study

Cross-over

n=16
7-day period 7-day period 7-day period
Start

n=12/16
7-day period 7-day period 7-day period
End of study
A. R.M. Anova global test n.s.

24 h SBP (mmHg)

24 h DBP (mmHg)

24 h HR (bpm)

Diurnal SBP (mmHg)

Diurnal DBP (mmHg)

Diurnal HR (bpm)

Nocturnal SBP (mmHg)

Nocturnal DBP (mmHg)

Nocturnal HR (bpm)

B. R.M. Anova global test n.s.

C. R.M. Anova global test n.s.

p=0.009

p=0.02

R.M. Anova global test n.s.
A R.M. Anova global test p=0.03

B R.M. Anova global test p=0.0002

C R.M. Anova global test p<0.0001

D R.M. Anova global test p=0.007

E R.M. Anova global test p=0.002

F R.M. Anova global test p=0.0007

Δ Urinary Na (mmol/h)

Δ Urinary K (mmol/h)
After thiazide administration

A  
SBP (mmHg)

B  
DBP (mmHg)

C  
HR (bpm)

D  
SBP (mmHg)

E  
DBP (mmHg)

F  
HR (bpm)

LSD  
NSD  
HSD

T0  
T3

p=0.02

After thiazide + amiloride administration

A  
SBP (mmHg)

B  
DBP (mmHg)

C  
HR (bpm)

D  
SBP (mmHg)

E  
DBP (mmHg)

F  
HR (bpm)

LSD  
NSD  
HSD

T0  
T3

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