VASCULAR EFFECTS OF SODIUM AND POTASSIUM INTAKE

LIEKE GIJSBERS

PROPOSITIONS

1. Blood pressure in older adult populations can be reduced by more than 10 mmHg when optimizing sodium and potassium intake.
   (this thesis)

2. Changes in blood pressure are not necessarily accompanied by changes in vascular function.
   (this thesis)

3. Waiting for medical ethical approval in infection disease outbreaks is unethical.

4. In life sciences, standardized collection, analysis and reporting of trial data is required to foster scientific progress.

5. Putting sustainability labels on foods wrongly suggests that unlabeled foods in that category are non-sustainable.

6. Statutes of limitations for criminal cases are outdated.

Propositions belonging to the thesis, entitled:
Vascular Effects of Sodium and Potassium Intake

Lieke Gijsbers
Wageningen, 13 September 2017

Vascular Effects of Sodium and Potassium Intake

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This research was conducted under the auspices of the Graduate School VLAG
(Advanced Studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences)

Vascular Effects of Sodium and Potassium Intake

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Thesis

submitted in fulfillment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 13 September 2017
at 11 a.m. in the Aula.

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Vascular Effects of Sodium and Potassium Intake.
162 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2017)
With references, with summary in English

ISBN 978-94-6343-626-7
DOI https://doi.org/10.18174/419525

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CHAPTER 1

General introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality. Annually, about 17.5 million people die from CVD, accounting for ~30% of deaths worldwide [1]. Elevated blood pressure (BP) is a major risk factor for CVD [2, 3] and the largest single contributor to global mortality, according to the World Health Organization [1]. In 2015, BP accounted for 9.2% of the Disability Adjusted Life Years (DALYs) in men and 7.8% of DALYs in women [4]. BP is a modifiable risk factor that is largely determined by lifestyle factors, including diet [5]. Dietary minerals, in particular sodium and potassium, play an important role in BP regulation [6]. While adverse effects of sodium and beneficial effects of potassium on BP have repeatedly been shown in human intervention studies, less is known about other vascular effects of these dietary minerals that could influence CVD risk. The present thesis aims at studying the BP effects of sodium and potassium intake in healthy humans in a broader (patho)physiological context, focusing also on endothelial function, arterial stiffness, body fluid balance and heart rate.

Sodium and Potassium Intake

The human body needs ~0.5 g/d of sodium for maintaining plasma volume, acid-base balance and cellular functions, including smooth muscle cells [7, 8]. Sodium is mostly consumed as table salt (sodium chloride) added to foods, with each 2.5 g of salt containing 1 g of sodium. Around the world people consume on average 9–12 g of salt on a daily basis, which is far beyond the recommended maximum intake of 5–6 g/d (1.5–2.4 g of sodium) [9-12]. In the Netherlands, about 80% of the salt in the diet is added by food manufacturers. An additional 5 to 10% is naturally present in foods, and 10 to 15% is added during cooking or at the table (i.e. discretionary salt) [13]. Around 90% of sodium consumed is excreted in urine [8]. Averaging multiple 24-h urine samples is considered the gold standard for estimating an individual's daily sodium intake. Methods based on reported food intake are inadequate because they fail to take discretionary salt use into account [14]. Also, they are hampered by the huge variability in sodium content in processed foods, depending on the manufacturer, which is not well captured in food composition databases [15].

Potassium is an essential nutrient, which is together with sodium involved in the distribution of body fluids, regulation of the acid-base balance, nerve impulse transmission and muscle contraction [16]. The main dietary sources of potassium are fruit and vegetables, legumes, whole grains, and dairy products [17]. Most countries lack specific guidelines for potassium intake, but the European Food and Safety Authority considers 3.5 g/d as an Adequate Intake for European populations [11]. A study of 21 countries across the world by Van Mierlo et al. [18] showed that the mean daily potassium intake ranged from 1.7 g in China to 3.7 g in Northern Europe. Adherence to the dietary guidelines, especially for fruit and vegetables, would increase current levels of potassium intake in Western populations to US guideline 4.7 g/d, as was also the target in the well-known DASH trial for hypertension prevention [16, 18, 19]. Of all potassium consumed, 77–92% of is excreted in urine [20]. As long as there is

no widespread use of potassium-containing salt substitutes by consumers or food industry, dietary assessment methods that make use of food composition tables can provide rather adequate estimations of daily potassium intake [21].

BP and Vascular Health

Hypertension, defined as a systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or use of antihypertensive medication [22], affects ~25% of the world's adult population [23]. Increasing BP increases the risk of cardiovascular morbidity and mortality, starting from levels as low as 115/75 mmHg [2]. It has been estimated that a population-wide reduction in systolic BP of 3 mmHg is associated with a ~13% lower risk of stroke death and ~10% lower risk of death from ischemic heart disease or other vascular causes in a middle-aged population [2]. Twenty-four-hour ambulatory BP, which includes repeated readings during day and night that are free from white-coat effect, may be superior in predicting cardiovascular morbidity and mortality compared to office measurements [24].

The endothelium is the thin layer of cells that covers the inner wall of blood vessels. It is involved in physiological functions related to vascular health, including regulation of vascular tone, coagulation and inflammatory processes [25]. Endothelial cells synthesize and release biologically active substances that play a role in these processes. An imbalance in these substances may result in endothelial dysfunction, which is characterized by impairment of endothelial-dependent vasodilation and proinflammatory, proliferative, and procoagulatory features [26]. Higher circulating concentrations of cytokines, adhesion molecules and regulators of thrombosis and coagulation markers may reflect dysfunction of the endothelium and more inflammatory activity. Endothelium-dependent vasodilation can be assessed by the noninvasive measurement of flow-mediated dilation (FMD). Using high-resolution ultrasound, this method quantifies the change in diameter of the brachial artery in response to reactive hyperemia [27]. FMD is the most accepted and established method to study endothelial function, also because it could have predictive value for CVD events [28, 29].

Another measure of vascular health is arterial stiffness, which is one of the earliest detectable manifestations of adverse structural changes within the vessel wall [30]. Pulse wave velocity (PWV) is the direct measure of arterial stiffness [31], and high PWV values have been associated with increased risk of CVD [32]. An indirect measure of arterial stiffness is augmentation index, which is deduced from a pulse wave analysis of the radial artery.

Sodium, Potassium and BP

Meta-analyses of randomized controlled trials (RCTs) have repeatedly shown that a reduced sodium and increased potassium intake lowers BP [33-38]. In a meta-analysis of 34 randomized trials with a minimum duration of 4 weeks, He et al. [38] showed reductions

of 4.2 mmHg in systolic BP and 2.1 mmHg in diastolic BP for a 75 mmol lower 24-h sodium excretion (i.e. 1.7 g sodium or 4.4 g salt per day) in adults not on antihypertensive medication. A significant dose-response relation was found, with larger effects in hypertensives than normotensives (-5.4 mmHg vs -2.4 mmHg for systolic BP).

In a meta-analysis of 22 trials by Aburto et al. [37], potassium supplementation (~2 g/d) reduced systolic/diastolic BP by 3.5/2.0 mmHg, an effect that was more pronounced in hypertensives. No clear dose-response relation was observed. Although not significantly different, the BP reduction was 6.9/2.9 mmHg when baseline sodium intake was high (> 4 g/d) compared to 2.0/2.0 mmHg at lower sodium intake (2–4 g/d). A larger BP response after potassium supplementation in populations with a high salt intake was also found in an earlier meta-analysis by Whelton et al. [36].

Sodium, Potassium and Other Vascular Outcomes

Endothelial function - Limited studies have addressed the effects of sodium and potassium intake on endothelial function. RCTs with sodium reductions ranging from 1.4 to 2.3 g/d for a period of 2–6 weeks showed improvements in brachial artery FMD of 1.5–2.4% [39-41]. Studies on the effects of sodium intake on biochemical markers have primarily focused on vasoconstrictor endothelin-1, showing inconclusive results [39, 41-44].

Supplemental potassium of 2.5 g/d for 4 weeks improved FMD, with larger effect for potassium chloride supplements (2.7%) than for potassium bicarbonate supplements (1.5%) [45]. An RCT with a potassium dose of 1.6 g/d for 6 weeks demonstrated no change in FMD [46]. RCTs in which potassium was increased for at least 4 weeks showed no effect on endothelin-1 [47], soluble adhesion molecules or inflammation marker C-reactive protein [46, 48].

Body fluid balance - A key aspect in long-term regulation of BP is fluid balance, which is regulated by means of osmoregulation and volume regulation [49]. Body fluid volume and electrolyte concentration are maintained within very narrow limits despite wide variations in dietary sodium and potassium intake [50]. The mechanisms involved in counterbalancing the BP raising effects of sodium have been investigated repeatedly. These mechanisms include suppression of the renin-angiotensin aldosterone system, resulting in a decreased tendency for sodium reabsorption [51], and stimulation of release of natriuretic peptides that promote sodium excretion [52, 53]. The BP-lowering effects of potassium were found to be more pronounced at higher levels of sodium intake. During sodium restriction, potassium has less effect on BP [37]. This suggests an interaction between potassium intake and sodium- and volume status. This interaction, however, has not been well characterized. In particular, the humoral mechanisms involved in the BP-lowering effects of potassium supplementation have been poorly documented.

Heart rate - Heart rate has been identified as a predictor of cardiovascular morbidity and mortality in population-based studies [54-56]. A meta-analysis of 7 prospective cohort studies showed that a high resting heart rate was associated with a 40% higher risk of heart failure compared to a low resting heart rate [56]. However, to what extent heart rate in healthy individuals can be modified by changes in sodium and potassium intake warrants further research.

THESIS OUTLINE

Research on sodium and potassium intake has mainly focused on BP. The aim of this thesis is to examine the effects of sodium and potassium intake on BP in healthy individuals in the broader context of cardiovascular health, by simultaneously studying the effects on vascular function, body fluid balance and heart rate (see Figure 1). In Chapter 2, the effects of sodium and potassium supplementation on office BP, 24-h ambulatory BP and arterial stiffness are examined in a randomized placebo-controlled crossover study of 36 untreated (pre)hypertensive Dutch individuals on a fully controlled diet. In the same study, the effects of sodium and potassium supplementation on the functional measure of endothelial function (FMD) and on a comprehensive set of biochemical markers of endothelial dysfunction and low-grade inflammation are evaluated in the same individuals (Chapter 3). To gain more mechanistic insight, the humoral effects of potassium supplementation during sodium-restriction are assessed using a panel of markers that are involved in osmoregulation and volume regulation. In addition, the effects of sodium supplementation, with surmised opposite changes in these markers are investigated (Chapter 4). The effects of potassium supplementation on heart rate are summarized by means of a meta-analysis of RCTs in healthy adults (Chapter 5). The BP effects of sodium and potassium as observed in RCTs may not be confirmed in observational research, which could be due to methodological issues. Chapter 6 focuses on the assessment of dietary sodium and potassium intake by different methods and how that affects BP associations in an observational study in healthy Dutch adults. Finally, in Chapter 7 the main findings and their clinical and public health implications are discussed.

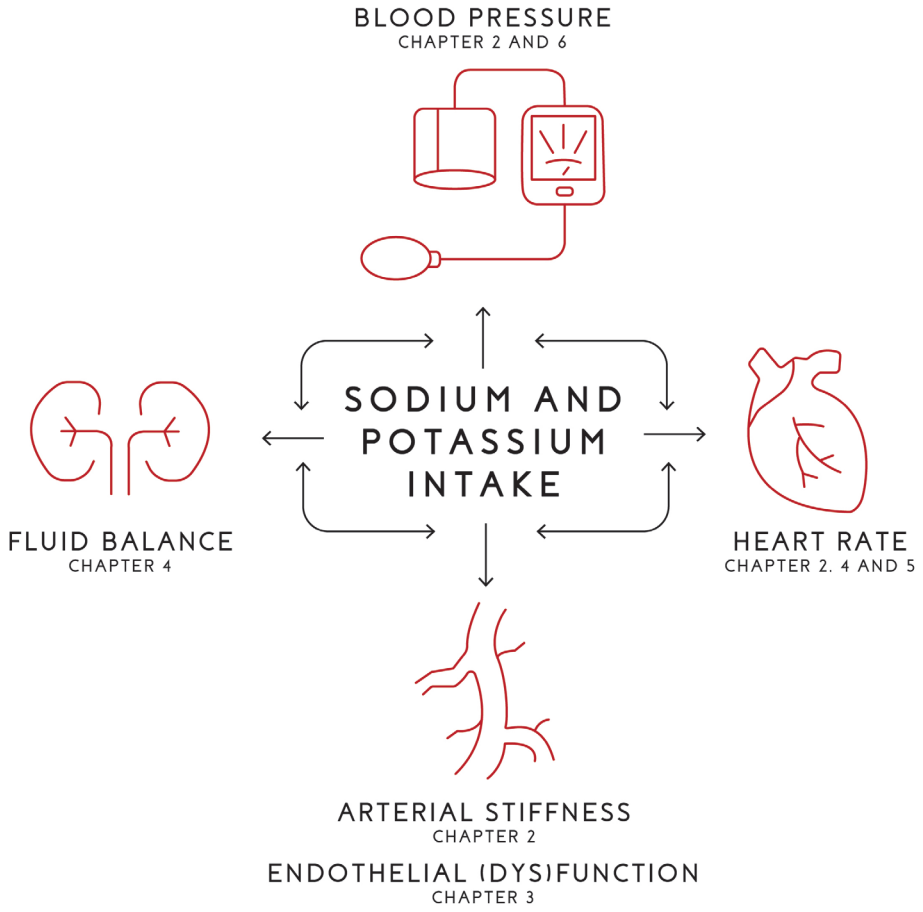

FIGURE 1.
Schematic overview of the vascular effects of sodium and potassium intake that are assessed in this thesis.

REFERENCES

1. World Health Organization. Global status report on noncommunicable diseases 2014. Geneva: WHO, 2014.
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002; 360:1903-13.
3. He J, Whelton PK. Elevated systolic blood pressure as a risk factor for cardiovascular and renal disease. J Hypertens Suppl. 1999; 17:S7-13.
4. Forouzanfar MH, Afshin A, Alexander LT, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388:1659-724.
5. Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. Hypertension. 2006; 47:296-308.
6. Adrogue HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. N Engl J Med. 2007; 356:1966-78.
7. Meneely GR. Plant Food Hum Nutr 1973; 23:3-31.
8. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr. 1984; 40:786-93.
9. World Health Organization. Salt Reduction Fact Sheet, 2016.
10. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. Int J Epidemiol. 2009; 38:791-813.
11. WHO. Guideline: Potassium intake for adults and children. Geneva, World Health Organization (WHO), 2012.
12. US Department of Agriculture and US Department of Health and Human Services.. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: US Government Printing Office, 2010.
13. van Rossum CTM, Buursma-Rethans EJM, Fransen HP, Verkaik-Kloosterman J, Hendriksen MAH. Zoutconsumptie van kinderen en volwassenen in Nederland: Resultaten uit de Voedselconsumptiepeiling 2007-2010 (Salt consumption of children and adults in the Netherlands: Results from the Dutch National Food Consumption Survey 2007-2010). Bilthoven, the Netherlands: National Institute for Public Health and Environment (RIVM). 2012.
14. McLean RM. Measuring population sodium intake: a review of methods. Nutrients. 2014; 6:4651-62.
15. Champagne CM, Cash KC. Assessment of salt intake: how accurate is it? Proc Nutr Soc. 2013; 72:342-7.
16. National Research Council. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, DC: The National Academies Press, 2005.

17. Hendriksen MAH, van Rossum CTM, van der A DL. Kalium inname: risico van hyperkaliëmie? Overzicht van beschikbare gegevens in Nederland. RIVM Briefrapport 2015-0009. National Institute for Public Health and Environment (RIVM). 2015.
18. van Mierlo LAJ, Greyling A, Zock PL, Kok FJ, Geleijnse JM. Suboptimal potassium intake and potential impact on population blood pressure. Arch Intern Med. 2010; 170:1501-2.
19. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med. 1997; 336:1117-24.
20. EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies), Scientific opinion on dietary reference values for potassium. EFSA Journal. 2016 ;14:4592
21. Freedman LS, Commins JM, Moler JE, Willett W, Tinker LF, Subar AF, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for potassium and sodium intake. American journal of epidemiology. 2015; 181:473-87.
22. Whitworth JA. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens. 2003; 21:1983-92.
23. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365:217-23.
24. Niiranen TJ, Maki J, Puukka P, Karanko H, Jula AM. Office, home, and ambulatory blood pressures as predictors of cardiovascular risk. Hypertension. 2014; 64:281-6.
25. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood. 1998; 91:3527-61.
26. Hadi HAR, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag. 2005; 1:183-98.
27. Al-Qaisi M, Kharbanda RK, Mittal TK, Donald AE. Measurement of endothelial function and its clinical utility for cardiovascular risk. Vasc Health Risk Manag. 2008; 4:647-52.
28. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010; 26:631-40.
29. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol. 2013; 168:344-51.
30. Cavalcante JL, Lima JAC, Redheuil A, Al-Mallah MH. Aortic stiffness: current understanding and future directions. J Am Coll Cardiol. 2011; 57:1511-22.
31. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006; 27:2588-605.
32. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010; 55:1318-27.
33. He FJ, MacGregor GA. Effect of longer-term modest salt reduction on blood pressure. Cochrane Database Syst Rev. 2004: CD004937.

34. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ. 2013; 346:f1326.
35. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens. 2003; 17:471-80.
36. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, et al. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. JAMA. 1997; 277:1624-32.
37. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013; 346:f1378.
38. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ. 2013; 346:f1325.
39. Dickinson KM, Clifton PM, Keogh JB. A reduction of 3 g/day from a usual 9 g/day salt diet improves endothelial function and decreases endothelin-1 in a randomised cross_over study in normotensive overweight and obese subjects. Atherosclerosis. 2014; 233:32-8.
40. Dickinson KM, Keogh JB, Clifton PM. Effects of a low-salt diet on flow-mediated dilatation in humans. Am J Clin Nutr. 2009; 89:485-90.
41. Jablonski KL, Racine ML, Geolfos CJ, Gates PE, Chonchol M, McQueen MB, et al. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J Am Coll Cardiol. 2013; 61:335-43.
42. Gates PE, Tanaka H, Hiatt WR, Seals DR. Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. Hypertension. 2004; 44:35-41.
43. Todd AS, Macginley RJ, Schollum JB, Johnson RJ, Williams SM, Sutherland WH, et al. Dietary salt loading impairs arterial vascular reactivity. Am J Clin Nutr. 2010; 91:557-64.
44. Todd AS, Macginley RJ, Schollum JBW, Williams SM, Sutherland WHF, Mann JI, et al. Dietary sodium loading in normotensive healthy volunteers does not increase arterial vascular reactivity or blood pressure. Nephrology. 2012; 17:249-56.
45. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD, et al. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension. 2010; 55:681-8.
46. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr. 2010; 104:1839-47.
47. Matthesen SK, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest. 2012; 72:78-86.
48. Graham UM, McCance DR, Young IS, Mullan KR. A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens. 2014; 28:333-9.
49. Farquhar WB, Edwards DG, Jurkovitz CT, Weintraub WS. Dietary sodium and health: More than just blood pressure. J Am Coll Cardiol. 2015; 65:1042-50.

50. Gumz ML, Rabinowitz L, Wingo CS. Disorders of fluids and electrolytes: An integrated view of potassium homeostasis. New England Journal of Medicine. 2015; 373:60-72.
51. Damkjaer M, Isaksson GL, Stubbe J, Jensen BL, Assersen K, Bie P. Renal renin secretion as regulator of body fluid homeostasis. Pflugers Archiv: European journal of physiology. 2013; 465:153-65.
52. Rose BD PT. Clinical physiology of acid-base and electrolyte disorders: United States of America: The McGraw-Hill Companies; 2001.
53. van Gastel MDA, Meijer E, Scheven LE, Struck J, Bakker SJL, Gansevoort RT. Modifiable Factors Associated With Copeptin Concentration: A General Population Cohort. Am J Kidney Dis. 2014.
54. Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham IM. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J. 2010; 159:612-9 e3.
55. Jensen MT, Marott JL, Allin KH, Nordestgaard BG, Jensen GB. Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. Eur J Prev Cardiol. 2012; 19:102-8.
56. Khan H, Kunutsor S, Kalogeropoulos AP, Georgiopoulou VV, Newman AB, Harris TB, et al. Resting heart rate and risk of incident heart failure: three prospective cohort studies and a systematic meta-analysis. J Am Heart Assoc. 2015; 4:e001364.

CHAPTER 2

Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study

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J Hum Hypertens. 2015;29(10):592-598

ABSTRACT

We performed a randomised, placebo-controlled, crossover study to examine the effects of sodium and potassium supplementation on blood pressure (BP) and arterial stiffness in untreated (pre)hypertensive individuals. During the study, subjects were on a fully controlled diet that was relatively low in sodium and potassium. After a 1-week run-in period, subjects received capsules with supplemental sodium (3 g d^{-1}, equals 7.6 g d^{-1} of salt), supplemental potassium (3 g d^{-1}) or placebo, for 4 weeks each, in random order. Fasting office BP, 24-h ambulatory BP and measures of arterial stiffness were assessed at baseline and every 4 weeks. Of 37 randomised subjects, 36 completed the study. They had a mean pre-treatment BP of 145/81 mm Hg and 69% had systolic BP ≥ 140 mm Hg. Sodium excretion was increased by 98 mmol per 24 h and potassium excretion by 63 mmol per 24 h during active interventions, compared with placebo. During sodium supplementation, office BP was significantly increased by 7.5/3.3 mm Hg, 24-h BP by 7.5/2.7 mm Hg and central BP by 8.5/3.6 mm Hg. During potassium supplementation, 24-h BP was significantly reduced by 3.9/1.6 mm Hg and central pulse pressure by 2.9 mm Hg. Pulse wave velocity and augmentation index were not significantly affected by sodium or potassium supplementation. In conclusion, increasing the intake of sodium caused a substantial increase in BP in subjects with untreated elevated BP. Increased potassium intake, on top of a relatively low-sodium diet, had a beneficial effect on BP. Arterial stiffness did not materially change during 4-week interventions with sodium or potassium.

INTRODUCTION

Hypertension is a key risk factor for renal and cardiovascular diseases (CVD) [1,2] and affects ~25% of the world's adult population [3]. Reducing population blood pressure (BP) through beneficial dietary and lifestyle changes may have important effects on CVD prevention. There is compelling evidence from randomised controlled trials that sodium reduction lowers BP [4-8]. In a meta-analysis of randomised trials with a minimum duration of 4 weeks, He et al. [7] showed reductions of 5.4 mm Hg in systolic BP (SBP) and 2.8 mm Hg in diastolic BP (DBP) in hypertensives for a 75 mmol lower 24-h sodium excretion (i.e. 1.7 g sodium or 4.4 g salt per day), with about half the effect in normotensives. Considering that most populations around the world have salt intakes higher than the recommended maximum intake of 5–6 g d^{-1} (equals 2.0–2.4 g sodium) [9-11], global reductions in salt intake could substantially reduce the burden of CVD [7, 12].

Increasing dietary potassium intake may favourably affect CVD risk. van Mierlo et al. [13] reported expected reductions of 1.7–3.2 mm Hg in population SBP when current potassium intakes in 21 countries (1.7–3.7 g d^{-1}) were increased to 4.7 g d^{-1}, as recommended by the US Institute of Medicine [14]. A recent meta-analysis of 21 randomised trials reported 3.5/2.0 mm Hg lower BP with an increased potassium intake, especially in hypertensives [15]. The BP reduction was 6.9/2.9 mm Hg when habitual sodium intake was high (> 4 g d^{-1}), compared with 2.0/2.0 mm Hg for sodium intake of 2–4 g d^{-1}. Whether potassium supplementation lowers BP in subjects who adhere to the dietary sodium recommendation has not extensively been investigated.

Arterial stiffness is an independent risk factor of CVD [16] and can be assessed non-invasively, using pulse wave analysis (PWA), or directly as pulse wave velocity (PWV) [17]. Limited studies have examined the effects of sodium or potassium on measures of arterial stiffness, with inconclusive results both for sodium [18-25] and potassium [26-29].

We performed a randomised, placebo-controlled, crossover study to examine the effects of sodium and potassium supplementation on office BP, ambulatory BP and arterial stiffness in Dutch subjects with untreated elevated BP. Supplementation took place while subjects were on a fully controlled diet that was relatively low in sodium and potassium, with all meals provided during the study.

MATERIALS AND METHODS

Subjects

Potential participants were recruited within a 10-km radius of the research centre from December 2011 to April 2012. Subjects filled out a medical questionnaire, underwent physical examination and provided one 24-h urine and a fasting blood sample. Eligible for participation were non-smoking men and women, aged 40–80 years, with a fasting supine SBP between 130 and 159 mm Hg. Subjects with diabetes mellitus or cardiovascular, gastrointestinal, liver or renal diseases were excluded based on the questionnaire data and laboratory parameters. Other exclusion criteria were body mass index > 40 kg m^{-2}; use of medication known to affect the cardiovascular system; use of nutritional supplements; an energy-restricted or medically prescribed diet; unstable body weight in past 2 months; alcohol use over 21 (women) or 28 (men) consumptions per week; and pregnancy or lactation (women).

The Medical Ethics Committee of Wageningen University approved the study. The trial was registered at ClinicalTrials.gov (NCT01575041). The study was conducted from March to August 2012 at the research centre of Wageningen University, Wageningen, The Netherlands. All subjects gave a written informed consent.

Study Design

We performed a randomised, double-blind, placebo-controlled crossover study, in which diet was fully controlled. During the 1-week run-in (to ensure energy balance and reach basal BP) and 3 consecutive intervention periods of 4 weeks (not separated by washout) each subject consumed a diet that was targeted to provide 2 g of sodium and 2 g of potassium per day for a 2500 kcal intake. At the end of the run-in ('baseline'), subjects were examined and randomly allocated to 1 of the 6 possible treatment orders, in strata of sex and SBP (130–139 and ≥ 140 mm Hg), by an independent person who used a computer-generated table. Treatments included 3 g of added sodium (equals 7.5 g salt) per day, 3 g of added potassium per day or placebo. Subjects were examined at baseline and at the end of each intervention. Examinations included ambulatory BP monitoring (ABPM), and in a fasting state anthropometric measurements, office BP, PWA and PWV and a blood sample. Subjects collected 24-h urine by discarding the first morning urine sample and collecting all urine for the next 24 h. Measurements were done at fixed time points of the day throughout the study. BP was re-measured 2 weeks after completion of the study, when subjects had returned to their usual diet.

Diet and Study Procedures

Individual energy needs were estimated using an FFQ [30], which was filled out during screening, combined with results of the Schofield equation [31]. The food and beverages that were supplied by the research institute covered 90% of daily energy needs. Remaining 10%

was chosen by the participant from a limited number of products that were low in sodium and potassium. These products and any deviations from the diet were recorded in a diary. Subjects were not allowed to add salt or salt-containing seasonings to their food. Maximum daily consumption levels were set for coffee (3 cups), alcohol (1 consumption, equalling 10–15 g of ethanol), fruit (2 portions) and liquorice (3 pieces).

During the trial, duplicates of each daily diet were collected, homogenised and analysed for energy, macronutrients and mineral content. The average composition of the diet (see Supplementary Table 1) was calculated from these duplicate diets and from free-choice items for which nutrient values were obtained from the Dutch food composition table [32]. A 2500-kcal diet provided a daily sodium and potassium intake of 2.4 and 2.3 g, respectively. We asked the subjects to maintain their usual level of physical activity during the study. Subjects were weighed twice a week and if needed, their energy intake was adjusted to keep body weight constant.

Experimental Treatment

Sodium and potassium intakes were increased through the daily use of capsules (Microz, Geleen, The Netherlands), while subjects were on the study diet. Depending on the intervention period, subjects had to ingest 8 sodium chloride capsules (in duplicate analysed content: 371 mg sodium per capsule, totalling 2968 mg), 8 potassium chloride capsules (353 mg potassium per capsule, totalling 2824 mg) or 8 placebo capsules (cellulose), distributed over the day with meals. Capsules were matched in size and colour and research staff and subjects were blinded to treatment. Compliance was checked through capsule counts and subjects' diaries. Subjects who ingested over 80% of the capsules for a given intervention period were considered compliant.

Measurements

Office BP and heart rate. Office BP and heart rate (HR) measurements were performed in a temperature-controlled (20–24 °C) quiet room by 1 trained staff member. BP and HR were measured in supine position after at least 10 min rest with 2-min intervals using an automated oscillometric device (Dinamap Pro 100, KP Medical, Houten, The Netherlands) with an appropriate cuff size on the left upper arm with the arm rested on the bed. The first measurement was discarded and the 3 subsequent measurements were averaged. Subjects remained blinded towards the BP and HR values until the end of the study.

Ambulatory BP and HR. Blinded ambulatory BP and HR monitoring was performed for 24 h using Spacelabs 90217 devices (Spacelabs Medical Inc. Redmond, WA, USA). Recordings were taken every 30 min at daytime (7 AM to 11 PM) and every 60 min at nighttime (11 PM to 7 AM) on the non-dominant arm ~2 cm above the antecubital fossa. Subjects were asked to maintain their normal daily activities during the recording period, to avoid intense exercise

and to register their activities in a diary. Subjects were instructed to perform ABPM 1 or 2 days before the end of each intervention period, at fixed times (i.e. same day of the week and time of the day). A weighted 24-h mean ambulatory BP and HR was calculated, as well as daytime (8 AM to 22 PM), nighttime (midnight to 6 AM) and early-morning (6 AM to 9 AM) means. Ambulatory BP and HR was based on at least 6 daytime and 4 nighttime recordings.

PWA and PWV. Radial artery PWA and carotid-femoral PWV were determined by applanation tonometry using the SphygmoCor system (version 8.0; AtCor Medical, Sydney, NSW, Australia) by the same staff member each occasion. Central aortic pressures and HR-corrected augmentation index (AIx), which are surrogate measures of arterial stiffness, were derived from PWA [17]. One subject was excluded in the PWA and PWV analysis as no reliable pressure wave could be recorded because of an irregular heartbeat.

Laboratory Analysis

Serum samples were stored at −80 °C until the end of the study for the determination of sodium, potassium, triglycerides, total cholesterol, HDL-cholesterol and creatinine. LDL-cholesterol was calculated from the Friedewald formula [33]. Twenty-four-hour urine samples were stored at −80 °C for the determination of sodium, potassium, creatinine (unprocessed samples) and calcium and magnesium (acidified samples). Serum and urinary sodium and potassium were determined using the ion-selective electrodes module on the Modular P of Roche (Roche Diagnostics, Mannheim, Germany) in a certified laboratory. Other serum and urine parameters were assessed using standard laboratory methods. Inter-assay coefficients of variation were < 3% for all biochemical measurements, except for calcium (5.0%).

Statistical Analysis

Double-data entry was performed and data were analysed according to the intention-to-treat principle, using a predefined protocol. Treatment codes were broken after data-analysis results had been verified by an independent statistician. For each outcome measure, mixed-effects model with covariance structure compound symmetry was used to estimate the effect of the active treatment compared to placebo. The effects on office SBP were defined as the primary outcomes. Differences in the occurrence of adverse events between treatments were assessed by the χ^2-test. Sensitivity analysis was conducted by excluding periods in which subjects were noncompliant (see Supplementary Table 2).

Values reported in text and tables are means with s.d. or treatment effects with 95% confidence interval. Two-sided P-values < 0.05 were regarded as statistically significant. Analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC, USA).

RESULTS

Subjects

Figure 1 shows the number of subjects screened, randomised and withdrawn during the study. Of 37 randomised Caucasian subjects, one dropped out because of gastrointestinal complaints due to capsule use. Baseline characteristics of the 24 men and 12 women who completed the study are reported in Table 1. Subjects were on average 65.8 y (range 47–80 y) and their body mass index was 27.2 kg m^{-2}.

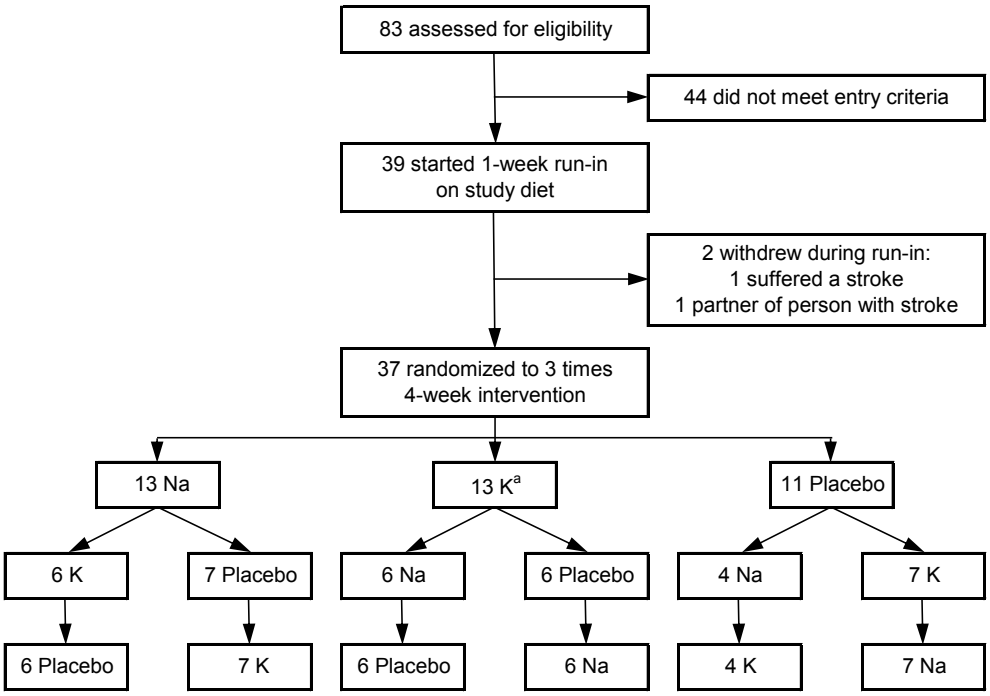

FIGURE 1. Flow chart of a randomized, double-blind, placebo-controlled crossover study in untreated (pre)hypertensive adults. Na, sodium; K, potassium. a One subject withdrew because of gastrointestinal complaints after the intake of capsules.

Sodium and Potassium Excretion

During screening, 24-h urinary excretion was 153.7 mmol for sodium and 81.8 mmol for potassium, which were reduced to 90.8 and 49.0 mmol, respectively, after run-in. Twenty-four-hour urinary sodium excretion was 105.1 mmol on placebo and 202.9 mmol on sodium supplementation, a mean difference of 97.6 mmol (equals 2.2 g sodium or 5.7 g salt). Urinary potassium excretion was increased from 55.3 mmol on placebo to 118.1 mmol on potassium

supplementation, i.e. by 62.9 mmol (equals 2.5 g) (Table 2). The molar sodium-to-potassium ratio was 2.0 during placebo, 4.0 during sodium supplementation and 0.9 during potassium supplementation (ratios based on weight: 1.2, 2.4 and 0.5, respectively).

Office BP and HR

During screening, subjects had a mean office BP of 145.3/80.6 mm Hg and 69% (25/36) had SBP ≥ 140 mm Hg. After run-in, BP was 133.4/75.7 mm Hg. Table 3 shows the treatment effects for office BP and HR. During sodium supplementation, SBP was increased by 7.5 mm Hg (3.8, 11.1), DBP by 3.3 mm Hg (1.5, 5.2) and pulse pressure (PP) by 4.1 mm Hg (1.5, 6.7) compared with placebo, with no effect on HR. Potassium supplementation resulted in a nonsignificantly lower SBP of 3.0 mm Hg (−0.6, 6.7), and significantly lower PP of 2.8 mm Hg (0.1, 5.4) compared with placebo, with no significant differences in DBP and HR. Two weeks after completion of the study, mean office BP was 132.1/74.8 mm Hg (data not shown), which was comparable to post-run-in values.

Ambulatory BP and HR

Figure 2 shows the mean unadjusted ambulatory SBP (A) and DBP (B) values, by treatment. Sodium supplementation resulted in a higher 24-h SBP of 7.5 mm Hg (4.4, 10.5) and DBP of 2.7 mm Hg (1.1, 4.2), compared with placebo. In 78% (28/36) of the subjects, 24-h SBP was higher during sodium supplementation than during placebo supplementation (Figure 3). For SBP, the effect did not essentially differ over the day, but for DBP a larger effect was seen during early-morning (4.1 mm Hg). Sodium supplementation did not significantly affect ambulatory HR (Table 3).

TABLE 1. Baseline characteristics of 36 subjects who completed the study

	Men (n=24)	Women (n=12)
Age (y)	66.0 ± 9.3	65.4 ± 8.2
Height (cm)	178.8 ± 8.9	168.8 ± 5.8
Weight (kg)	87.9 ± 19.0	77.2 ± 16.0
BMI (kg m−2)	27.3 ± 4.8	27.0 ± 4.6
Waist circumference (cm)	103.0 ± 15.0	93.8 ± 12.9
Pre-run-in SBP (mm Hg)	147.7 ± 10.3	140.4 ± 11.7
Pre-run-in DBP (mm Hg)	82.8 ± 7.7	76.2 ± 7.0
Post-run-in SBP (mm Hg)	136.4 ± 14.7	127.5 ± 13.3
Post-run-in DBP (mm Hg)	77.8 ± 8.0	71.4 ± 7.6

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Values are mean ± s.d.

TABLE 2. Effects of sodium and potassium supplementation on urinary and serum parameters in 36 untreated (pre)hypertensive adults

	Values after 4 weeks of intervention[a]			Treatment effect[b]			
	Sodium	Potassium	Placebo	Sodium vs placebo	P	Potassium vs placebo	P
Urinary parameters							
Volume (mL per 24 h)	1931 ± 826	1786 ± 785	1910 ± 793	9 (-224, 242)	0.94	-128 (-361, 104)	0.28
Sodium (mmol per 24 h)[c]	202.9 ± 54.8	96.5 ± 39.0	105.1 ± 39.7	97.6 (81.0, 114.1)	< 0.001	-8.9 (-25.4, 7.6)	0.29
Potassium (mmol per 24 h)[d]	53.2 ± 16.6	118.1 ± 32.2	55.3 ± 16.7	-2.2 (-10.2, 5.7)	0.58	62.9 (54.9, 70.8)	< 0.001
Calcium (mmol per 24 h)	5.45 ± 2.51	4.05 ± 2.15	4.28 ± 1.91	1.16 (0.70, 1.61)	< 0.001	-0.24 (-0.69, 0.21)	0.30
Magnesium (mmol per 24 h)[e]	4.35 ± 1.21	4.37 ± 1.29	4.06 ± 1.11	0.28 (-0.06, 0.63)	0.11	0.30 (-0.05, 0.65)	0.088
Creatinine (mmol per 24 h)[f]	12.6 ± 3.6	12.7 ± 3.1	11.8 ± 3.0	0.75 (-0.06, 1.56)	0.068	0.82 (0.01, 1.63)	0.048
Fasting serum parameters							
Sodium (mmol l^{-1})	143.8 ± 1.5	142.7 ± 1.5	143.4 ± 1.2	0.39 (-0.07, 0.84)	0.096	-0.68 (-1.13, -0.23)	0.004
Potassium (mmol l^{-1})	4.18 ± 0.34	4.41 ± 0.30	4.29 ± 0.32	-0.10 (-0.18, -0.02)	0.012	0.13 (0.05, 0.20)	0.002
Creatinine (µmol l^{-1})	77.3 ± 11.4	81.1 ± 13.0	80.8 ± 13.6	-3.7 (-6.1, -1.2)	0.003	0.2 (-2.2, 2.6)	0.86
Total cholesterol (mmol l^{-1})	5.47 ± 1.02	5.55 ± 0.93	5.66 ± 1.08	-0.19 (-0.36, -0.02)	0.032	-0.11 (-0.28, 0.06)	0.21
HDL-cholesterol (mmol l^{-1})	1.49 ± 0.36	1.44 ± 0.35	1.48 ± 0.34	0.01 (-0.04, 0.06)	0.80	-0.04 (-0.09, 0.01)	0.15
LDL-cholesterol (mmol l^{-1})	3.71 ± 0.94	3.80 ± 0.83	3.89 ± 0.98	-0.18 (-0.34, -0.01)	0.036	-0.08 (-0.25, 0.08)	0.32
Total-to-HDL-cholesterol ratio	3.85 ± 0.96	4.02 ± 0.98	3.98 ± 0.97	-0.13 (-0.28, 0.02)	0.089	0.05 (-0.11, 0.20)	0.55
Triglycerides (mmol l^{-1})	1.41 ± 0.63	1.56 ± 0.70	1.49 ± 0.63	-0.08 (-0.20, 0.03)	0.15	0.06 (-0.05, 0.18)	0.28

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a] Unadjusted mean ± SD. [b] Data are mean differences (95% CI) obtained from linear mixed-effect models for repeated measurements using the compound symmetry covariance structure. [c] To convert sodium in mmol per 24 h to mg per 24 h multiply by 23. [d] To convert potassium in mmol per 24 h to mg per 24 h multiply by 39. [e] One subject was excluded from the analyses due to an outlying value of 13.2 mmol per 24 h during potassium supplementation; treatment effects with inclusion of the outlier were 0.24 mmol per 24 h (-0.20, 0.68; P = 0.28) for sodium and 0.47 mmol per 24 h (0.03, 0.91; P = 0.038) for potassium. [f] One subject was excluded from the analyses due to an outlying value of 45.5 mmol per 24 h during potassium supplementation; treatment effects with inclusion of the outlier were 0.73 mmol per 24 h (-0.77, 2.24; P = 0.33) for sodium and 1.75 mmol per 24 h (0.25, 3.25; P = 0.023) for potassium.

TABLE 3. Effects of sodium and potassium supplementation on blood pressure, heart rate, and arterial stiffness in 36 untreated (pre)hypertensive adults

	Values after 4 weeks of intervention[a]			Treatment effect[b]			
	Sodium	Potassium	Placebo	Sodium vs placebo	P	Potassium vs placebo	P
Office BP and HR							
SBP (mm Hg)	132.9 ± 17.6	122.2 ± 15.3	125.1 ± 15.0	7.5 (3.8, 11.1)	<0.001	-3.0 (-6.7, 0.6)	0.10
DBP (mm Hg)	75.7 ± 7.5	72.0 ± 8.2	72.3 ± 7.7	3.3 (1.5, 5.2)	<0.001	-0.3 (-2.1, 1.6)	0.77
PP (mm Hg)	57.2 ± 14.3	50.1 ± 11.5	52.8 ± 12.6	4.1 (1.5, 6.7)	0.002	-2.8 (-5.4, -0.1)	0.039
HR (b.p.m.)	58.5 ± 8.3	59.8 ± 8.0	59.2 ± 8.0	-0.7 (-2.5, 1.0)	0.42	0.6 (-1.1, 2.4)	0.47
Ambulatory BP and HR							
24-hour SBP (mm Hg)	136.8 ± 14.4	125.6 ± 13.3	129.4 ± 14.1	7.5 (4.4, 10.5)	<0.001	-3.9 (-6.9, -0.9)	0.013
24-hour DBP (mm Hg)	79.2 ± 8.9	74.9 ± 7.8	76.5 ± 8.3	2.7 (1.1, 4.2)	<0.001	-1.6 (-3.2, -0.1)	0.039
24-hour HR (b.p.m.)	64.8 ± 9.8	68.4 ± 8.6	65.8 ± 9.4	-1.0 (-2.5, 0.6)	0.21	2.6 (1.1, 4.1)	<0.001
Daytime SBP (mm Hg)	141.5 ± 14.6	130.2 ± 15.2	134.5 ± 15.0	7.1 (3.7, 10.5)	<0.001	-4.4 (-7.8, -0.9)	0.013
Daytime DBP (mm Hg)	82.5 ± 9.6	78.6 ± 8.6	80.3 ± 8.9	2.1 (0.3, 4.0)	0.025	-1.8 (-3.6, 0.1)	0.062
Daytime HR (b.p.m.)	68.6 ± 10.3	73.4 ± 9.5	70.6 ± 11.1	-1.9 (-3.9, 0.1)	0.063	2.7 (0.8, 4.7)	0.007
Nighttime SBP (mm Hg)	126.0 ± 14.2	115.3 ± 11.1	118.8 ± 15.0	7.4 (3.9, 10.8)	<0.001	-3.6 (-7.1, -0.1)	0.043
Nighttime DBP (mm Hg)	71.6 ± 8.8	67.2 ± 7.2	68.7 ± 8.8	3.1 (1.1, 5.0)	0.003	-1.5 (-3.5, 0.4)	0.12
Nighttime HR (b.p.m.)	58.3 ± 9.8	61.1 ± 10.0	58.9 ± 8.6	-0.5 (-2.3, 1.4)	0.60	2.2 (0.4, 4.0)	0.020
Early-morning SBP (mm Hg)	132.6 ± 17.4	121.6 ± 15.1	124.7 ± 15.1	7.8 (3.6, 12.0)	<0.001	-3.2 (-7.5, 1.0)	0.13
Early-morning DBP (mm Hg)	77.9 ± 11.1	73.6 ± 9.0	73.7 ± 9.9	4.1 (1.4, 6.8)	0.003	-0.2 (-2.9, 2.5)	0.87
Early-morning HR (b.p.m.)	62.9 ± 11.9	64.8 ± 11.0	62.2 ± 9.0	0.7 (-2.0, 3.5)	0.59	2.6 (-0.1, 5.4)	0.059
PWA[c]							
Central AIx (%)[d]	24.7 ± 8.3	23.5 ± 8.3	23.7 ± 8.4	1.0 (-0.4, 2.4)	0.16	-0.2 (-1.6, 1.2)	0.74
Central SBP (mm Hg)	126.0 ± 18.7	114.2 ± 16.2	117.2 ± 16.3	8.5 (4.8, 12.2)	<0.001	-3.0 (-6.6, 0.7)	0.11
Central DBP (mm Hg)	76.6 ± 7.8	72.7 ± 8.1	72.8 ± 7.7	3.6 (1.8, 5.4)	<0.001	-0.2 (-2.0, 1.7)	0.86
Central PP (mm Hg)	49.4 ± 15.1	41.5 ± 12.5	44.4 ± 13.7	4.8 (2.1, 7.4)	<0.001	-2.9 (-5.5, -0.3)	0.031
PWV (m s^{-1})[c]	13.1 ± 2.9	12.7 ± 2.6	13.1 ± 3.0	0.03 (-0.48, 0.53)	0.91	-0.35 (-0.85, 0.16)	0.17

Abbreviations: AIx, augmentation index; BP, blood pressure; b.p.m., beats per minute; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; PP, pulse pressure; PWA, pulse wave analysis; PWV, pulse wave velocity. [a]Unadjusted mean ± s.d. [b]Data are mean differences (95% CI) obtained from linear mixed-effect models for repeated measurements using the compound symmetry covariance structure. [c]Results are based on 35 subjects as one subject had an irregular heartbeat. [d]Adjusted to a standard HR of 75 b.p.m.

Potassium supplementation resulted in a lower 24-h SBP of 3.9 mm Hg (0.9, 6.9) and 24-h DBP of 1.6 mm Hg (0.1, 3.2) compared with placebo (Figure 2, Table 3). For SBP, the effect did not essentially differ over the day, but for DBP no effect was seen during early-morning. In 67% (24/36) of the subjects, 24-h SBP was lower during potassium supplementation than during placebo supplementation (Figure 3). During potassium supplementation, 24-h HR was 2.6 beats per minute (1.1, 4.1) higher than during placebo.

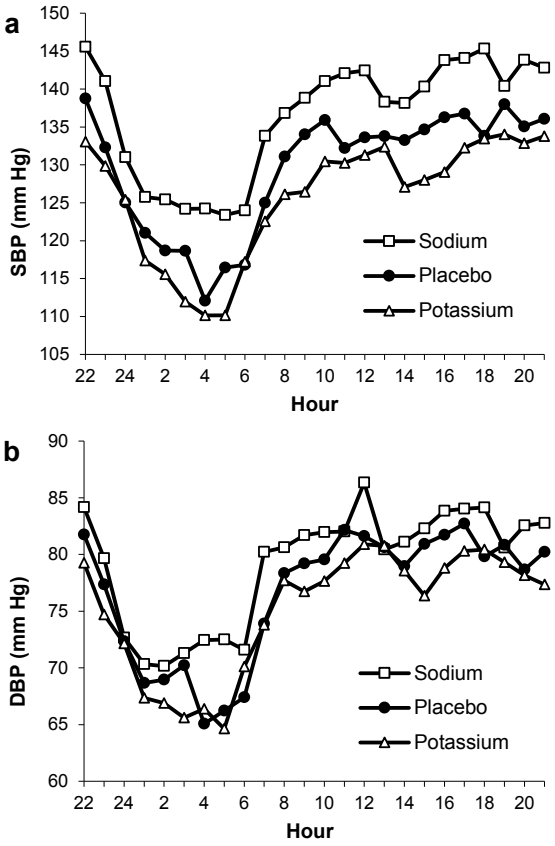

FIGURE 2. Unadjusted mean ambulatory systolic (a) and diastolic (b) blood pressure for each hour over 24 h after 4-week supplementation with sodium, potassium or placebo in 36 untreated (pre)hypertensive adults. DBP, diastolic blood pressure; SBP, systolic blood pressure.

PWA and PWV

Sodium supplementation resulted in a significantly higher central SBP of 8.5 mm Hg, central DBP of 3.6 mm Hg and central PP of 4.8 mm Hg compared with placebo. PWV was unaffected by sodium supplementation (Table 3). Potassium supplementation resulted in a significantly lower central PP of 2.9 mm Hg, and a nonsignificantly lower central SBP and DBP of 3.0 and

0.2 mm Hg, respectively. PWV was nonsignificantly decreased by 0.35 m s^{-1}. Central HR-corrected AIx was unaffected by sodium or potassium supplementation (Table 3).

Serum and Urine Parameters, and Body Weight

Sodium supplementation resulted in a nonsignificantly higher serum sodium of 0.39 mmol l^{-1}, and significantly lower serum potassium of 0.10 mmol l^{-1}, serum creatinine of 3.7 µmol l^{-1}, total cholesterol of 0.19 mmol l^{-1} and LDL-cholesterol of 0.18 mmol l^{-1}, compared with placebo. Serum HDL-cholesterol, total-to-HDL-cholesterol ratio and triglycerides did not differ significantly between sodium and placebo supplementation. Twenty-four-hour urinary calcium excretion was significantly higher by 1.16 mmol (equals 46.4 mg) during sodium supplementation than during placebo. Urinary magnesium and creatinine excretion were not significantly affected (Table 2).

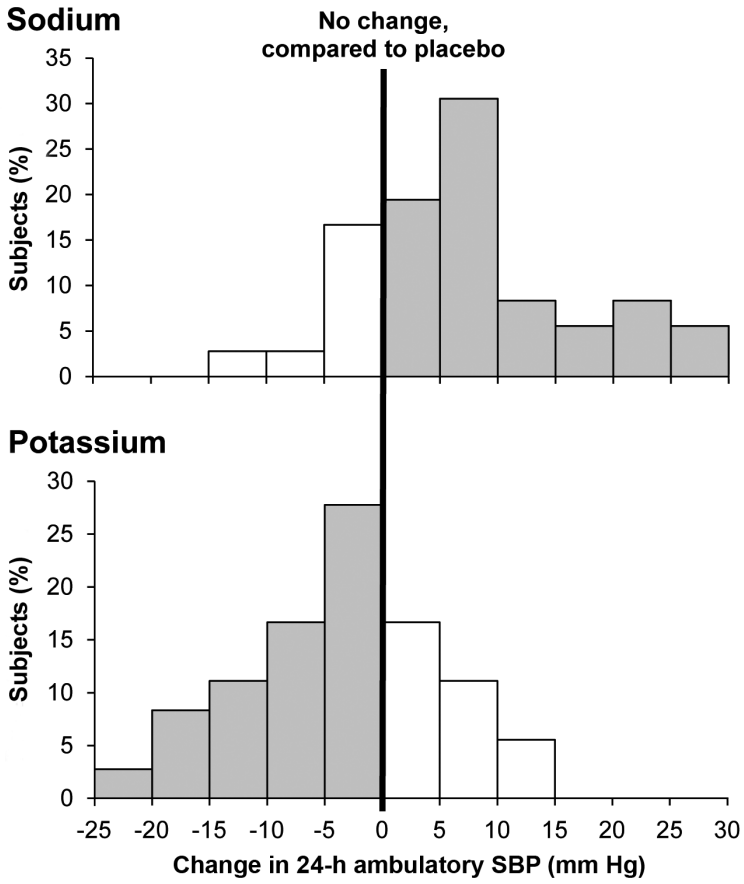

FIGURE 3. Effects of 4-week sodium and potassium supplementation on 24-h ambulatory systolic blood pressure in 36 untreated (pre)hypertensive adults, compared with placebo. SBP, systolic blood pressure.

Potassium supplementation resulted in a significantly lower serum sodium of 0.68 mmol l^{-1}, higher serum potassium of 0.13 mmol l^{-1} and higher urinary creatinine of 0.82 mmol per 24 h compared with placebo. Other serum and urinary parameters did not differ significantly (Table 2). Body weight was kept constant during the study through adjustments in caloric intake, and did not differ between the intervention periods.

Adverse Events

Reported side effects in subjects' diaries indicated that 19 persons experienced gastrointestinal complaints during sodium, 21 during potassium and 8 during placebo supplementation (P = 0.004). Other side effects including dizziness, headache, illness, shortness of breath and oedema were not significantly different among the 3 treatments.

DISCUSSION

In Dutch adults with untreated elevated BP, increasing the intake of sodium by 3.0 g d^{-1} (equals 7.6 g d^{-1} of salt) strongly raised office, ambulatory and central SBP by ~8 mm Hg. Increasing the potassium intake by 2.8 g d^{-1} significantly lowered ambulatory SBP by 4 mm Hg in these individuals on a relatively low-sodium diet. Measures of arterial stiffness did not materially change after 4 weeks of sodium or potassium supplementation.

In most Western societies, mean sodium intakes are above recommended levels [9], whereas potassium intakes are relatively low [13]. This could have a major impact on population health, including risk of CVD. A 7.5-mm Hg lower SBP, in our study achieved by decreasing sodium intake from a level common in Western societies to the recommended level, would be associated with a 30% lower risk of stroke mortality and 22% lower risk of ischaemic heart disease in a middle-aged population [2]. Increasing the intake of potassium, even when subjects adhere to guidelines for dietary salt intake, may further reduce the risk.

A major strength of the present study is the fully controlled diet, which strongly reduced the intra-individual variability in BP resulting from dietary influences (for example, use of alcohol, coffee and salt) and thereby increasing power to demonstrate effects on BP. Subjects were also instructed to keep other lifestyle behaviours, such as physical activity, constant. Fasting BP was repeatedly measured at the research centre using a strict protocol, at fixed times in the morning. All subjects underwent ABPM, which is considered a better predictor of CVD than office BP [34]. Because we provided a (relatively) low-sodium, low-potassium diet, combined with capsules that contained ~3 g of sodium or potassium, we were able to achieve large contrasts in sodium and potassium intake. Eighty-six percent of the subjects were compliant during all periods, as also reflected in 24-h urinary excretions.

Our study showed a 7.5 mm Hg increase in SBP with an increase in 24-h urinary sodium of 98 mmol. Assuming a linear relation, this is equivalent to an effect of 0.7-0.8 mm Hg per 10 mmol change in 24-h urinary sodium. This finding is comparable to the result of a meta-analysis of randomised trials of at least 4 weeks duration, in which a reduction in 24-h urinary sodium of 75 mmol decreased SBP by 5.4 mm Hg (0.7 mm Hg per 10 mmol) in hypertensives [7].
Potassium supplementation in our study increased 24-h urinary potassium by 63 mmol and reduced SBP by 3–4 mm Hg. In a meta-analysis of 16 randomised controlled trials [15], potassium lowered SBP by ~5 mm Hg in hypertensives. The smaller effect in our study may result from the inclusion of prehypertensives (31% of our subjects) who may show smaller BP responses. Also, our subjects consumed a relatively low-sodium diet (2.2 g d^{-1}). In the meta-analysis, BP reductions after increased potassium intake depended on sodium intake, i.e. SBP was reduced by 7 mm Hg for sodium intake of > 4 g d^{-1} and by 2 mm Hg for sodium intake of 2–4 g d^{-1} [15]. Therefore, the beneficial effect of increased potassium intake on BP in individuals with Western, high-salt diets may be greater than observed in the present study.

We found significant effects of sodium and potassium on BP, but not on arterial stiffness as measured by PWV and the surrogate measure AIx. The differences in several indices of central BP may have resulted from changes in brachial artery pressure, which was used in the algorithm to estimate the central pressures [17]. Since no effect was found for PWV, a direct indicator of arterial stiffness, we consider effects of sodium and potassium on arterial stiffness in our study unlikely. The 4-week duration of the intervention periods may have been too short to induce changes in the vascular structure. However, short-term interventions may affect arterial stiffness by influencing functional properties, such as vascular tone and endothelial function [35]. Moreover, other studies with a 4–6-week duration did find effects of sodium intake on PWV in (pre)hypertensives [21, 22, 24], although 2 studies in normotensives [19, 25] did not. Therefore, further studies of longer duration in subjects with untreated hypertension are warranted to assess the effects of sodium intake on arterial stiffness.

For potassium supplementation, limited data are available on the effects of arterial stiffness and results are inconsistent. A randomised controlled trial in 42 untreated hypertensives showed a significant reduction of 0.8 m s^{-1} in PWV for 2.5 g d^{-1} higher potassium intake [28]. Another trial in 40 subjects at increased CVD risk with the same potassium dose found a reduction in PWV of 0.4 m s^{-1} [27]. A trial in 48 early hypertensives with lower doses of potassium, however, found no effect [26]. In our study, PWV was 0.35 m s^{-1} lower during potassium but this finding was not statistically significant, despite a high potassium dose. Our study had ample power (> 80%) to detect an effect of 0.7 m s^{-1} in PWV, which has been associated with a 11% lower risk of mortality [16].

Sodium supplementation had no significant effect on HR. Potassium supplementation, however, significantly increased ambulatory HR, although no effect was seen for office HR.

The reason for this finding remains unclear. During high potassium intake, serum potassium was increased by 0.13 mmol l^{-1} but levels of all participants remained within the normal range, not posing them at increased risk for hyperkalaemia or cardiac rhythm disturbances. Possibly, decreases in plasma volume contributed to the effect on HR. Another trial in healthy humans reporting the effects of 4-week potassium supplementation of 3.9 g d^{-1}, however, found no differences in ambulatory HR [29]. Because an effect was found only on 24-h and not on office HR in our subjects, we cannot exclude the possibility that our finding was due to chance.

Sodium supplementation resulted in lower total and LDL-cholesterol levels of 3.4 and 4.6%, respectively, in our study. Other studies showed raised serum total or LDL-cholesterol during sodium restriction or use of thiazide diuretics, which is in line with these findings [36]. In a meta-analysis of sodium interventions that lasted 4 weeks or more, differences in serum cholesterol were not significant, suggesting a transient response [37]. Urinary calcium excretion in our subjects was higher during sodium supplementation, as has also been reported by others [38]. When urinary calcium losses occur, calcium mobilisation from bone may be increased. It has been suggested that this side effect of high sodium intake, if sustained over time, may lead to osteoporosis [38].

In conclusion, the current study demonstrates that increasing the intake of sodium from a recommended level to a level that is common in Western societies, has a strong adverse effect on BP in untreated (pre)hypertensive individuals. Increasing potassium intake, however, lowers BP even when people are on a relatively low-sodium diet. Measures of arterial stiffness were not materially affected by sodium or potassium supplementation. Our findings support the recommendations to reduce sodium intake and to increase potassium intake, which will likely lower BP in older individuals with untreated elevated BP, and the burden of CVD in Western societies.

Acknowledgements
We gratefully acknowledge the assistance of Harrie Robins, Sarah Mount and Danielle Schoenaker in the conduct of the study. We thank the dieticians for dietary calculations and catering, Karin Borgonjen-van den Berg for randomisation and blinding procedures, Anita Bruggink-Hoopman and Diana Emmen-Benink for blood sampling, Marlies Diepeveen-de Bruin for analysing duplicate diets and Hendriek Boshuizen for statistical support.

Funding
The research is funded by TI Food and Nutrition, a public–private partnership on pre-competitive research in food and nutrition. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures
The authors declare no conflict of interest.

REFERENCES

1. He J, Whelton PK. Elevated systolic blood pressure as a risk factor for cardiovascular and renal disease. J Hypertens Suppl 1999; 17: S7–13.
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002; 360: 1903–1913.
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: Analysis of worldwide data. Lancet 2005; 365: 217–223.
4. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ 2013; 346: f1326
5. Cutler JA, Follmann D, Allender PS. Randomised trials of sodium reduction: an overview. Am J Clin Nutr 1997; 65: 643S–651S.
6. Geleijnse JM, Kok FJ, Grobbee DE. Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens 2003; 17: 471–480.
7. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ 2013; 346: f1325.
8. Midgley JP, Matthew AG, Greenwood CM, Logan AG. Effect of reduced dietary sodium on blood pressure: a meta-analysis of randomised controlled trials. JAMA 1996; 275: 1590–1597.
9. Brown IJ, Tzoulaki I, Candeias V, Elliott P. Salt intakes around the world: implications for public health. Int J Epidemiol 2009; 38: 791–813.
10. WHO. Guideline: Sodium intake for adults and children. Geneva, World Health Organization (WHO), 2012.
11. US Department of Agriculture and US Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: US Government Printing Office, 2010.
12. He FJ, MacGregor GA. How far should salt intake be reduced? Hypertension 2003;42: 1093–1099.
13. van Mierlo LAJ, Greyling A, Zock PL, Kok FJ, Geleijnse JM. Suboptimal potassium intake and potential impact on population blood pressure. Arch Intern Med 2010; 170: 1501–1502.
14. National Research Council. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, DC: The National Academies Press, 2005.
15. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ 2013; 346: f1378.
16. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010; 55: 1318–1327.
17. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006; 27: 2588–2605.

18. Dickinson KM, Keogh JB, Clifton PM. Effects of a low-salt diet on flow-mediated dilatation in humans. Am J Clin Nutr 2009; 89: 485–490.
19. Dickinson KM, Clifton PM, Keogh JB. A reduction of 3 g/day from a usual 9 g/day salt diet improves endothelial function and decreases endothelin-1 in a randomised crossover study in normotensive overweight and obese subjects. Atherosclerosis 2014; 233: 32–38.
20. Gates PE, Tanaka H, Hiatt WR, Seals DR. Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. Hypertension 2004; 44: 35–41.
21. He FJ, Marciniak M, Visagie E, Markandu ND, Anand V, Dalton RN et al. Effect of modest salt reduction on blood pressure, urinary albumin, and pulse wave velocity in white, black, and asian mild hypertensives. Hypertension 2009; 54: 482–488.
22. Jablonski KL, Fedorova OV, Racine ML, Geolfos CJ, Gates PE, Chonchol M et al. Dietary sodium restriction and association with urinary marinobufagenin, blood pressure, and aortic stiffness. Clin J Am Soc Nephrol 2013; 8: 1952–1959.
23. Seals DR, Tanaka H, Clevenger CM, Monahan KD, Reiling MJ, Hiatt WR et al. Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. J Am Coll Cardiol 2001; 38: 506–513.
24. Todd AS, Macginley RJ, Schollum JB, Johnson RJ, Williams SM, Sutherland WH et al. Dietary salt loading impairs arterial vascular reactivity. Am J Clin Nutr 2010; 91: 557–564.
25. Todd AS, Macginley RJ, Schollum JBW, Williams SM, Sutherland WHF, Mann JI et al. Dietary sodium loading in normotensive healthy volunteers does not increase arterial vascular reactivity or blood pressure. Nephrology (Carlton) 2012; 17: 249–56.
26. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in uk men and women with early hypertension: a randomised controlled trial. Br J Nutr 2010; 104: 1839–1847.
27. Graham UM, McCance DR, Young IS, Mullan KR. A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens 2014; 28: 333–339.
28. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD et al. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension 2010; 55: 681–688.
29. Matthesen SK, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest 2012; 72: 78–86.
30. Siebelink E, Geelen A, de Vries JHM. Self-reported energy intake by ffq compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr 2011; 106: 274–281.
31. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39: 5–41.
32. NEVO-tabel 2011, RIVM/Voedingscentrum, Den Haag 2011.
33. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499–502.

34. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S et al. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. Hypertension 2005; 46: 156–161.
35. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am J Clin Nutr 2011; 93: 446–454.
36. He FJ, Campbell NRC, Macgregor GA. Reducing salt intake to prevent hypertension and cardiovascular disease. Rev Panam Salud Publica 2012; 32: 293–300.
37. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane Database Syst Rev 2011, CD004022.
38. He FJ, MacGregor GA. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. J Hum Hypertens 2009; 23: 363–384.

SUPPLEMENTAL RESULTS

SUPPLEMENTAL TABLE 1. Composition of the background study diet

	Average daily menu[a]	Average daily menu, standardised to 2500 kcal
Energy intake (kcal d^{-1})	2774	
Energy intake (MJ d^{-1})	11.6	
Protein (g d^{-1})	91.2	82.2
Protein (en%)	13.4	13.4
Fat (g d^{-1})	110.9	100.0
Fat (en%)	35.4	35.4
Carbohydrates (g d^{-1})	327.9	295.5
Carbohydrates (en%)	48.0	48.0
Cholesterol (mg MJ^{-1})	23.5	23.5
Dietary fiber (g MJ^{-1})	2.0	2.0
Alcohol (g d^{-1})	6.5	5.8
Alcohol (en%)	1.6	1.6
Sodium (mg d^{-1})	2700	2433
Salt (g d^{-1})	6.9	6.2
Potassium (mg d^{-1})	2506	2258
Calcium (mg d^{-1})	1119	1008
Magnesium (mg d^{-1})	305	275

[a] Based on chemical analysis of daily duplicate diets, plus free-choice items for which the nutrient content was obtained from the Dutch food composition table 2011 (NEVO) [1].

SUPPLEMENTAL TABLE 2. Effects of sodium and potassium supplementation on urinary parameters, blood pressure, heart rate and arterial stiffness in 36 untreated (pre)hypertensive adults, after the exclusion of periods in which subjects were noncompliant[a]

	Treatment effect[b]			
	Sodium vs placebo	P	Potassium vs placebo	P
Urinary parameters				
Sodium (mmol per 24 h)[c]	100.7 (84.0, 117.3)	<0.001	-11.3 (-28.1, 5.5)	0.18
Potassium (mmol per 24 h)[d]	-2.1 (-8.7, 4.6)	0.53	68.8 (62.1, 75.5)	<0.001
Office BP and HR				
SBP (mm Hg)	7.2 (3.3, 11.0)	<0.001	-3.4 (-7.3, 0.6)	0.092
DBP (mm Hg)	3.4 (1.5, 5.4)	<0.001	-0.4 (-2.3, 1.6)	0.72
PP (mm Hg)	3.7 (1.0, 6.5)	0.008	-3.0 (-5.7, -0.2)	0.035
HR (b.p.m.)	-0.7 (-2.6, 1.2)	0.46	0.7 (-1.2, 2.6)	0.47
Ambulatory BP and HR				
24-h SBP (mm Hg)	7.4 (4.3, 10.5)	<0.001	-4.6 (-7.7, -1.5)	0.005
24-h DBP (mm Hg)	2.7 (1.1, 4.3)	0.001	-1.9 (-3.5, -0.2)	0.025
24-h HR (b.p.m.)	-1.0 (-2.6, 0.6)	0.23	2.6 (1.0, 4.2)	0.002
Daytime SBP (mm Hg)	7.0 (3.6, 10.5)	<0.001	-5.0 (-8.6, -1.5)	0.005
Daytime DBP (mm Hg)	2.2 (0.2, 4.1)	0.029	-1.9 (-3.9, 0.0)	0.052
Daytime HR (b.p.m.)	-1.7 (-3.8, 0.3)	0.10	2.8 (0.7, 4.9)	0.010
Nighttime SBP (mm Hg)	7.8 (4.2, 11.4)	<0.001	-4.0 (-7.7, -0.4)	0.030
Nighttime DBP (mm Hg)	3.2 (1.2, 5.3)	0.002	-1.7 (-3.8, 0.4)	0.10
Nighttime HR (b.p.m.)	-0.5 (-2.4, 1.5)	0.64	1.9 (0.0, 3.8)	0.053
Early-morning SBP (mm Hg)	8.1 (3.7, 12.5)	<0.001	-3.9 (-8.3, 0.6)	0.086
Early-morning DBP (mm Hg)	4.6 (1.9, 7.4)	0.001	-0.3 (-3.1, 2.5)	0.82
Early-morning HR (b.p.m.)	0.7 (-2.2, 3.5)	0.65	3.1 (0.2, 6.0)	0.035
PWA[e]				
Central AIx (%)[f]	0.7 (-0.7, 2.2)	0.32	-0.2 (-1.7, 1.2)	0.75
Central SBP (mm Hg)	8.1 (4.3, 12.0)	<0.001	-3.3 (-7.2, 0.6)	0.10
Central DBP (mm Hg)	3.7 (1.8, 5.6)	<0.001	-0.2 (-2.2, 1.7)	0.81
Central PP (mm Hg)	4.4 (1.6, 7.1)	0.002	-3.1 (-5.9, -0.3)	0.029
PWV (m s^{-1})[e]	0.09 (-0.43, 0.62)	0.73	-0.35 (-0.88, 0.18)	0.19

Abbreviations: AIx, augmentation index; BP, blood pressure; b.p.m., beats per minute; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; PP, pulse pressure; PWA, pulse wave analysis; PWV, pulse wave velocity.
[a] Excluded were two periods of sodium supplementation, three periods of potassium supplementation and one period of placebo supplementation. [b] Data are mean differences (95% CI) obtained from linear mixed-effect models for repeated measurements using the compound symmetry covariance structure. [c] To convert sodium in mmol per 24 h to mg per 24 h multiply by 23. [d] To convert potassium in mmol per 24 h to mg per 24 h multiply by 39. [e] One subject was excluded as no reliable pressure wave could be recorded due to an irregular heartbeat. [f] Adjusted to a standard HR of 75 b.p.m.

SUPPLEMENTAL REFERENCE

1. NEVO-tabel 2011, RIVM/Voedingscentrum, Den Haag, 2011.

CHAPTER 3

Effects of sodium and potassium supplementation on endothelial function: a fully controlled dietary intervention study

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Br J Nutr. 2015;114:1419-1426

ABSTRACT

High Na and low K intakes have adverse effects on blood pressure, which increases the risk for CVD. The role of endothelial dysfunction and inflammation in this pathophysiological process is not yet clear. In a randomised placebo-controlled cross-over study in untreated (pre) hypertensives, we examined the effects of Na and K supplementation on endothelial function and inflammation. During the study period, subjects were provided with a diet that contained 2.4 g/d of Na and 2.3 g/d of K for a 10 460 kJ (2500 kcal) intake. After 1-week run-in, subjects received capsules with supplemental Na (3.0 g/d), supplemental K (2.8 g/d) or placebo, for 4 weeks each, in random order. After each intervention, circulating biomarkers of endothelial function and inflammation were measured. Brachial artery flow-mediated dilation (FMD) and skin microvascular vasomotion were assessed in sub-groups of twenty-two to twenty-four subjects. Of thirty-seven randomised subjects, thirty-six completed the study. Following Na supplementation, serum endothelin-1 was increased by 0.24 pg/ml (95% CI 0.03, 0.45), but no change was seen in other endothelial or inflammatory biomarkers. FMD and microvascular vasomotion were unaffected by Na supplementation. K supplementation reduced IL-8 levels by 0.28 pg/ml (95% CI 0.03, 0.53), without affecting other circulating biomarkers. FMD was 1.16% (95% CI 0.37, 1.96) higher after K supplementation than after placebo. Microvascular vasomotion was unaffected. In conclusion, a 4-week increase in Na intake increased endothelin-1, but had no effect on other endothelial or inflammatory markers. Increased K intake had a beneficial effect on FMD and possibly IL-8, without affecting other circulating endothelial or inflammatory biomarkers.

INTRODUCTION

Excess Na intake and low K intake have been associated with detrimental effects on blood pressure (BP) and CVD risk [1, 2], as recently confirmed by us in thirty-six adults who had a 7.5/2.7 mmHg higher 24 h BP after Na supplementation and 3.9/1.6 mmHg lower 24 h BP after K supplementation [3]. The vascular endothelium has been suggested to play a key role in BP homeostasis [4]. However, limited well-controlled studies have examined the effects of Na and K intake on endothelial function.

Endothelial function can be measured by circulating blood biomarkers that are expressed by activation of the endothelium [5]. For Na intake, randomised controlled trials with an intervention duration of 4 weeks or more mainly focused on the vasoconstrictor endothelin-1 and showed inconsistent results [6-10]. Similar studies on K intake showed no effect on endothelin-1 [11] or on soluble adhesion molecules [12, 13]. Effects of Na and K intake on low-grade inflammation, closely related to endothelial function [14], are largely unknown. High-sensitivity C-reactive protein (CRP) did not respond to the intake of Na [8-10] or K [12, 13] in randomised controlled trials. Flow-mediated dilation (FMD), the dilation of conduit arteries in response to blood flow-induced increases in shear stress, is a functional biomarker of endothelial function [15]. In randomised controlled trials, modest Na reductions ranging from 1.4 to 2.3 g/d for 2–6 weeks improved brachial artery FMD by 1.5–2.4% [6, 8, 16]. FMD has also been shown to improve after increased K intake of 2.5 g/d for 4 weeks [17]. A randomised controlled trial with a lower dose of K (i.e. 1.6 g/d) for 6 weeks demonstrated no effect [12]. Microvascular vasomotion, the periodic oscillations of microvessel diameter, is thought to be partly dependent on endothelial function [18]. Microvascular vasomotion, assessed via spectral analysis of skin laser Doppler flowmetry (LDF) tracing, has not yet been studied in relation to Na and K intakes.

In a double-blind placebo-controlled cross-over study, we performed a comprehensive assessment of Na and K supplementation on endothelial function and low-grade inflammation in subjects with untreated elevated BP. In addition, we assessed microvascular vasomotion as an exploratory secondary outcome.

METHODS

Study Population

The details of this study have been published previously [3]. Potential subjects were recruited from within a 10 km radius of the research centre through subject email databases and advertisements. Non-smoking men and women, aged between 40 and 80 years, with a fasting supine systolic BP (SBP) between 130 and 159 mmHg were eligible to participate. Exclusion

criteria included a history of diabetes mellitus or cardiovascular, gastrointestinal, liver or renal diseases based on questionnaire data and laboratory parameters; BMI > 40 kg/m^2; use of medication known to affect the cardiovascular system; use of nutritional supplements; an energy-restricted or medically prescribed diet; unstable body weight in the past 2 months; alcohol use over 21 units for women and 28 units for men/week (1 unit equalling 10–15 g of ethanol); and pregnant or lactating women.

Of the thirty-nine subjects who started the 1-week run-in, thirty-seven were randomised. One randomised subject dropped out because of gastrointestinal complaints due to the capsules, leaving thirty-six subjects who completed the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving human subjects were approved by the Medical Ethics Committee of Wageningen University. Written informed consent was obtained from all the subjects. The study was registered at ClinicalTrials.gov (registration no. NCT01575041).

Study Design

The study was a randomised, double-blind, placebo-controlled cross-over trial in which diet was fully controlled, as described previously [3]. In brief, during the 1-week run-in and three consecutive intervention periods of 4 weeks, not separated by washout, subjects were provided with a relatively low-Na, low-K diet that contained 2.4 g/d of Na and 2.3 g/d of K for a 10 460 kJ (2500 kcal) intake. At the end of the run-in (baseline), subjects were randomly allocated to one of the six possible treatment orders, based on sex and SBP (130–139 and ≥ 140 mmHg). Treatments were daily consumption of eight sodium chloride capsules (Na: 3.0 g), eight potassium chloride capsules (K: 2.8 g) and eight placebo (cellulose) capsules (Microz), for 4 weeks each, in random order. Body weight was kept constant during the study period through adjustments in energy intake, and subjects were asked to maintain their usual level of physical activity.

At baseline and at the end of each intervention period, subjects underwent 24 h ambulatory BP monitoring and collected 24 h urine by discarding the first morning urine and collecting all urine secretions for the next 24 h. Subjects also underwent anthropometric measurements, blood sampling, and office BP, FMD and microvascular vasomotion assessment. The measurements were taken following an overnight fast (from 20.00 hours) at fixed time points in the morning, in a temperature-controlled (20–24°C) quiet room at the research centre.

Measurements

Biochemical analysis

Fasting blood samples collected in EDTA- and sodium citrate-containing tubes were centrifuged at 1550 g for 15 min at 4 and 20°C, respectively. Blood samples collected in

heparin-coated tubes were centrifuged at 800 g for 20 min at 4°C. Aliquots were stored at −80°C until the end of the study for analysis. Concentrations of soluble E-selectin, soluble thrombomodulin, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), CRP, serum amyloid A, TNF-α, monocyte chemoattractant protein-1, IL-1β, IL-6 and IL-8 were measured in EDTA plasma using a multi-array detection system (SECTOR Imager 2400; Meso Scale Discovery) in the laboratory of the Maastricht University Medical Center, as previously described [19]. Endothelin-1 and von Willebrand factor (vWf) were determined by ELISA in the laboratory of the Maastricht University Medical Center from EDTA plasma and citrate plasma, respectively. Levels of vWf were expressed as a percentage of vWf detected in pooled citrated plasma of healthy volunteers [19]. Heparin plasma levels of nitric oxide (NO) were determined at the RIKILT Institute of Food Safety by estimating NO by the chemiluminescence formed after the release of NO from NO_2, NO_2^- and nitrosated and nitrosylated species, as described previously [20]. Intra-assay CV were <10% for all biomarker measurements, except for IL-1β (10.4%). Inter-assay CV were <10% for all biomarker measurements, except for sVCAM-1 (12.8%), IL-1β (12.4%) and NO (18.6%). Na and K levels were determined in 24 h urine samples in a certified laboratory using ion-selective electrodes module on the Modular P of Roche.

Flow-mediated dilation and blood pressure

FMD was measured by a trained staff member according to established guidelines [21]. After rest of at least 20 min in the supine position, longitudinal ultrasonographic images of the right brachial artery were continuously recorded. Baseline arterial diameter was recorded for 3 min, after which a pressure cuff on the forearm was inflated to 200 mmHg for 5 min to induce reactive hyperaemia. After cuff release, images were recorded for 5 min for the determination of the maximum arterial diameter. Images were processed automatically using custom-written software (DuplexFMD, Department of Biomedical Engineering, Maastricht University). FMD was calculated as the percentage change in arterial diameter from baseline to the maximum value after cuff release. Endothelium-independent dilation (EID) was assessed as the maximum change in arterial diameter over a 5 min period following sublingual administration of nitroglycerin (400 μg). Before data analysis, recordings of insufficient quality due to movements of subjects or unclear images of the arterial wall were excluded, leaving twenty-two subjects for the analysis of FMD for Na v. placebo supplementation and twenty-four subjects for K v. placebo supplementation.

Office brachial BP was measured in the supine position after at least 10 min of rest using an automated oscillometric device (Dinamap Pro 100). Ambulatory BP monitoring was performed for 24 h using Spacelabs 90217 devices (Spacelabs Medical Inc.), as described previously [3].

Microvascular vasomotion

Skin blood flow was measured using an LDF system (PeriFlux 5000; Perimed) with a laser Doppler probe (PF 457; Perimed) at approximately 2 cm distal to the wrist on the back of the left hand. Subjects were in the supine position, and after a rest of at least 20 min skin temperature was set at 30°C and skin blood flow was recorded. Spectral analysis was performed using Fast-Fourier transform analysis of skin LDF (Perisoft for Windows version 2.5; Perimed) to determine the power spectral density of the LDF signal. The power density was calculated in the total frequency spectrum of 0.01–1.60 Hz and in five frequency sub-intervals to determine the contribution of oscillations of endothelial (0.01–0.02 Hz), neurogenic (0.02–0.06 Hz), myogenic (0.06–0.15 Hz), respiratory (0.15–0.40 Hz) and heart beat origin (0.40–1.60 Hz) to microvascular vasomotion [22]. Data were expressed as arbitrary perfusion units. Recordings of insufficient quality due to movements of subjects were excluded before data analysis, leaving twenty-three subjects for the analysis of Na v. placebo supplementation and twenty-three subjects for K v. placebo supplementation.

Statistical Analysis

Data were analysed according to the intention-to-treat principle, using a predefined statistical analysis plan. FMD was defined as the primary outcome. The present study had 80% power to detect a FMD difference of 1.0% for a SD of 1.7% and a two-sided α of 0.05. Circulating biomarkers with a skewed distribution were transformed taking the natural logarithm. After analyzing biomarkers individually, overall Z scores were created for a set of biomarkers of endothelial function and of low-grade inflammation (Supplemental Methods) [19]. For each outcome measure, a mixed-effects model with covariance structure compound symmetry was used to estimate the effect of the active treatment compared with placebo. 'Treatment' and 'period' were included as fixed effects and 'subject' as the random effect. In the sensitivity analysis, analyses were repeated after the exclusion of intervention periods in which subjects were non-compliant. Values reported in text and tables are expressed as mean with standard deviation, median with interquartile range for skewed variables or treatment effect with 95% CI. Two-sided P values < 0.05 were considered statistically significant. Analyses were performed using SAS software version 9.2 (SAS Institute).

RESULTS

Subjects and Compliance

Baseline characteristics of the twenty-four men and twelve women who completed the study are reported in Table 1. Subjects were on average 65.8 years old and their BMI was 27.2 kg/m2. During screening, their 24 h urinary excretion was 153.7 (SD 63.6) mmol for Na and 81.8 (SD 25.6) mmol for K. This decreased to 90.8 (SD 26.6) mmol and 49.0 (SD 13.4 mmol),

TABLE 1. Baseline characteristics of the 36 subjects who completed the study

	Total (n=36)	Men (n=24)	Women (n=12)
Age, y	65.8 ± 8.8	66.0 ± 9.3	65.4 ± 8.2
Height, cm	175.5± 9.3	178.8 ± 8.9	168.8 ± 5.8
Weight, kg	84.3 ± 18.5	87.9 ± 19.0	77.2 ± 16.0
Body mass index, kg/m^2	27.2 ± 4.7	27.3 ± 4.8	27.0 ± 4.6
Waist circumference, cm	99.9 ± 14.8	103.0 ± 15.0	93.8 ± 12.9
Pre-run-in sodium excretion, mmol/24h	153.7 ± 63.6	160.2 ± 71.4	140.8 ± 44.1
Pre-run-in potassium excretion, mmol/24h	81.8 ± 25.6	82.2 ± 28.7	81.0 ± 19.3
Post-run-in sodium excretion, mmol/24h	90.8 ± 26.6	98.3 ± 26.3	75.9 ± 20.8
Post-run-in potassium excretion, mmol/24h	49.0 ± 13.4	50.5 ± 13.6	46.0 ± 13.0
Pre-run-in SBP, mmHg	145.3 ± 11.2	147.7 ± 10.3	140.4 ± 11.7
Pre-run-in DBP, mmHg	80.6 ± 8.0	82.8 ± 7.7	76.2 ± 7.0
Post-run-in SBP, mmHg	133.4 ± 14.7	136.4 ± 14.7	127.5 ± 13.3
Post-run-in DBP, mmHg	75.7 ± 8.3	77.8 ± 8.0	71.4 ± 7.6

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.
Values are mean ± SD.

respectively, after 1-week run-in. Subjects had a mean office SBP/diastolic BP of 145.3/80.6 mmHg, and 69% of them (25/36) had an SBP ≥ 140 mmHg during screening. BP was 133.4/75.7 mmHg after the 1-week run-in period on the low-Na, low-K diet. Baseline characteristics of the subjects with FMD or vasomotion data did not essentially differ from the overall study population (Supplemental Tables 1 and 2, respectively). Based on returned capsules and diary entries, 86% (31/36) of the subjects were compliant, ingesting over 80% of the capsules during each intervention period.

Sodium Supplementation

Na supplementation increased urinary Na excretion by 97.6 mmol/24 h (95% CI 81.0, 114.1; P < 0.001) and 24 h SBP by 7.5 mmHg (95% CI 4.4, 10.5; P < 0.001) compared with placebo (Table 2). After Na supplementation, endothelin-1 levels were 0.24 pg/ml (95% CI 0.03, 0.45; P = 0.023) higher compared with placebo. Other individual biomarkers of endothelial function and the aggregate Z score of endothelial function (0.080; 95% CI −0.029, 0.189; P = 0.15) did not change. In addition, markers of low-grade inflammation and the aggregate Z score (−0.047; 95% CI −0.189, 0.094; P = 0.51) were unaffected. Differences in FMD are depicted in Figure 1, with, on average, no effect of Na supplementation on FMD (0.06%; 95% CI −0.75, 0.86; P = 0.89). Moreover, EID (−0.01%; 95% CI −1.77, 1.75; P = 0.99) was unaffected. No effects of Na supplementation were seen on the power densities of the different frequency intervals determining microvascular vasomotion (Table 2).

TABLE 2. Effects of 4–week supplementation with sodium (3 g/d) or placebo on urinary, clinical and blood parameters, flow–mediated dilation and microvascular vasomotion in untreated pre–hypertensive and hypertensive adults

	Values after 4 weeks of intervention[a]		Treatment effect[b]	
	Sodium	Placebo	Sodium vs placebo	P
Urinary parameters				
Sodium, mmol/24h	202.9 ± 54.8	105.1 ± 39.7	97.6 (81.0, 114.1)	<0.001
Potassium, mmol/24h	53.2 ± 16.6	55.3 ± 16.7	-2.2 (-10.2, 5.7)	0.58
Weight, kg	82.5 ± 18.3	82.5 ± 18.3	-0.1 (-0.7, 0.5)	0.71
Office BP and HR				
SBP, mmHg	132.9 ± 17.6	125.1 ± 15.0	7.5 (3.8, 11.1)	<0.001
DBP, mmHg	75.7 ± 7.5	72.3 ± 7.7	3.3 (1.5, 5.2)	<0.001
HR, bpm	58.5 ± 8.3	59.2 ± 8.0	-0.7 (-2.5, 1.0)	0.42
Blood parameters[c]				
Nitric oxide, nmol/L	62.4 (54.0-83.6)	59.9 (48.1-86.0)		
Ln nitric oxide, nmol/L	4.20 ± 0.38	4.17 ± 0.49	0.03 (-0.09, 0.15)	0.62
Endothelin-1, pg/ml	2.47 ± 0.73	2.22 ± 0.63	0.24 (0.03, 0.45)	0.023
Soluble E-selectin, ng/ml	10.6 ± 4.9	10.6 ± 4.5	0.05 (-0.77, 0.87)	0.90
Soluble thrombomodulin, ng/ml	3.89 ± 0.71	3.88 ± 0.77	0.01 (-0.08, 0.10)	0.85
von Willebrand factor,%[d]	136.0 ± 54.3	129.8 ± 34.6	5.7 (-5.8, 17.3)	0.33
Soluble vascular cellular adhesion molecule-1, ng/ml	367.8 ± 78.5	366.2 ± 73.7	1.5 (-11.4, 14.4)	0.82
Soluble intercellular adhesion molecule-1, ng/ml	227.2 ± 45.9	232.7 ± 45.0	-5.2 (-13.3, 3.0)	0.21
Tumor necrosis factor-α, pg/ml	9.22 ± 2.61	9.09 ± 2.10	0.15 (-0.27, 0.58)	0.48
C-reactive protein, µg/ml	1.29 (0.61-2.34)	1.43 (0.76-2.88)		
Ln C-reactive protein, µg/ml	0.23 ± 1.04	0.33 ± 1.01	-0.10 (-0.33, 0.14)	0.43
Serum Amyloid A, µg/ml	1.46 (0.94-2.76)	2.15 (1.00-3.70)		
Ln serum Amyloid A, µg/ml	0.44 ± 0.80	0.61 ± 0.81	-0.17 (-0.35, 0.01)	0.064

Monocyte chemoattractant protein-1, pg/ml	207.0 (192.0-236.3)	219.3 (195.8-243.2)		
Ln monocyte chemoattractant protein-1, pg/ml	5.39 ± 0.21	5.42 ± 0.23	-0.03 (-0.07, 0.01)	0.099
Interleukin-1β, pg/ml	0.41 (0.18-0.55)	0.45 (0.20-0.62)		
Ln interleukin-1β, pg/ml	-1.18 ± 1.01	-1.15 ± 1.01	-0.04 (-0.13, 0.05)	0.41
Interleukin-6, pg/ml	1.42 (1.09-2.00)	1.31 (1.07-1.94)		
Ln interleukin-6, pg/ml	0.46 ± 0.72	0.45 ± 0.65	0.02 (-0.16, 0.20)	0.81
Interleukin-8, pg/ml[d]	3.30 ± 0.99	3.55 ± 1.14	-0.25 (-0.50, 0.00)	0.052
Z-score endothelial function[f]	0.083 ± 0.692	0.000 ± 0.640	0.080 (-0.029, 0.189)	0.15
Z-score low-grade inflammation[g]	-0.051 ± 0.563	0.000 ± 0.546	-0.047 (-0.189, 0.094)	0.51
Flow-mediated dilation[h]				
Baseline brachial artery diameter, mm	4.59 ± 0.90	4.56 ± 0.78	0.03 (-0.11, 0.17)	0.64
Post-release brachial artery diameter, mm	4.71 ± 0.90	4.69 ± 0.78	0.04 (-0.11, 0.18)	0.61
Flow-mediated dilation, mm	0.13 ± 0.08	0.13 ± 0.09	0.00 (-0.03, 0.04)	0.80
Flow-mediated dilation,%	2.94 ± 1.88	3.01 ± 2.15	0.06 (-0.75, 0.86)	0.89
Vasomotion - Power density, AU[i]				
Endothelial frequency interval	2.22 ± 1.25	2.46 ± 1.09	-0.21 (-0.83, 0.40)	0.49
Neurogenic frequency interval	3.76 ± 1.96	3.83 ± 1.63	-0.05 (-1.03, 0.93)	0.92
Myogenic frequency interval	4.10 ± 2.42	3.62 ± 2.41	0.57 (-0.65, 1.78)	0.35
Respiratory frequency interval[j]	6.04 ± 3.34	6.55 ± 6.04	-0.26 (-2.59, 2.07)	0.82
Heart beat frequency interval	23.5 ± 14.9	23.3 ± 13.8	0.09 (-6.94, 7.13)	0.98
Total	39.6 ± 20.9	39.7 ± 21.4	0.06 (-10.77, 10.88)	0.99

Abbreviations: BP, blood pressure; AU, arbitrary units. [a] Unadjusted mean values and standard deviations; medians and interquartile ranges; mean differences and 95% confidence intervals). [b] Data are obtained from linear mixed models for repeated measurements using the compound symmetry covariance structure. [c] Based on thirty-six subjects, for difference in urinary Na excretion of 97.6 mmol/24 h (95% CI 81.0, 114.1; P < 0.001). [d] Treatment effect after excluding one outlying value of 366% during Na supplementation was −1.1% (95% CI −6.8, 4.5; P = 0.70). [e] Treatment effect after excluding one outlying value of 8.25 pg/ml during placebo supplementation was −0.13 pg/ml (95% CI −0.33, 0.07; P = 0.19). [f] Included were endothelin-1, soluble E-selectin, soluble thrombomodulin, von Willebrand factor, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1. [g] Included were soluble intercellular adhesion molecule-1, C-reactive protein, serum amyloid A, TNF-α, monocyte chemoattractant protein-1, IL-1β, IL-6 and IL-8. [h] Based on twenty-two subjects, for difference in urinary Na excretion of 99.6 mmol/24 h (95% CI 78.6, 120.6; P < 0.001). [i] Based on twenty-three subjects, for difference in urinary Na excretion of 102.7 mmol/24 h (95% CI 81.4, 124.0; P < 0.001). [j] Treatment effect after excluding one outlying value of 30.48 AU during placebo supplementation was 0.67 AU (95% CI −1.25, 2.59; P = 0.49).

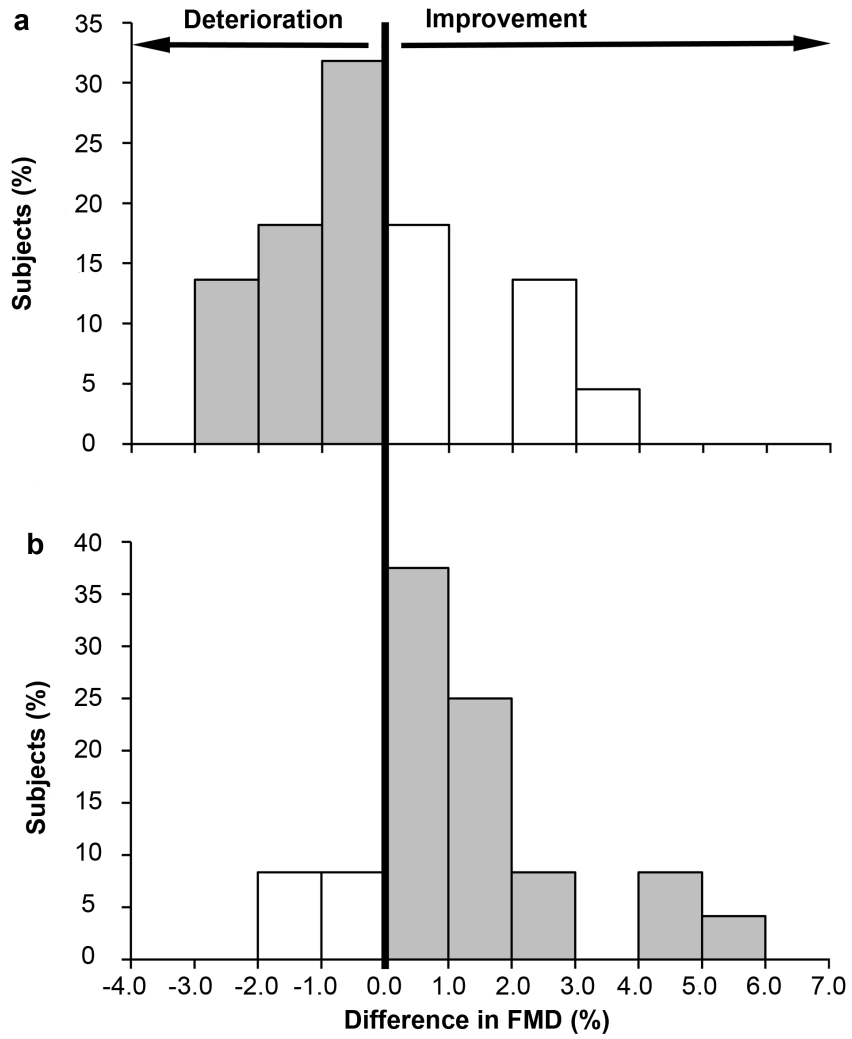

FIGURE 1. Effects of 4-week sodium (a) and potassium (b) supplementation on flow-mediated dilation (FMD) in untreated pre-hypertensive and hypertensive adults, compared with placebo.

Potassium Supplementation

K supplementation increased urinary K excretion by 62.9 mmol/24 h (95% CI 54.9, 70.8; P < 0.001) and decreased 24 h SBP by 3.9 mmHg (95% CI 0.9, 6.9; P = 0.013), compared with placebo (Table 3). K supplementation resulted in a lower IL-8 of 0.28 pg/ml (95% CI 0.03, 0.53; P = 0.031). In the sensitivity analysis, after the exclusion of intervention periods in which subjects were non-compliant, the effect on IL-8 was no longer significant (−0.24 pg/ml; 95% CI −0.50, 0.03, P = 0.080). K supplementation had no effect on other individual biomarkers,

on the Z score of endothelial function (0.007; 95% CI −0.102, 0.115; P = 0.90) or low-grade inflammation (−0.032; 95% CI −0.173, 0.109; P = 0.66). However, in the sensitivity analysis, K supplementation increased vWf by 12.5% (95% CI 1.0, 23.9, P = 0.033) compared with placebo. FMD was 1.16% (95% CI 0.37, 1.96; P = 0.005) higher after K supplementation compared with placebo, with FMD being improved in 83% (20/24) of the subjects after K supplementation compared with placebo (Figure 1). K supplementation had no effect on EID (0.51%; 95% CI −1.17, 2.19; P = 0.54). The power densities of the frequency intervals related to activities determining vasomotion were unaffected (Table 3).

DISCUSSION

In untreated pre-hypertensive and hypertensive adults on a fully controlled diet that was relatively low in Na and K, 4 weeks of Na supplementation had no effect on endothelial function or low-grade inflammation, except for an increase in endothelin-1. K supplementation lowered the inflammatory marker IL-8 and increased the functional biomarker FMD.

A major strength of the present study is the comprehensive assessment of endothelial function and low-grade inflammation by an extensive set of circulating biomarkers, the functional biomarker FMD and microvascular vasomotion. All measurements were performed in a fasting state at fixed times of the day using a strict protocol. We used high-sensitivity assay techniques, and eleven circulating biomarkers were assessed simultaneously to minimise between-assay variation. Our study had limited variability in diet and lifestyle behaviours due to the provision of a fully controlled diet and the instruction to keep other lifestyle factors such as physical activity constant. In addition, a large contrast in Na and K intake was achieved, the compliance was high and the drop-out rate was low. Moreover, although the number of subjects in this study was limited, the study had ample power (80%) to show a clinically relevant effect on FMD of 1.0% [23, 24].

Our study showed that for an increase in Na intake of 2.2 g/d (based on urinary excretions), the potent vasoconstrictor and pro-inflammatory peptide endothelin-1 increased by 0.24 pg/ml. Other circulating biomarkers of endothelial function and low-grade inflammation were unaffected. These findings are in line with the results of a randomised controlled trial, in which lowering Na intake moderately for 6 weeks resulted in a decrease in endothelin-1 and no changes in ICAM-1, VCAM-1 and E-selectin [6]. Other randomised controlled trials also indicated lower levels of endothelin-1 at lower Na intakes, but findings were not significant [7, 9]. In contrast, in seventeen adults with moderately elevated BP, endothelin-1 levels were higher after 4 weeks of Na restriction than after normal Na intake (6.3 v. 5.9 pg/ml); however, this was not statistically significant [8]. Although not as comprehensively assessed as in our study, other randomised controlled trials with a 4-week intervention also observed no significant effects of Na intake on inflammatory markers [8–10].

TABLE 3. Effects of 4-week supplementation with potassium (3 g/d) or placebo on urinary, clinical and blood parameters, flow-mediated dilation and microvascular vasomotion in untreated pre-hypertensive and hypertensive adults

	Values after 4 weeks of intervention[a]		Treatment effect[b]	
	Potassium	Placebo	Potassium vs placebo	P
Urinary parameters				
Sodium, mmol/24h	96.5 ± 39.0	105.1 ± 39.7	-8.9 (-25.4, 7.6)	0.29
Potassium, mmol/24h	118.1 ± 32.2	55.3 ± 16.7	62.9 (54.9, 70.8)	<0.001
Weight, kg	82.3 ± 18.2	82.5 ± 18.3	-0.3 (-0.9, 0.2)	0.28
Office BP and HR				
SBP, mmHg	122.2 ± 15.3	125.1 ± 15.0	-3.0 (-6.7, 0.6)	0.10
DBP, mmHg	72.0 ± 8.2	72.3 ± 7.7	-0.3 (-2.1, 1.6)	0.77
HR, bpm	59.8 ± 8.0	59.2 ± 8.0	0.6 (-1.1, 2.4)	0.47
Blood parameters[c]				
Nitric oxide, nmol/L	59.7 (50.8-81.2)	59.9 (48.1-86.0)		
Ln nitric oxide, nmol/L	4.15 ± 0.37	4.17 ± 0.49	-0.02 (-0.14, 0.10)	0.70
Endothelin-1, pg/ml	2.14 ± 0.61	2.22 ± 0.63	-0.09 (-0.29, 0.12)	0.42
Soluble E-selectin, ng/ml	10.5 ± 4.3	10.6 ± 4.5	-0.13 (-0.94, 0.69)	0.76
Soluble thrombomodulin, ng/ml	3.92 ± 0.77	3.88 ± 0.77	0.03 (-0.06, 0.12)	0.47
von Willebrand factor,%[d]	138.0 ± 44.1	129.8 ± 34.6	8.1 (-3.4, 19.6)	0.17
Soluble vascular cellular adhesion molecule-1, ng/ml	363.0 ± 73.6	366.2 ± 73.7	-2.9 (-15.8, 10.0)	0.66
Soluble intercellular adhesion molecule-1, ng/ml	231.0 ± 47.9	232.7 ± 45.0	-1.6 (-9.7, 6.6)	0.71
Tumor necrosis factor-α, pg/ml	9.40 ± 2.84	9.09 ± 2.10	0.31 (-0.11, 0.74)	0.15
C-reactive protein, µg/ml	1.73 (0.53-2.58)	1.43 (0.76-2.88)		
Ln C-reactive protein, µg/ml	0.33 ± 1.09	0.33 ± 1.01	0.00 (-0.24, 0.24)	0.99
Serum Amyloid A, µg/ml	1.92 (1.09-2.73)	2.15 (1.00-3.70)		
Ln serum Amyloid A, µg/ml	0.61 ± 0.75	0.61 ± 0.81	0.01 (-0.17, 0.19)	0.94

Monocyte chemoattractant protein-1, pg/ml	211.4 (192.9-233.1)	219.3 (195.8-243.2)		
Ln monocyte chemoattractant protein-1, pg/ml	5.39 ± 0.22	5.42 ± 0.23	-0.02 (-0.06, 0.01)	0.21
Interleukin-1β, pg/ml	0.40 (0.23-0.62)	0.45 (0.20-0.62)		
Ln interleukin-1β, pg/ml	-1.13 ± 1.01	-1.15 ± 1.01	0.01 (-0.08, 0.11)	0.80
Interleukin-6, pg/ml	1.40 (1.02-2.02)	1.31 (1.07-1.94)		
Ln interleukin-6, pg/ml	0.42 ± 0.54	0.45 ± 0.65	-0.03 (-0.21, 0.15)	0.74
Interleukin-8, pg/ml[d]	3.27 ± 0.78	3.55 ± 1.14	-0.28 (-0.53, -0.03)	0.031
Z-score endothelial function[f]	0.008 ± 0.669	0.000 ± 0.640	0.007 (-0.102, 0.115)	0.90
Z-score low-grade inflammation[g]	-0.033 ± 0.524	0.000 ± 0.546	-0.032 (-0.173, 0.109)	0.66
Flow-mediated dilation[h]				
Baseline brachial artery diameter, mm	4.54 ± 0.76	4.60 ± 0.80	-0.06 (-0.19, 0.08)	0.41
Post-release brachial artery diameter, mm	4.72 ± 0.78	4.73 ± 0.79	0.00 (-0.15, 0.14)	0.98
Flow-mediated dilation, mm	0.18 ± 0.09	0.12 ± 0.09	0.05 (0.02, 0.09)	0.006
Flow-mediated dilation,%	4.04 ± 1.82	2.85 ± 2.13	1.16 (0.37, 1.96)	0.005
Vasomotion - Power density, AU[i]				
Endothelial frequency interval	2.28 ± 0.95	2.39 ± 1.04	-0.18 (-0.80, 0.43)	0.55
Neurogenic frequency interval	3.80 ± 1.58	3.83 ± 1.63	-0.06 (-1.04, 0.91)	0.90
Myogenic frequency interval	3.52 ± 1.86	3.64 ± 2.40	-0.07 (-1.28, 1.14)	0.90
Respiratory frequency interval[j]	5.92 ± 3.50	6.38 ± 6.03	-0.59 (-2.91, 1.74)	0.62
Heart beat frequency interval	22.1 ± 9.8	23.6 ± 14.0	-1.60 (-8.62, 5.43)	0.65
Total	37.6 ± 13.3	39.8 ± 21.4	-2.78 (-13.58, 8.03)	0.61

Abbreviations: BP, blood pressure; AU, arbitrary units. [a] Unadjusted mean values and standard deviations; medians and interquartile ranges; mean differences and 95% confidence intervals). [b] Data are obtained from linear mixed models for repeated measurements using the compound symmetry covariance structure. [c] Based on thirty-six subjects, for difference in urinary K excretion of 62.9 mmol/24 h (95% CI 54.9, 70.8; P < 0.001). [d] Treatment effect after excluding one outlying value of 366% during Na supplementation was 8.3% (95% CI 2.7, 13.9; P = 0.004). [e] Treatment effect after excluding one outlying value of 8.25 pg/ml during placebo supplementation was −0.17 pg/ml (95% CI −0.37, 0.03; P = 0.10). [f] Included were endothelin-1, soluble E-selectin, soluble thrombomodulin, von Willebrand factor, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1. [g] Included were soluble intercellular adhesion molecule-1, C-reactive protein, serum amyloid A, TNF-α, monocyte chemoattractant protein-1, IL-1β, IL-6 and IL-8. [h] Based on twenty-four subjects, for difference in urinary K excretion of 67.7 mmol/24 h (95% CI 57.5, 78.0; P < 0.001). [i] Based on twenty-three subjects, for difference in urinary K excretion of 65.0 mmol/24 h (95% CI 54.4, 75.6; P < 0.001). [j] Treatment effect after excluding one outlying value of 30.48 AU during placebo supplementation was 0.43 AU (95% CI −1.49, 2.35; P = 0.66).

We found no effect of Na supplementation on FMD. Other cross-over studies demonstrated improvements in FMD of 1.5–2.4% after Na reductions for 2–6 weeks [6, 8, 16]. In these studies, in contrast to our study, K intake was not reduced. Moreover, our subjects had, on average, a high baseline brachial artery diameter and a low FMD compared with these studies. We excluded FMD recordings of low quality, but baseline characteristics of the subjects included in the FMD analysis were similar to all thirty-six subjects and randomisation was maintained.

In our study, doubling the intake of K from 2.2 to 4.6 g/d had no effect on circulating biomarkers of endothelial function, whereas the inflammatory marker IL-8 was reduced. Other randomised controlled trials with durations of 4 weeks or more also observed no effect of increased K intake on endothelial biomarkers [11–13]. To our knowledge, in randomised controlled trials, the effects of supplemental K on inflammatory biomarkers have only been investigated by measuring high-sensitivity CRP, which, in line with our study, was not affected [12, 13]. As other cytokines did not change, we cannot exclude the possibility that our findings for IL-8 were per chance because of the large number of outcomes that we examined. Moreover, in the sensitivity analysis, the effect on IL-8 was no longer significant after the exclusion of intervention periods in which subjects were non-compliant.

The functional biomarker FMD was improved by 1.16% following K supplementation, which may contribute to cardiovascular risk reduction. In the meta-analyses, each 1% increase in FMD was associated with a 8–13% lower risk of cardiovascular events [23, 24]. A randomised controlled trial investigating the effects of increased K intake using potassium chloride supplements revealed in forty-two untreated hypertensives a significant increase of 2.7% in FMD for an increased urinary K excretion of 45 mmol/24 h [17]. This study also showed a 1.5% increase in FMD after 4-week supplemental potassium bicarbonate. In contrast, increasing K intake for 6 weeks through potassium citrate supplements and fruit and vegetables with a maximum increase in urinary K of 27 mmol/24 h resulted in no effect on FMD in forty-eight early hypertensives [12]. Possibly, a minimum dose of K is required to improve FMD. Skin microvascular vasomotion, which is thought to be partly dependent on endothelial function, was unaffected by K supplementation. It is uncertain to what extent endothelial function of the microvessel is similar to that of macrovessels (e.g. brachial artery). Moreover, our data on microvascular vasomotion should be considered exploratory, and need confirmation by others using similar methods.

In the present cross-over study, we have previously shown that 24 h BP was 7.5/2.7 mmHg higher after Na supplementation and 3.9/1.6 mmHg lower after K supplementation [3]. The results of the present investigation suggest that supplemental Na for 4 weeks can increase BP without altering endothelial function. The endothelium may play a role in the BP effects of K. However, we saw no effect of K intake on the circulating biomarkers of endothelial function, while FMD was affected. Endothelial function is a complex process involving a number of

factors and it needs to be determined which factors are directly related to K intake-induced changes in FMD. Furthermore, we cannot conclude whether the improvement in endothelial function preceded or followed BP reduction, or whether these changes are independent.

In conclusion, a 4-week increase in Na intake had, besides an increase in endothelin-1, no effect on endothelial function and low-grade inflammation in subjects with untreated elevated BP. Increasing K intake improved endothelial function as assessed by FMD, but did not affect other indicators of endothelial function or low-grade inflammation. This suggests that K intake may have protective effects on endothelial function. Other studies replicating these findings and further studies about the mechanisms underlying the effect of K intake on endothelial function are warranted.

Acknowledgements
The authors gratefully acknowledge the assistance of Harrie Robins, Sarah Mount and Danielle Schoenaker for conducting the study. The authors also thank Jos op 't Roodt, Peter Joris and Diederik Esser for the training in the performance and analysis of FMD measurements, and Marjo van de Waarenburg and Dini Venema for performing the biochemical assays.

Funding
The research is funded by TI Food and Nutrition, a public–private partnership on pre-competitive research in food and nutrition. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Disclosures
There are no conflicts of interest to declare.

REFERENCES

1. Aburto NJ, Hanson S, Gutierrez H, et al. (2013) Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ 346, f1378.
2. He FJ, Li J & Macgregor GA (2013) Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ 346, f1325.
3. Gijsbers L, Dower JI, Mensink M, et al. (2015) Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens (Epublication ahead of print version 12 February 2015).
4. Bautista LE (2003) Inflammation, endothelial dysfunction, and the risk of high blood pressure: epidemiologic and biological evidence. J Hum Hypertens 17, 223–230.
5. Deanfield JE, Halcox JP & Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. Circulation 115, 1285–1295.
6. Dickinson KM, Clifton PM & Keogh JB (2014) A reduction of 3 g/day from a usual 9 g/day salt diet improves endothelial function and decreases endothelin-1 in a randomised cross-over study in normotensive overweight and obese subjects. Atherosclerosis 233, 32–38.
7. Gates PE, Tanaka H, Hiatt WR, et al. (2004) Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. Hypertension 44, 35–41.
8. Jablonski KL, Racine ML, Geolfos CJ, et al. (2013) Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J Am Coll Cardiol 61, 335–343.
9. Todd AS, Macginley RJ, Schollum JB, et al. (2010) Dietary salt loading impairs arterial vascular reactivity. Am J Clin Nutr 91, 557–564.
10. Todd AS, Macginley RJ, Schollum JBW, et al. (2012) Dietary sodium loading in normotensive healthy volunteers does not increase arterial vascular reactivity or blood pressure. Nephrology (Carlton) 17, 249–256.
11. Matthesen SK, Larsen T, Vase H, et al. (2012) Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest 72, 78–86.
12. Berry SE, Mulla UZ, Chowienczyk PJ, et al. (2010) Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr 104, 1839–1847.
13. Graham UM, McCance DR, Young IS, et al. (2014) A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens 28, 333–339.
14. Zhang C (2008) The role of inflammatory cytokines in endothelial dysfunction. Basic Res Cardiol 103, 398–406.
15. Al-Qaisi M, Kharbanda RK, Mittal TK, et al. (2008) Measurement of endothelial function and its clinical utility for cardiovascular risk. Vasc Health Risk Manag 4, 647–652.

16. Dickinson KM, Keogh JB & Clifton PM (2009) Effects of a low-salt diet on flow-mediated dilatation in humans. Am J Clin Nutr 89, 485–490.
17. He FJ, Marciniak M, Carney C, et al. (2010) Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension 55, 681–688.
18. Rossi M, Carpi A, Galetta F, et al. (2008) Skin vasomotion investigation: a useful tool for clinical evaluation of microvascular endothelial function? Biomed Pharmacother 62, 541–545.
19. van Bussel BCT, Henry RMA, Schalkwijk CG, et al. (2011) Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation during a 6-year follow-up. J Nutr 141, 1719–1725.
20. Appeldoorn MM, Venema DP, Peters THF, et al. (2009) Some phenolic compounds increase the nitric oxide level in endothelial cells in vitro. J Agric Food Chem 57, 7693–7699.
21. Corretti MC, Anderson TJ, Benjamin EJ, et al. (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 39, 257–265.
22. Stefanovska A, Bracic M & Kvernmo HD (1999) Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. IEEE Trans Biomed Eng 46, 1230–1239.
23. Inaba Y, Chen JA & Bergmann SR (2010) Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging 26, 631–640.
24. Ras RT, Streppel MT, Draijer R, et al. (2013) Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol 168, 344–351.

SUPPLEMENTAL METHODS

Calculation of Overall Z Scores for Endothelial Function and Low–Grade Inflammation

To reduce the influences of biological variability of each individual measure, we created overall *Z* scores for both endothelial function and low-grade inflammation. These overall *Z* scores were calculated as follows: first, for each individual biomarker a *Z* score was calculated according to the formula: (individual value – study population mean after placebo supplementation) / study population standard deviation after placebo supplementation. The overall *Z* score for either endothelial function or low-grade inflammation was then calculated by averaging the individual *Z* scores [1]. The endothelial function *Z* score consisted of the biomarkers endothelin-1, soluble E-selectin, soluble thrombomodulin, von Willebrand factor, soluble vascular cellular adhesion molecule-1 and soluble intercellular adhesion molecule-1. The low-grade inflammation overall *Z* score consisted of soluble intercellular adhesion molecule-1, C-reactive protein, serum amyloid A, TNF-α, monocyte chemoattractant protein-1, IL-1β, IL-6 and IL-8. Soluble intercellular adhesion molecule-1 was included in both overall *Z* scores, because monocytes and the endothelium express soluble intercellular adhesion molecule-1 [2]. Nitric oxide was not included in the endothelial function *Z* score, because plasma nitric oxide levels may not always represent endothelium-derived nitric oxide [3].

SUPPLEMENTAL RESULTS

SUPPLEMENTAL TABLE 1. Baseline characteristics of subjects in flow–mediated dilation analysis[a]

	Sodium vs placebo (n=22)	Potassium vs placebo (n=24)
Men/women	15/7	17/7
Age (y)	62.8 ± 8.5	63.8 ± 8.8
Height (cm)	177.7 ± 8.2	177.5 ± 7.9
Weight (kg)	88.0 ± 18.9	86.9 ± 18.4
Body mass index (kg/m^2)	27.7 ± 4.8	27.4 ± 4.7
Waist circumference (cm)	101.9 ± 15.9	101.5 ± 15.3
Pre-run-in office SBP (mmHg)	142.9 ± 11.6	143.7 ± 11.6
Pre-run-in office DBP (mmHg)	80.0 ± 7.8	79.8 ± 7.6
Post-run-in office SBP (mmHg)	129.2 ± 15.6	130.8 ± 16.3
Post-run-in office DBP (mmHg)	74.0 ± 7.8	74.3 ± 7.5

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are mean ± SD. [a] Flow-mediated dilation recordings of insufficient quality were excluded, leaving 22 subjects for analysis of flow-mediated dilation for sodium versus placebo supplementation and 24 subjects for potassium versus placebo supplementation.

SUPPLEMENTAL TABLE 2. Baseline characteristics of subjects in vasomotion analysis[a]

	Sodium vs placebo (n=23)	Potassium vs placebo (n=23)
Men/women	16/7	16/7
Age (y)	64.5 ± 9.6	64.0 ± 9.2
Height (cm)	176.6 ± 10.5	177.0 ± 10.6
Weight (kg)	85.6 ± 18.4	86.3 ± 18.2
Body mass index (kg/m^2)	27.2 ± 4.1	27.3 ± 4.1
Waist circumference (cm)	101.2 ± 14.0	101.3 ± 14.0
Pre-run-in office SBP (mmHg)	146.3 ± 11.7	146.2 ± 11.6
Pre-run-in office DBP (mmHg)	80.9 ± 9.0	81.3 ± 8.9
Post-run-in office SBP (mmHg)	133.0 ± 16.5	132.7 ± 16.0
Post-run-in office DBP (mmHg)	75.6 ± 9.0	75.9 ± 9.2

Values are mean ± SD. Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure. [a] Vasomotion recordings of insufficient quality were excluded, leaving 23 subjects for analysis of vasomotion for sodium versus placebo supplementation and 23 subjects for potassium versus placebo supplementation.

SUPPLEMENTAL REFERENCES

1. van Bussel BCT, Henry RMA, Schalkwijk CG et al. (2011) Fish consumption in healthy adults is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation during a 6-year follow-up. J Nutr 141, 1719-1725.
2. Schram MT, Stehouwer CDA (2005) Endothelial dysfunction, cellular adhesion molecules and the metabolic syndrome. Horm Metab Res 37 Suppl 1, 49-55.
3. Rassaf T, Feelisch M, Kelm M (2004) Circulating NO pool: assessment of nitrite and nitroso species in blood and tissues. Free Radic Biol Med 36, 413-422.

CHAPTER 4

Effects of potassium supplementation on markers of osmoregulation and volume regulation: results of a fully controlled dietary intervention study

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J Hypertens. 2016 Feb;34:215-220

ABSTRACT

Objective

Lifestyle measures including dietary sodium restriction and increased potassium intake are recognized to lower blood pressure (BP). Potassium was found to be effective in reducing BP at higher levels of sodium intake, but to have little effect when sodium intake is restricted. The humoral mechanisms underlying these sodium intake dependent effects of potassium are unknown. We investigated the effects of potassium supplementation on top of a fully controlled sodium-restricted diet on markers of osmoregulation and volume regulation.

Methods

In this *post-hoc* analysis, we included 35 (pre)hypertensive individuals participating in a randomized, double-blind, placebo-controlled crossover trial. Individuals received capsules containing sodium [3.0 g (130 mmol)/day], potassium [2.8 g (72 mmol)/day], or placebo for three four-week periods. Linear mixed-effect models were used to estimate the effects of potassium supplementation compared with placebo. Skewed data were ln-transformed before analysis.

Results

Increased potassium intake was associated with a significant decrease in 24-h BP (-3.6/-1.6 mmHg). Furthermore, we found a significant decrease in ln MRproANP {-0.08 [95% confidence interval (95% CI)] (-0.15, -0.01) pmol/l, P = 0.03} and significant increases in 24-h heart rate [2.5 (0.9, 4.0) bpm, P = 0.002], ln plasma copeptin [0.11 (0.01, 0.20) pmol/l, P=0.02], ln renin [0.34 (0.08, 0.60) μIU/ml, P = 0.01], and ln aldosterone [0.14 (0.07, 0.22) nmol/l, P < 0.001] compared with placebo.

Conclusions

We found that potassium has BP-lowering effects during sodium restriction. These BP-lowering effects, however, seem mitigated by several counter regulatory mechanisms (i.e. increased secretion of vasopressin, stimulation of RAAS, and increased heart rate) that were activated to maintain volume homeostasis and counterbalance the decrease in BP.

INTRODUCTION

Given the high prevalence of hypertension and the concomitant increased risks for cardiovascular and renal disease [1, 2], hypertension is an important worldwide public-health challenge [3]. Lifestyle measures including dietary sodium restriction and increased potassium intake are recognized to lower blood pressure (BP) and cardiovascular risk [4–6]. A key aspect in long-term regulation of BP is fluid balance, which is precisely regulated by means of osmoregulation and volume regulation. Any increase in plasma osmolarity, which is mainly determined by the plasma sodium concentration, is normally counterbalanced by an increase in vasopressin to maintain osmotic homeostasis [7]. Multiple effectors are involved in volume regulation including the renin–angiotensin–aldosterone system (RAAS) that stimulates sodium reabsorption [8], natriuretic peptides that promote sodium excretion [9], and vasopressin that stimulates water reabsorption in the kidneys [7, 10].

Previous studies, including meta-analyses of randomized controlled trials, have suggested that the BP-lowering effects of potassium are more pronounced at higher levels of sodium intake [6, 11, 12]. During sodium restriction, potassium intake was found to have little or no effect on BP [13]. To date, it is not known whether a modest BP-lowering effect of potassium supplementation during sodium restriction is biologically plausible. Also, the humoral mechanisms involved in the BP-lowering effects of potassium supplementation have poorly been described. Therefore, our aim was to investigate whether there could be biological plausibility for a modest BP-lowering effect of potassium supplementation during sodium restriction. To this end, we investigated the humoral effects of potassium supplementation during a fully controlled sodium-restricted diet using a panel of markers that are involved in osmoregulation and volume regulation. We additionally investigated the effects of sodium supplementation, with surmised opposite changes in markers of osmoregulation and volume regulation.

MATERIALS AND METHODS

Study Protocol and Individuals

The current study is a post-hoc analysis of a randomized, double-blind, placebo-controlled crossover trial in which individuals were on a fully controlled diet for a period of 13 weeks, as described previously [14]. The study was designed to examine the effects of sodium and potassium supplementation on BP and arterial stiffness in untreated (pre)hypertensive individuals (i.e. individuals with a fasting office SBP between 130 and 159 mmHg) [14]. In brief, at the end of a one-week run-in period (‘baseline’), individuals were randomized to take eight sodium chloride capsules [i.e. 3.0 g (130 mmol) sodium], eight potassium chloride capsules [i.e. 2.8 g (72 mmol) potassium] or eight placebo capsules (cellulose) daily, for four weeks each, while they were provided with the fully controlled diet. The fully controlled diet

provided on average 2.4 g (104 mmol) of sodium, based on the recommended maximum sodium intake of 2.0 – 2.4 g per day (which equals 87 – 104 mmol sodium or 5 – 6 g salt per day) [15, 16], and 2.3 g (59 mmol) of potassium per day for a 2500-kcal intake. Individuals were weighed twice a week and if needed, their energy intake was adjusted to keep body weight constant.

Nonsmoking men and women aged 40 – 80 years, who had a fasting office SBP between 130 and 159 mmHg were eligible for the study. Exclusion criteria were a history of diabetes mellitus, cardiovascular, gastrointestinal, liver, or renal diseases; BMI more than 40 kg/m^2; use of medication known to affect the cardiovascular system; use of nutritional supplements; an energy-restricted or medically prescribed diet; unstable body weight in the preceding two months; alcohol use over 21 (women) or 28 (men) consumptions per week; and pregnant or lactating women. Of the 37 Whites that were included and randomized in the study, 36 completed the study. One individual withdrew because of experiencing gastrointestinal complaints. Given the effects of trauma or severe infection on plasma copeptin [17], one individual was excluded from all analyses because of severe trauma during the placebo study period and one individual was excluded only from analyses on the effects of potassium because of severe infection during the potassium intervention period. Written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of Wageningen University and was in adherence to the Declaration of Helsinki. The trial was registered at ClinicalTrials.gov (registration no. NCT01575041).

Measurements

At baseline and at the end of each four-week intervention, participants collected a 24-h urine sample and underwent 24-h ambulatory BP and heart rate monitoring (Spacelabs 90127 devices; Spacelabs Medical Inc. Redmond, Washington, USA). At the research center, in a fasting state, anthropometrics and BP were measured, and blood was sampled. Individuals rested at least 10 min before office brachial BP was assessed in the supine position with an automated oscillometric device (Dinamap Pro 100; KP Medical, Houten, The Netherlands).
Serum and urinary sodium and potassium concentrations were measured using the ion-selective electrodes module on the Roche Modular P (Roche Diagnostics, Mannheim, Germany); plasma copeptin and MR-proANP using an automated sandwich immunoassay (KRYPTOR; BRAHMS GmbH, Hennigsdorf/Berlin, Germany); plasma NT-proBNP using the Roche Modular E170 (Roche Diagnostics, Mannheim, Germany); and plasma renin and aldosterone concentrations using an automated sandwich immunochemiluminescent assay (LIAISON, Diasorin; DiaSorin Ltd, Schiphol Rijk, The Netherlands). Other serum and urinary parameters were assessed using standard laboratory methods. Serum osmolarity was calculated using the Eq. 1.9 * ([Na] + [K]) + [glucose] + 0.5 * [urea] + 5 [18]. The creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation was used to estimate glomerular filtration rate [19].

Statistical Analysis

Analyses were performed using SAS 9.2 software (SAS Institute, Cary, North Carolina, USA) and SPSS version 22.0 for Windows (IBM Corporation, Chicago, Illinois, USA). Data are reported as mean with standard deviation for variables with a normal distribution or geometric mean with 95% confidence interval for variables with a skewed distribution. Nominal data are presented as the number of individuals with percentage [n (%)]. A two-sided P-value less than 0.05 was considered to indicate statistical significance. To estimate the effects of potassium and sodium supplementation compared with placebo on clinical parameters, we used linear mixed-effect models for repeated measurements, using the compound symmetry covariance structure with 'treatment' and 'period' as fixed effects and 'individual' as random effect. Skewed data were logarithmically transformed before statistical analysis.

RESULTS

Baseline Characteristics

During screening, mean 24-h urinary sodium excretion was 152 mmol (i.e. 3.5 g sodium or 8.7 g of salt), which reduced to 91 mmol after one-week run-in (i.e. baseline). Mean 24-h potassium excretion during screening was 82 mmol (i.e. 3.2 g potassium), which reduced to 49 mmol after run-in. Average office BP was 146/81 mmHg during screening and 134/76 mmHg after the one-week run-in period on controlled diet. Baseline characteristics of the study participants are shown in Table 1.

Effects of Potassium and Sodium Supplementation

The effects of potassium and sodium supplementation on clinical parameters are shown in Table 2. After four weeks of potassium supplementation, 24-h BP and plasma MR-proANP decreased significantly, whereas plasma copeptin, renin, aldosterone, serum urea, and 24-h heart rate increased significantly compared with placebo (Table 2). After four weeks of sodium supplementation, we found significant increases in 24-h BP and plasma concentrations of copeptin and natriuretic peptides (i.e. NT-proBNP and MR-proANP), whereas plasma renin and aldosterone concentrations decreased significantly compared with placebo (Table 2).

The percentage change in several markers for osmoregulation and volume regulation after potassium and sodium supplementation compared with placebo are depicted in Figure 1. Changes in serum osmolarity and serum sodium concentrations were minor (i.e. ranging between -2.0 and +1.7% for serum osmolarity and -2.1 and +2.1% for serum sodium; Figure 1). The percentage change in serum potassium, BP, and heart rate were more pronounced and interindividual variability was higher, but all percentage changes remained within the range of -20 to +25% (Figure 1). The changes in plasma copeptin, NT-proBNP, MR-proANP, renin, and aldosterone were much more pronounced, with distinct interindividual differences (Figure 1).

TABLE 1. Baseline characteristics of the study participants

	All participants[a] (n=35)
Demographics	
Male sex [n, (%)]	23 (66)
Age (years)	66 ± 9
Clinical measurements	
BMI (kg/m^2)	27.4 ± 4.7
Body weight (kg)	84.9 ± 18.5
SBP (mmHg)	134 ± 15
DBP (mmHg)	76 ± 8
Heart rate (bpm)	61 ± 6
Fasting serum/plasma parameters	
Sodium (mmol/L)	143 ± 2
Potassium (mmol/L)	4.3 ± 0.3
Serum osmolarity (mmol/L)	294 ± 3
Total cholesterol to HDL ratio	3.9 ± 1.0
Renal function parameters	
Serum urea (mmol/L)	5.4 ± 1.0
Serum creatinine (µmol/L)	81 ± 13
eGFR (mL/min per 1.73m^2)	79 ± 12
ACR (mg/mmol)	0.41 (0.28-0.59)
Urinary parameters	
Sodium excretion (mmol/24 h)[b]	91 ± 27
Potassium excretion (mmol/24 h)[c]	49 ± 14

Abbreviations: ACR, urinary albumin to creatinine ratio; bpm, beats per minute, eGFR, estimated glomerular filtration rate. [a] Data are presented as mean ± SD, geometric mean (95% confidence interval), or number (percentage). [b] To convert sodium in mmol/24 h to mg/24 h multiply by 23. [c] To convert potassium in mmol/24 h to mg/24 h multiply by 39.

TABLE 2. Effects of potassium and sodium supplementation in 35 untreated (pre)hypertensive adults

	Values after 4 weeks of intervention[a]			Treatment effect[b]			
	Potassium[c]	Sodium	Placebo	Potassium vs placebo[c]	P	Sodium vs placebo	P
Urinary parameters							
Sodium (mmol/24 h)[d]	95 ± 39	201 ± 55	102 ± 36	-7 (-23, 10)	0.4	99 (83, 115)	<0.001
Potassium (mmol/24 h)[e]	116 ± 32	53 ± 16	54 ± 16	62 (54, 70)	<0.001	-2 (-10, 6)	0.6
Volume (mL/24 h)	1745 ± 775	1928 ± 838	1900 ± 803	-133 (-370, 104)	0.3	20 (-214, 255)	0.9
Clinical measurements							
24-h SBP (mmHg)	125.9 ± 13.6	137.2 ± 14.4	129.3 ± 14.3	-3.6 (-6.7, -0.6)	0.02	8.0 (5.0, 11.1)	<0.001
24-h DBP (mmHg)	75.1 ± 8.0	79.3 ± 9.0	76.5 ± 8.4	-1.6 (-3.2, -0.04)	0.04	2.9 (1.3, 4.5)	<0.001
24-h heart rate (bpm)	69.2 ± 8.2	65.4 ± 9.3	66.3 ± 9.1	2.5 (0.9, 4.0)	0.002	-0.8 (-2.4, 0.7)	0.3
Fasting serum/plasma parameters							
Sodium (mmol/L)	142.7 ± 1.5	143.8 ± 1.5	143.4 ± 1.2	-0.7 (-1.2, -0.2)	0.003	0.4 (-0.1, 0.9)	0.09
Potassium (mmol/L)	4.41 ± 0.31	4.17 ± 0.34	4.28 ± 0.32	0.13 (0.05, 0.21)	0.003	-0.10 (-0.19, -0.02)	0.01
Serum osmolarity (mmol/L)	293 ± 3	294 ± 3	294 ± 2	-1 (-2, 0)	0.04	0 (0, 1)	0.3
Copeptin (pmol/L)	7.2 (5.9-8.9)	7.6 (6.3-9.2)	6.4 (5.4-7.6)				
Ln Copeptin (pmol/L)	1.98 ± 0.60	2.03 ± 0.55	1.86 ± 0.51	0.11 (0.01, 0.20)	0.02	0.18 (0.08, 0.27)	<0.001
NT-proBNP (ng/L)	68 (48-96)	102 (72-144)	73 (51-105)				
Ln NT-proBNP (ng/L)	4.22 ± 0.98	4.62 ± 1.00	4.29 ± 1.06	-0.08 (-0.24, 0.08)	0.3	0.33 (0.17, 0.49)	<0.001
MR-proANP (pmol/L)	83 (73-94)	100 (86-116)	89 (77-103)				
Ln MR-proANP (pmol/L)	4.42 ± 0.37	4.60 ± 0.43	4.49 ± 0.41	-0.08 (-0.15, -0.01)	0.03	0.11 (0.04, 0.18)	0.002
Renin (µIU/mL)	16.1 (11.3-22.9)	6.1 (4.2-8.9)	11.5 (8.1-16.1)				
Ln renin (µIU/mL)	2.78 ± 1.02	1.81 ± 1.08	2.44 ± 1.00	0.34 (0.08, 0.60)	0.01	-0.62 (-0.87, -0.36)	<0.001
Aldosterone (nmol/L)	0.24 (0.22-0.26)	0.18 (0.16-0.20)	0.21 (0.18-0.23)				
Ln aldosterone (nmol/L)	-1.44 ± 0.26	-1.72 ± 0.28	-1.58 ± 0.34	0.14 (0.07, 0.22)	<0.001	-0.14 (-0.22, -0.06)	<0.001
Renal function parameters							
Serum urea (mmol/L)	5.62 ± 1.41	5.16 ± 1.09	5.31 ± 1.13	0.30 (0.002, 0.59)	0.05	-0.15 (-0.44, 0.14)	0.3
Serum creatinine (µmol/L)	82.0 ± 12.8	77.5 ± 11.5	81.4 ± 13.3	0.5 (-2.0, 3.0)	0.7	-4.0 (-6.4, -1.6)	0.001
eGFR (mL/min per 1.73m^2)	78.5 ± 11.7	82.7 ± 11.1	79.2 ± 11.6	-0.4 (-2.8, 1.9)	0.7	3.5 (1.2, 5.9)	0.003
ACR (mg/mmol)	0.33 (0.24-0.44)	0.47 (0.30-0.72)	0.47 (0.33-0.66)				
Ln ACR (mg/mmol)	-1.11 ± 0.86	-0.76 ± 1.25	-0.76 ± 1.00	-0.36 (-0.66, -0.06)	0.02	0.004 (-0.29, 0.30)	0.9

ACR, urinary albumin to creatinine ratio; bpm, beats per minute; eGFR, estimated glomerular filtration rate. [a] Data are presented as unadjusted mean ± SD or geometric mean (95% CI). Variables with a skewed distribution were ln-transformed before analyses. [b] Data are mean differences (95% CI) obtained from linear mixed-effect models for repeated measurements using the compound symmetry covariance structure. [c] N=34. [d] To convert sodium in mmol/24 h to mg/24 h multiply by 23. [e] To convert potassium in mmol/24 h to mg/24 h multiply by 39.

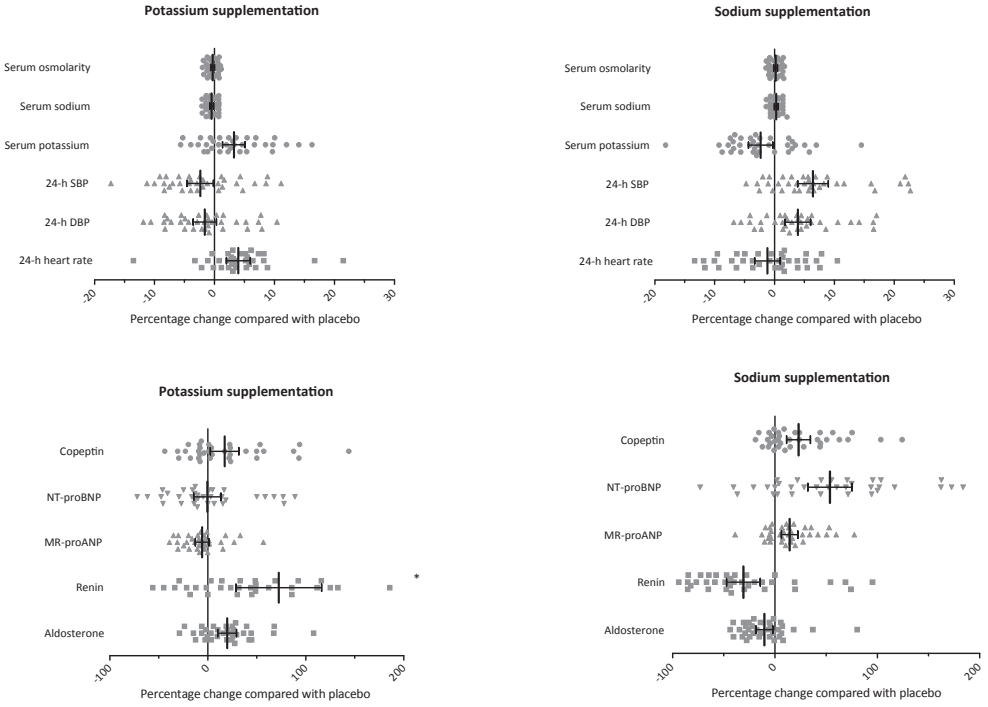

FIGURE 1. Effects of potassium and sodium supplementation on markers of osmoregulation and volume regulation in 35 (pre)hypertensive subjects.
*3 data points (percentage change in renin after potassium supplementation of 281, 380, and 569%, respectively) are outside the x-axis limits.

DISCUSSION

The present study is, to the best of our knowledge, the first to investigate the humoral effects of potassium supplementation during sodium restriction and provides a biologically plausible explanation for the diminished BP-lowering effects of potassium supplementation during sodium restriction. In this *post-hoc* analysis of a fully controlled dietary intervention study, we found that BP decreased significantly after four weeks of potassium supplementation, indicating that potassium has BP-lowering effects, albeit relatively small, during sodium restriction. The BP-lowering effects of potassium during sodium restriction seem mitigated by activation of several counter regulatory mechanisms (i.e. increased secretion of vasopressin, stimulation of RAAS, and increased heart rate) in order to maintain volume homeostasis and counterbalance the decrease in BP.

The BP-lowering effects of potassium have been established in several randomized clinical trials [6, 14]. Potassium is suggested to exert its BP-lowering effects, at least in part, through

stimulation of natriuresis [20, 21]. A dietary potassium load was reported to induce a rapid natriuresis [22, 23], which is most likely explained by deactivation of the NaCl cotransporter that occurs independent of plasma aldosterone concentrations [23]. Moreover, potassium was reported to be more effective in reducing BP at higher levels of sodium intake [6, 11]. High potassium intake is suggested to blunt the BP increasing effects of high sodium intake [24], which could be explained by enhanced natriuretic effects of potassium during high sodium intake [22]. A previous randomized crossover trial that examined the effects of increased potassium intake on top of a sodium-restricted diet (i.e., 70 mmol/24 h) showed little or no effect of potassium on BP in individuals with mild or moderate hypertension [13]. It was hypothesized that potassium has either less of a natriuretic effect or less effect on renin suppression when sodium intake is restricted [13]. In the present study, we found a significant decrease in BP after potassium supplementation, indicating that potassium can have BP-lowering effects when sodium intake is restricted. Because the BP-lowering effects of potassium are suggested to depend on the level of sodium intake [6, 11], differences in background sodium intake (i.e. ~100 mmol/24 h in the present study versus ~70 mmol/ 24 h in the study of Smith et al. [13]) may explain the differences in the observed effects of potassium supplementation on BP.

Interestingly, the BP-lowering effects of potassium during sodium restriction seem mitigated by counter regulatory effects of hormones involved in maintenance of volume and BP homeostasis. In line, we found significant increases in both plasma renin and aldosterone. In hyperkalemia, aldosterone secretion is increased, while secretion of renin and angiotensin II is suppressed, resulting in electrochemical sodium reabsorption that promotes kaliuresis [25]. In contrast, secretion of both renin and aldosterone is increased in effective circulating volume depletion, in which aldosterone and angiotensin II act synergistically to promote maximal sodium reabsorption [25]. The significant increase in serum urea is likely to correspond with increased tubular sodium reabsorption, because reabsorption of filtered urea is passively linked to that of sodium and water [7]. In addition, we found a significant increase in plasma copeptin, a surrogate for vasopressin. Vasopressin stimulates water reabsorption, which raises the extracellular volume toward normal [17]. Furthermore, we found a significant increase in 24-h heart rate, which may indicate that the decrease in effective circulating volume is counterbalanced by an increase in heart rate to increase cardiac output.

The effects of increased sodium intake on RAAS and natriuretic peptides are well known. In line with previous studies, we found that increased sodium intake is associated with suppression of RAAS [26, 27] and increases in natriuretic peptides [7, 26]. However, limited data are available on the effects of increased dietary sodium intake on copeptin, or vasopressin, concentrations. In line with a recent study of Tasevska et al. [28], we found that increased sodium intake was associated with an increase in plasma copeptin concentrations.

We found that percentage changes in serum sodium concentration after sodium and potassium supplementation, compared with placebo, are minor (i.e. less than 2%). This suggests that, in line with a recent study of Zhang et al. [29], serum sodium concentrations are tightly regulated around a subject-specific set point.

We acknowledge that this study has several limitations. The main limitation of the present study is the relatively small sample size. However, the fact that we found anticipated effects of sodium supplementation and opposing effects of potassium intake makes our data robust. Furthermore, we studied relatively short-term effects of potassium and sodium intake on osmoregulation and volume regulation in (pre)hypertensive individuals. It would be of interest to investigate whether long-term effects of potassium and sodium intake on osmoregulation and volume regulation would alter the risk of cardiovascular and renal disease end points. A major strength of our study is the fully controlled diet, which strongly reduced the intraindividual variability resulting from dietary influences (for example, use of alcohol, coffee, and salt) and thereby increasing the power to demonstrate effects that are exclusively attributable to potassium and sodium intake. The power of our study is furthermore strengthened by its design as a crossover study, allowing paired data analysis.

In conclusion, in this *post-hoc* analysis of a fully controlled dietary intervention study, we demonstrated that potassium has BP-lowering effects during sodium restriction. These BP-lowering effects of potassium, however, seem mitigated by activation of several counter regulatory mechanisms (i.e. increased secretion of vasopressin, stimulation of RAAS, and increased heart rate) to maintain volume homeostasis and counterbalance the decrease in BP. Our study provides biological plausibility for the observation that BP-lowering effects of potassium supplementation are diminished during sodium restriction.

Funding
The research is funded by TI Food and Nutrition, a public-private partnership on precompetitive research in food and nutrition. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures
There are no conflicts of interest.

REFERENCES

1. He J, Whelton PK. Elevated systolic blood pressure as a risk factor for cardiovascular and renal disease. J Hypertens Suppl 1999; 17:S7–13.
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002; 360:1903–1913.
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet 2005; 365:217–223.
4. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ 2013; 346:f1326.
5. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: cochrane systematic review and meta-analysis of randomised trials. BMJ 2013; 346:f1325.
6. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ 2013; 346:f1378.
7. Rose BD, Post TW. Clinical physiology of acid-base and electrolyte disorders. United States of America: The McGraw-Hill Companies; 2001
8. Damkjaer M, Isaksson GL, Stubbe J, Jensen BL, Assersen K, Bie P. Renal renin secretion as regulator of body fluid homeostasis. Pflugers Arch 2013; 465:153–165.
9. Zois NE, Bartels ED, Hunter I, Kousholt BS, Olsen LH, Goetze JP. Natriuretic peptides in cardiometabolic regulation and disease. Nat Rev Cardiol 2014; 11:403–412.
10. van Gastel MD, Meijer E, Scheven LE, Struck J, Bakker SJ, Gansevoort RT. Modifiable factors associated with copeptin concentration: a general population cohort. Am J Kidney Dis 2015; 65:719–727.
11. Whelton PK, He J, Cutler JA, Brancati FL, Appel LJ, Follmann D, Klag MJ. Effects of oral potassium on blood pressure. Meta-analysis of randomized controlled clinical trials. JAMA 1997; 277:1624–1632.
12. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. N Engl J Med 2014; 371:601–611.
13. Smith SJ, Markandu ND, Sagnella GA, MacGregor GA. Moderate potassium chloride supplementation in essential hypertension: is it additive to moderate sodium restriction? Br Med J (Clin Res Ed) 1985; 290:110–113.
14. Gijsbers L, Dower JI, Mensink M, Siebelink E, Bakker SJ, Geleijnse JM. Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens 2015; 29:592–598.
15. US Department of Agriculture and US Department of Health and Human Services. Dietary Guidelines for Americans, 2010, 7th edition. Washington, DC: US Government Printing Office, 2010.
16. WHO. Guideline: Sodium intake for adults and children. Geneva, World Health Organization (WHO), 2012.

17. Morgenthaler NG. Copeptin: a biomarker of cardiovascular and renal function. Congest Heart Fail 2010; 16 (Suppl 1):S37–44.
18. Fazekas AS, Funk GC, Klobassa DS, Ruther H, Ziegler I, Zander R, Semmelrock HJ. Evaluation of 36 formulas for calculating plasma osmolality. Intensive Care Med 2013; 39:302–308.
19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604–612.
20. Smith SR, Klotman PE, Svetkey LP. Potassium chloride lowers blood pressure and causes natriuresis in older patients with hypertension. J Am Soc Nephrol 1992; 2:1302–1309.
21. Fujita T, Ando K. Hemodynamic and endocrine changes associated with potassium supplementation in sodium-loaded hypertensives. Hypertension 1984; 6:184–192.
22. van Buren M, Rabelink AJ, Bijlsma JA, Koomans HA. Natriuretic and kaliuretic response to potassium load: modulation by sodium intake. Nephrol Dial Transplant 1993; 8:495–500.
23. Sorensen MV, Grossmann S, Roesinger M, Gresko N, Todkar AP, Barmettler G, et al. Rapid dephosphorylation of the renal sodium chloride cotransporter in response to oral potassium intake in mice. Kidney Int 2013; 83:811–824.
24. Rodrigues SL, Baldo MP, Machado RC, Forechi L, Molina Mdel C, Mill JG. High potassium intake blunts the effect of elevated sodium intake on blood pressure levels. J Am Soc Hypertens 2014; 8:232–238.
25. Seva Pessoa B, van der Lubbe N, Verdonk K, Roks AJ, Hoorn EJ, Danser AH. Key developments in renin-angiotensin-aldosterone system inhibition. Nat Rev Nephrol 2013; 9:26–36.
26. Pimenta E, Gaddam KK, Oparil S, Aban I, Husain S, Dell'Italia LJ, Calhoun DA. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. Hypertension 2009; 54:475–481.
27. Laragh JH, Baer L, Brunner HR, Buhler FR, Sealey JE, Vaughan ED Jr. Renin angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. Am J Med 1972; 52:633–652.
28. Tasevska I, Enhorning S, Burri P, Melander O. High salt intake increases copeptin but salt sensitivity is associated with fluid induced reduction of copeptin in women. Int J Hypertens 2014; 2014:641587.
29. Zhang Z, Duckart J, Slatore CG, Fu Y, Petrik AF, Thorp ML, Cohen DM. Individuality of the plasma sodium concentration. Am J Physiol Renal Physiol 2014; 306:F1534–F1543.

CHAPTER 5

Potassium supplementation and heart rate: a meta-analysis of randomized controlled trials

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Nutr Metab Cardiovasc Dis. 2016;26:674-82

ABSTRACT

Background and aims

Increasing the intake of potassium has been shown to lower blood pressure, but whether it also affects heart rate (HR) is largely unknown. We therefore assessed the effect of potassium supplementation on HR in a meta-analysis of randomized controlled trials.

Methods and results

We searched PubMed (1966–October 2014) for randomized, placebo-controlled trials in healthy adults with a minimum duration of two weeks in which the effect of increased potassium intake on HR was assessed. In addition, reference lists from meta-analysis papers on potassium and blood pressure were hand-searched for publications. Two investigators independently extracted the data. We performed random effects meta-analyses, subgroup and meta-regression analyses for characteristics of the study (e.g. design, intervention duration, potassium dose and salt type, change in potassium excretion, sodium excretion during intervention) and study population (e.g. gender, age, hypertensive status, pre-study HR, pre-study potassium excretion). A total of 22 trials (1086 subjects), with a median potassium dose of 2.5 g/day (range: 0.9–4.7 g/day), and median intervention duration of 4 weeks (range: 2–24 weeks) were included. The meta-analysis showed no overall effect of increased potassium intake on HR (0.19 bpm, 95% CI: –0.44, 0.82). Stratified analyses yielded no significant effects of potassium intake on HR in subgroups, and there was no evidence for a dose-response relationship in meta-regression analyses.

Conclusion

A chronic increase in potassium intake with supplemental doses of 2–3 g/day is unlikely to affect HR in apparently healthy adults.

INTRODUCTION

Elevated resting heart rate (HR) has been identified as a predictor of cardiovascular morbidity and mortality in population-based studies [1–3]. In a meta-analysis of 7 prospective cohort studies a high resting HR was associated with a 40% higher risk of heart failure compared to a low resting HR [3]. HR can be affected by cardiac drug therapy, e.g. use of beta-blockers [3–8], and by non-pharmacological factors such as stress [9], physical activity [10–12], smoking [13], and alcohol use [14, 15]. To what extent HR can be modified by diet, however, is largely unknown. García-López et al. [16] showed that baseline adherence to the Mediterranean diet, characterized by high consumption of fruits and vegetables, olive oil, legumes, whole grain cereals, moderate consumption of fish, poultry and dairy products, and low consumption of red and processed meats, was associated with a lower average HR. No association was found for repeated measurements of adherence during follow up and no difference in HR was observed among the dietary intervention groups in the PREDIMED trial [16]. Mozaffarian et al. [17], however, showed in a meta-analysis of 30 randomized controlled trials (RCTs) that fish oil significantly reduced HR by 1.6 beats per minute (bpm), particularly in subjects with high pre-study HR and after longer treatment duration.

Increased potassium intake favorably affects BP. A meta-analysis of 21 RCTs in healthy adults showed a 3.5 mmHg lower systolic BP and 2.0 mmHg lower diastolic BP after potassium supplementation (~2 g/day), an effect that was most pronounced in hypertensives and in those with a high sodium intake [18]. In a recent crossover study, we found a 4.0/1.7 mmHg lower 24-h BP in 36 subjects with untreated elevated BP who received 3 g/day of potassium for 4 weeks on top of a fully controlled, reduced-sodium diet (2.4 g per 2500 kcal) [19]. In that study, we noted that potassium supplementation significantly increased 24-h HR by 2.6 bpm, without affecting resting office HR. In a 4-week trial in 21 healthy adults with a high sodium intake, however, no effect of 4 g/day of potassium on 24-h HR was seen [20].

To clarify the role of potassium intake in determining HR, and possible interaction with sodium intake or other factors, we performed a meta-analysis of RCTs of potassium supplementation and HR (mostly as a secondary study outcome) in healthy adults.

METHODS

Search Strategy

A systematic literature search was performed for RCTs of potassium supplementation evaluating the effect on HR or BP in PubMed (1966 through 31 October 2014), using terms and algorithms as presented in Supplemental Table 1. References from previous meta-analyses and reviews evaluating the effect of potassium on BP were screened for additional publications. No restrictions were imposed on language.

Study Selection

An overview of the study selection is given in Figure 1. After screening titles and abstracts, full-text articles were screened according to predefined criteria. Inclusion criteria were (1) randomized design; (2) effect of increased potassium intake on HR or BP assessed; (3) placebo-controlled study; (4) subjects of 18 years or older; (5) apparently healthy individuals; (6) treatment effect could be appointed to increased potassium intake alone; and (7) intervention period was 2 weeks or longer. After excluding duplicate publications, 34 potential relevant studies remained. If not reported, authors were contacted to provide HR data. For 12 studies HR data were not obtained, either because (1) no HR measurements were performed [21–23]; (2) HR data were not accessible [24–28]; or (3) no contact could be established with the author [29–32], leaving 22 RCTs [19, 20, 33–52] that were eligible and included in the meta-analysis.

Data Extraction and Risk of Bias Assessment

Two investigators (LG and FJMM) extracted the following data from each study using a standardized extraction sheet: study design; primary outcome of the study; intervention duration; potassium type and dose; data on potassium and sodium excretion; method of HR assessment; data on HR and variance measures; and data on BP. Data on sample size and characteristics of the study population including mean baseline age, sex, hypertensive status, and use of antihypertensive medications were also collected. When the outcome was measured multiple times, data from the latest time point were extracted. Discrepancies were resolved by discussion with a third investigator (JMG). When data were missing, authors were requested or data were calculated using published data. None of the 22 RCTs reported the HR effect estimate with the standard error (SE) of this treatment effect. For 5 RCTs [19, 45, 48, 50, 52], HR effects with SEs were available upon author request. For 17 RCTs [20, 33–44, 46, 47, 49, 51], the HR effect and SE were calculated using published data, of which for one RCT [44] HR data were derived from graphs. If pre-study characteristics were missing, placebo values were extracted if the protocol aimed to maintain usual dietary patterns during the study period. Eventually, for one study [34] data on pre-study HR, for two studies [42, 49] data on potassium excretion after potassium supplementation, and for one study [42] data on sodium excretion after potassium supplementation were missing. Mean age was not reported for one study [39] and we took the midpoint of the age range.

We assessed the risk of bias of the included studies as being low, unclear, or high using the Cochrane Collaboration's tool [53], taking into account method of sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment and incomplete outcome data. Selective reporting was not taken into account, because authors were requested for additional HR data equalizing and minimalizing reporting bias between all studies. Studies considered to be high in risk of bias were the studies graded as high in risk for the method of sequence generation, allocation concealment and additionally in one of the blinding procedures or incomplete outcome data.

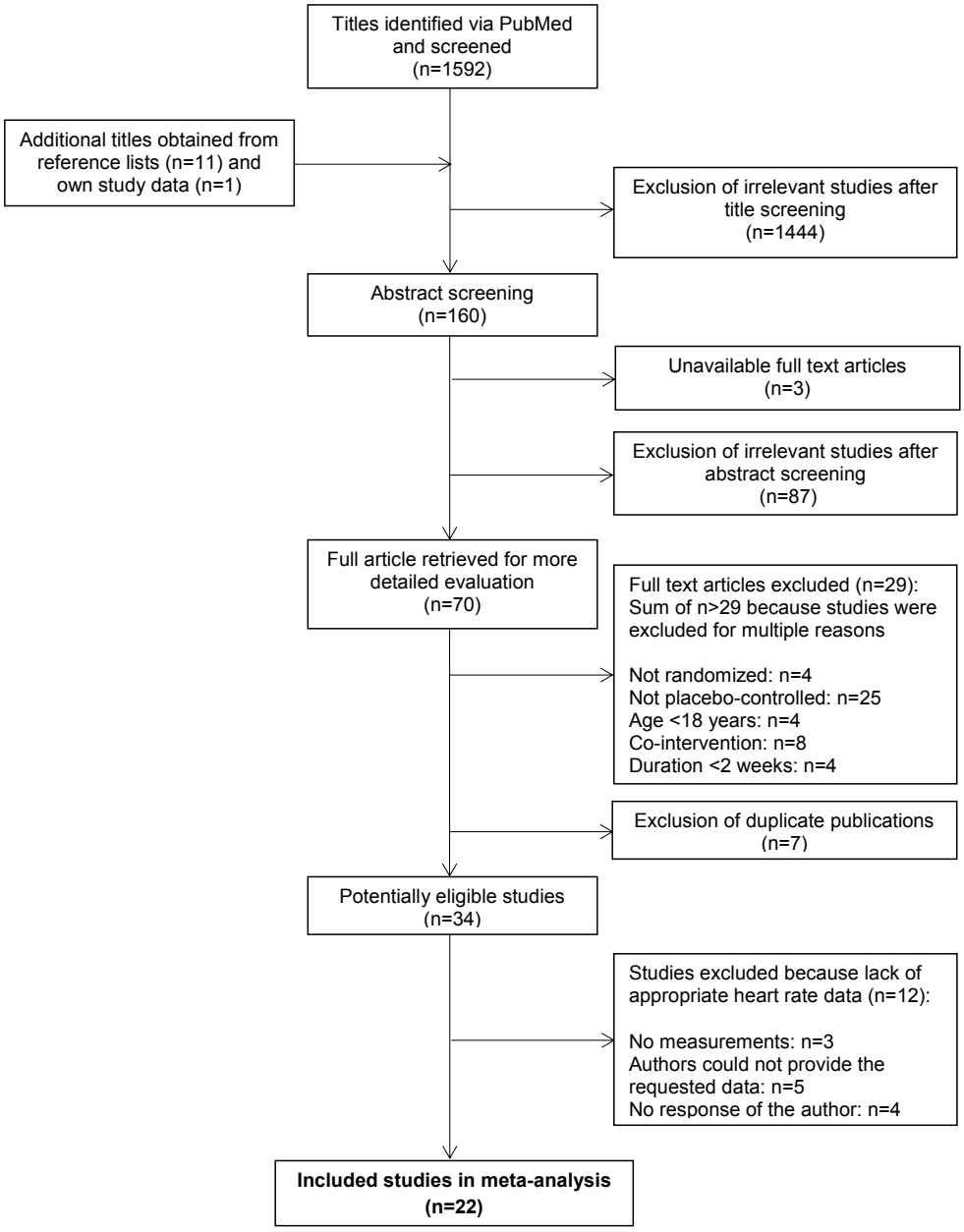


FIGURE 1. Flow chart of study selection.

Statistical Analysis

Our primary outcome was the effect of potassium supplementation on HR, as compared to a placebo-controlled situation. For crossover RCTs, the treatment effect was calculated as HR after potassium intervention minus HR after placebo intervention. For parallel RCTs, the treatment effect was calculated as the HR change from baseline to end in the potassium intervention group minus the HR change from baseline to end in the control group. If not obtained, the SE of the HR effect was estimated following the method as described by Streppel et al. [54]. For this estimation, a correlation of 0.50 between baseline and end HR (parallel design) or HR after intervention and placebo period (crossover design) was assumed, according to Follmann et al. [55]. Considering a correlation coefficient of 0.00 and less conservative correlation coefficients of 0.65 and 0.80 yielded similar results. For one parallel RCT [41] only SEs for baseline HR were reported and it was assumed that end SEs were similar. An overview of the methods to impute SE is given in the supplemental material.

Meta-analyses were performed in STATA (version 11.0; STATA Corp, College Station, TX) through the use of random-effects model which takes both within and between study variance into account [56], with each study weighted by the inverse of its variance. Heterogeneity between studies was evaluated with the I^2 test statistic and the Cochran's Q-test [57].

For RCTs with different types of potassium-salts [43, 47, 51], the potassium-chloride data were included in the main meta-analyses. When HR was measured in various body positions [19, 33, 36, 37, 41, 43, 45, 46, 50], HR data in supine position were included, or if not available we used seated, standing, or 24-h ambulatory monitoring measurements, consecutively.

To investigate potential sources of heterogeneity and to explore effect modification, we conducted predefined stratified and meta-regression analyses for study design (parallel vs crossover), gender (men vs women vs mixed), age (≤ 45 vs > 45 years), hypertensive status of the study population (normotensive vs hypertensive [SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg] vs mixed), intervention duration (≤ 4 vs > 4 weeks), potassium dose (≤ 2.5 vs > 2.5 g/day), potassium salt type (potassium chloride vs potassium citrate vs potassium bicarbonate), HR measurement (office supine vs office standing vs office seated vs 24-h ambulatory), pre-study HR (≤ 70 bpm vs > 70 bpm), pre-study potassium excretion (≤ 65 vs > 65 mmol/24 h), urinary potassium excretion during potassium intervention (≤ 120 vs > 120 mmol/24 h), change in potassium excretion (≤ 50 vs > 50 mmol/24 h), urinary sodium excretion during potassium intervention (≤ 140 vs > 140 mmol/24 h) and reduction in SBP (≤ 3 mmHg vs > 3 mmHg). In the stratification analyses of potassium salt type and HR measurement, some RCTs were included in multiple subgroups, because in these RCTs [43, 47, 51] multiple potassium-salt types were administered or HR was measured in multiple positions [19, 33, 36, 37, 41, 43, 45, 46, 50], respectively. For subgroup analysis, a minimum of three studies was required per strata.

We performed sensitivity analysis by excluding one study at a time from the analysis. We planned to do a sensitivity analysis to examine the effect of removing studies at high risk of bias from the analysis, but none of the studies appeared to be high in risk. Publication bias was assessed using funnel plots and Egger's test [58]. Two- sided p-values < 0.05 were regarded as statistically significant.

RESULTS

Study Characteristics

Table 1 provides an overview of 22 included RCTs (5 parallel, 17 crossover) of potassium supplementation and HR, which had a duration of 2–24 weeks (median: 4 weeks.) The overall meta-analysis included 1086 subjects (64% men) with a mean pre-study HR of 70 bpm and BP of 143/89 mmHg (Supplemental Table 2). Subjects received potassium doses between 0.9 and 4.7 g/day (median: 2.5 g/day), with resulting changes in urinary potassium excretion between 17 and 108 mmol/24 h (median: 53 mmol/24 h). The effect of potassium supplementation on SBP ranged from –13.7 mmHg to +2.0 mmHg (median: –3.0 mmHg). None of the 22 studies were considered to be high in risk of bias (Supplemental Table 3).

Effect on Heart Rate

The meta-analysis showed no overall effect of increased potassium intake on HR (0.19 bpm, 95% CI: –0.44, 0.82; P = 0.56), and there was no evidence for heterogeneity (I^2 = 0, P = 0.56) (Figure 2). Table 2 shows the results of the stratified and meta-regression analyses, and in Supplemental Table 4 the descriptive characteristics of the subgroups are given. HR was reduced by 0.50 bpm in short-term RCTs (≤ 4 weeks), and increased by 0.85 bpm in longer-term RCTs, but the estimates were not statistically significant. Moreover, meta-regression analysis indicated no linear relationship of intervention duration with the effect on HR (β = 0.08 bpm per one week increase in intervention duration; P = 0.19) (Table 2 and Supplemental Figure 1). Stratified analyses also suggested a difference in effect (P for interaction = 0.050) for pre-study HR: in subjects with a lower HR (≤ 70 bpm), potassium supplementation increased HR by 0.70 bpm, whereas in subjects with a higher HR (> 70 bpm) potassium reduced HR by 0.85 bpm, albeit these effects were not significant. Moreover, meta-regression analysis indicated no linear relationship of pre-study HR with the effect on HR (Table 2 and Supplemental Figure 2). In the stratification for the way in which HR measurement was performed, potassium intake tended to increase 24-h ambulatory HR by 0.99 bpm, but results were only based on 4 studies and not statistically significant (P = 0.26) (Supplemental Figure 3). The effect of potassium on HR was not associated with the concomitant 24-h urinary sodium excretion (β = 0.07 bpm per 10 mmol/24 h, P = 0.50; Table 2 and Supplemental Figure 4). The effect of potassium on HR was inversely related to SBP reduction (β = –0.24 bpm per mmHg, P = 0.019; Table 2 and Supplemental Figure 5).

TABLE 1. Overview of 22 randomized controlled trials evaluating the effect of increased potassium intake on heart rate

Author	Year	Country	Trial design	Duration (weeks)	No. of subjects	Men (%)	Mean age (yr)	Hypertensive	Dose (g/day)	Type K[a]	HR[b] measurement	Treatment effect HR ± SE (bpm)
Barden et al. [37]	1986	Australia	XO	4	43	0	32	No	3.1	K-chloride	Office supine	-1.3 ± 2.3[c]
											Office standing	-1.6 ± 2.3[c]
Berry et al. [50]	2010	UK	XO	6	48	48	45	Mixed	1.6	K-citrate	Office supine	0.9 ± 0.9
											24-h ambulatory	0.2 ± 1.0
Fotherby and Potter [46]	1992	UK	XO	4	18	28	75	Yes	2.3	K-chloride	Office supine	-1.0 ± 2.4[c]
											Office standing	-2.0 ± 2.5[c]
											24-h ambulatory	-1.0 ± 2.3[c]
Gijsbers et al. [19]	2014	The Netherlands	XO	4	36	67	66	Mixed	2.8	K-chloride	Office supine	0.6 ± 0.9
											24-h ambulatory	2.6 ± 0.8
Graham et al. [52]	2013	North Ireland	XO	6	40	80	55	Mixed	2.5	K-chloride	Office supine	1.1 ± 1.1
Grobbee et al. [40]	1987	The Netherlands	XO	6	40	85	24	Yes	2.8	Unspecified	Office supine	2.0 ± 2.0[c]
He et al. [51]	2010	UK	XO	4	42	71	51	Yes	2.5	K-chloride	Office seated	0.0 ± 1.3[c]
										K-bicarbonate		0.0 ± 1.3[c]
MacGregor et al. [33]	1982	UK	XO	4	23	52	45	Yes	2.5	K-chloride	Office supine	-1.0 ± 2.0[c]
											Office standing	-1.0 ± 2.0[c]
Matlou et al. [38]	1986	South-Africa	XO	6	32	0	51	Yes	2.5	K-chloride	Office seated	-2.0 ± 2.0[c]
Matthesen et al. [20]	2012	Denmark	XO	4	21	43	26	No	3.9	K-chloride	24-h ambulatory	0.0 ± 2.8[c]
Mullen and O'Connor [43]	1990	USA	XO	2	24	100	25	No	2.9	K-chloride	Office supine	-1.0 ± 2.0[c]
											Office standing	-1.0 ± 3.0[c]
										K-citrate	Office supine	-2.0 ± 2.0[c]
											Office standing	-4.0 ± 3.0[c]
Naismith and Braschi [49]	2003	UK	P	6	59	56	43	Mixed	0.9	K-chloride	Office seated	-1.0 ± 1.5[c]

Overlack et al. [44]	1991	Germany	XO	8	12	67	37	Yes	4.7	Mix of K-citrate and bicarbonate	Office supine	2.0 ± 3.3[c]
Overlack et al. [47]	1995	Germany	XO	8	25	72	48	Mixed	4.7	K-chloride	Office seated	2.3 ± 1.7[c]
										K-citrate		3.4 ± 1.5[c]
Poulter and Sever [39]	1986	Kenya	XO	2	19	100	33	No	2.5	K-chloride	Office seated	2.2 ± 2.3[c]
Siani et al. [41]	1987	Italy	P	15	37	62	45	Yes	1.9	Unspecified	Office supine	5.1 ± 3.9[c]
											Office standing	0.8 ± 4.5[c]
Smith et al. [34]	1985	UK	XO	4	20	55	53	Yes	2.5	K-chloride	Office supine	-1.0 ± 3.1[c]
Sundar et al. [35]	1985	India	P	4	50	58	46	Mixed	2.3	Mix of K-chloride and bicarbonate	Office supine	-3.0 ± 1.1[c]
Svetkey et al. [42]	1987	USA	P	8	101	85	51	Yes	4.7	K-chloride	Office seated	0.0 ± 2.4[c]
Valdés et al. [45]	1991	Chile	XO	4	24	54	50	Yes	2.5	K-chloride	Office supine	-0.1 ± 1.5
											Office standing	1.3 ± 1.3
Whelton et al. [48]	1995	USA	P	24	353	72	43	No	2.3	K-chloride	Office seated	1.1 ± 1.0
Zoccali et al. [36]	1985	Scotland	XO	2	19	53	38	Yes	3.9	K-chloride	Office supine	0.0 ± 2.0[c]

Abbreviations: K: potassium; HR: heart rate; SE: standard error; P: parallel; XO: crossover; UK: United Kingdom; USA: United States of America. [a] For studies evaluating different types of potassium salts, potassium chloride data were included in the main meta-analyses. [b] For studies measuring HR in various body positions, HR data in supine position were included above seated, standing, and 24-hr ambulatory monitoring, consecutively. [c] SE was estimated following the method of Streppel et al. [54] (see Supplemental Methods).

However, after excluding the study of Sundar et al. [35], the relationship was no longer present (β = –0.10 bpm per mmHg, P = 0.43). When single studies were excluded from the overall analysis, the overall HR effect ranged from 0.08 bpm (95% CI: –0.60, 0.75; P = 0.83) when the study of Whelton et al. [48] was excluded to 0.49 bpm (95% CI: –0.17, 1.15; P = 0.15) when the study of Sundar et al. [35] was excluded. The funnel plot (Supplemental Figure 6) and the Egger's test (P = 0.94) indicated no evidence of publication bias.

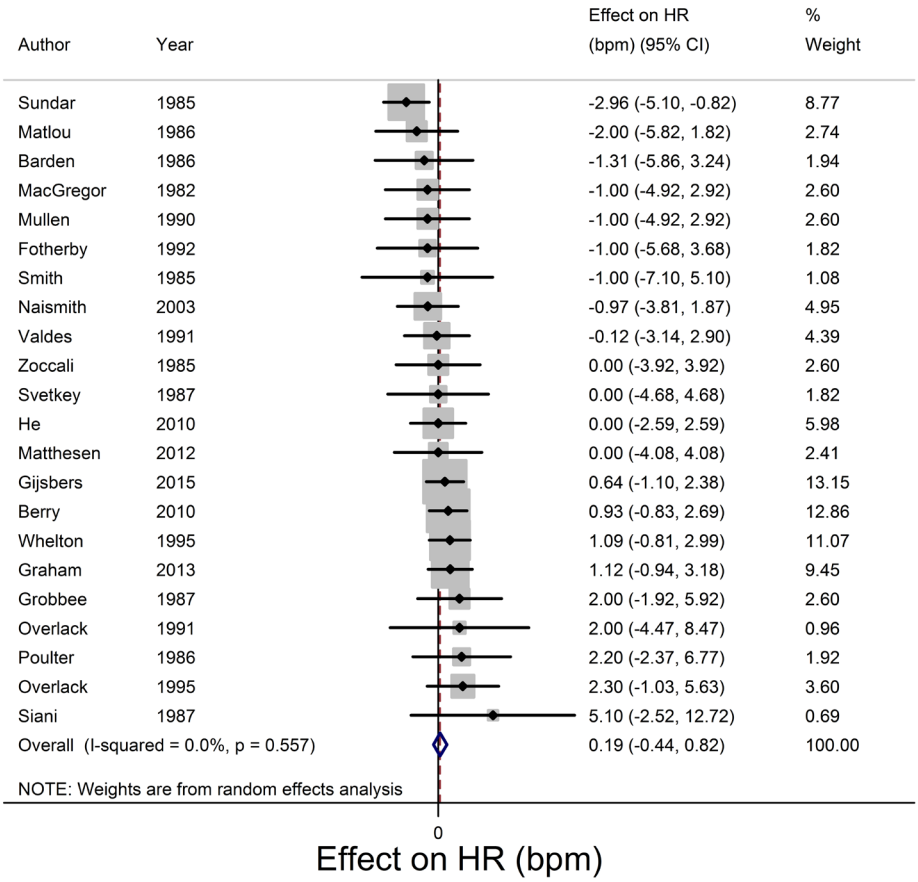


FIGURE 2. Forest plot of 22 randomized controlled trials evaluating the effect of potassium on heart rate. Black bullets indicate the point estimate for each trial, with the horizontal lines representing the 95% CIs. The size of the square is proportional to the weight of the study, using a random-effects model. The overall pooled treatment effect is indicated by the dotted line and the width of the diamond corresponds with the 95% CI of the overall effect. Abbreviation: HR, heart rate.

TABLE 2. Effect of potassium supplementation on HR according to predefined study characteristics

	Number of studies	Effect on HR (bpm)		Heterogeneity test		P for interaction
		Estimate (95% CI)	P	I^2 (%)	P	
Overall	22	0.19 (-0.44, 0.82)	0.56	0.0	0.56	
Study design						
Parallel	5	-0.38 (-2.56, 1.80)	0.73	60.0	0.041	0.22
Crossover	17	0.47 (-0.28, 1.21)	0.22	0.0	0.96	
Gender						
Men	2	0.38 (-2.73, 3.48)	0.81	7.9	0.30	ref
Women	2	-1.71 (-4.64, 1.21)	0.25	0.0	0.82	0.43
Mixed	18	0.28 (-0.39, 0.94)	0.41	0.0	0.48	0.94
Per% men	22	0.03 (0.00, 0.07)	0.087			
Age[a]						
≤ 45 years	12	0.56 (-0.36, 1.48)	0.24	0.0	0.86	0.36
> 45 years	10	-0.18 (-1.25, 0.89)	0.74	25.6	0.21	
Per 5 years	22	-0.01 (-0.36, 0.34)	0.96			
Hypertensive population						
No	5	0.56 (-0.86, 1.98)	0.44	0.0	0.71	ref
Mixed	6	0.13 (-1.28, 1.53)	0.86	59.2	0.13	0.82
Yes[b]	11	-0.03 (-1.24, 1.18)	0.96	0.0	0.91	0.54
Intervention duration[a]						
≤ 4 weeks	12	-0.50 (-1.40, 0.40)	0.28	0.0	0.65	0.055
> 4 weeks	10	0.85 (-0.04, 1.74)	0.060	0.0	0.71	
Per week	22	0.08 (-0.04, 0.21)	0.19			
Potassium dose[a]						
≤ 2.5 grams/day	12	0.04 (-0.90, 0.99)	0.93	23.6	0.21	0.71
> 2.5 grams/day	10	0.38 (-0.69, 1.46)	0.49	0.0	0.85	
Per gram/day	22	0.34 (-0.56, 1.24)	0.44			
Potassium salt type[c]						
Potassium chloride	17	0.30 (-0.44, 1.03)	0.43	0.0	0.96	ref
Potassium citrate	3	1.01 (-1.46, 3.48)	0.42	57.2	0.097	0.34
Potassium bicarbonate	1	0.00 (-2.59, 2.59)	0.99			
HR measurement						
Office supine	14	0.04 (-0.80, 0.89)	0.92	8.8	0.36	ref
Office standing	7	-0.11 (-1.65, 1.44)	0.89	0.0	0.86	0.82
Office seated HR	7	0.45 (-0.67, 1.56)	0.43	0.0	0.56	0.64
24-hr ambulatory HR	4	0.99 (-0.73, 2.71)	0.26	46.5	0.13	0.29
Pre-study HR[d]						
≤ 70 bpm	13	0.70 (-0.08, 1.49)	0.077	0.0	0.91	0.050
> 70 bpm	8	-0.85 (-2.12, 0.42)	0.19	17.1	0.30	
Per 5 bpm	21	-0.53 (-1.14, 0.08)	0.086			
Pre-study potassium excretion[e]						
≤ 65 mmol/24h	10	-0.32 (-1.67, 1.02)	0.64	28.6	0.18	0.31
> 65 mmol/24h	9	0.48 (-0.44, 1.39)	0.31	0.0	0.82	
Per 5 mmol/24h	19	0.16 (-0.16, 0.48)	0.31			

TABLE 2. Continued

	Number of studies	Effect on HR (bpm)		Heterogeneity test		P for interaction
		Estimate (95% CI)	P	I^2 (%)	P	
Potassium excretion during potassium intervention[f]						
≤ 120 mmol/24h	12	0.07 (-0.92, 1.06)	0.89	29.6	0.16	0.62
> 120 mmol/24h	8	0.50 (-0.77, 1.78)	0.44	0.0	0.89	
Per 10 mmol/24h	20	0.15 (-0.13, 0.44)	0.28			
Change in potassium excretion[g]						
≤ 50 mmol/24h	10	0.16 (-0.98, 1.29)	0.79	34.7	0.13	
> 50 mmol/24h	10	0.34 (-0.70, 1.38)	0.52	0.0	0.84	0.87
Per 10 mmol/24h	20	0.11 (-0.23, 0.45)	0.51			
Sodium excretion during potassium intervention[h]						
≤ 140 mmol/24h	9	-0.07 (-1.24, 1.10)	0.91	30.1	0.18	0.58
> 140 mmol/24h	12	0.42 (-0.49, 1.34)	0.36	0.0	0.76	
Per 10 mmol/24h	21	0.07 (-0.14, 0.27)	0.50			
Reduction in SBP[a]						
≤ 3.0 mmHg	12	0.63 (-0.19, 1.46)	0.13	0.0	0.98	0.13
> 3.0 mmHg	10	-0.39 (-1.65, 0.88)	0.55	31.6	0.16	
Per mmHg	22	-0.24 (-0.43, -0.04)	0.019			

Abbreviations: HR, heart rate; SBP, systolic blood pressure [a] Cut-off point is based on median. [b] SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. [c] Studies using another potassium salt [35, 44] or not reporting which type of potassium salt used [40, 41] are excluded. [d] Data on pre-study HR is missing for Smith et al. [34]. [e] Data on pre-study potassium excretion is missing for Smith et al. [34], Berry et al. [50], and Svetkey et al. [42]. [f] Data on potassium excretion during potassium intervention is missing for Naismith and Braschi [49], and Svetkey et al. [42]. [g] Data on the change in potassium excretion is missing for Naismith and Braschi [49], and Svetkey et al. [42]. [h] Data on sodium excretion during potassium intervention is missing for Svetkey et al. [42].

DISCUSSION

In this meta-analysis of 22 RCTs, increasing potassium intake for at least two weeks had no effect on resting HR in apparently healthy adults. To our knowledge, this is the first meta-analysis evaluating the effects of an increased potassium intake on HR, based on double-blind, placebo-controlled trials with a high internal validity. None of the RCTs, however, examined HR as the primary outcome of interest and it may therefore be that these studies were underpowered to find an effect on HR. There was no evidence for publication bias, as indicated by the funnel plot that showed reasonable symmetry. For 5 published RCTs, we obtained data on HR after contacting the authors. However, the SEs of treatment effects, which were needed to compute weighing factors, had to be imputed for 17 RCTs. We assumed a correlation of 0.50 between baseline and end HR (parallel design) or HR after intervention and placebo period (crossover design), according to Follmann et al. [55]. This assumption may have influenced our pooled estimate, as the weight of the individual studies in the meta-

analysis is based on its SE. Also the precision of the pooled estimate may have been affected, as it is partly determined by the precision of the individual studies. However, similar results were obtained when correlation coefficients of 0.00, 0.65 or 0.80 were used.

In a recent potassium trial at our research center, we found a 4-mmHg lower 24-h systolic BP in healthy adults with untreated elevated BP [19], in line with previous meta-analyses of RCTs [18]. In that study, we also found a substantial and significant 2.6-bpm increase in 24-h HR [19]. Based on these findings, we hypothesized that an increase in HR may be caused by a shift in plasma electrolyte balance after increased potassium intake [59, 60] or that it could be a compensatory response to a reduction in effective circulating volume [61, 62]. The latter, however, was not confirmed by results from the present meta-analysis, because HR responses stratified by the magnitude of SBP reduction were small and not significantly different. Moreover, the effect of potassium on HR was not influenced by concomitant 24-h urinary sodium excretion. The observed increase in 24-h HR in our recent potassium trial, therefore, may be considered a chance finding.

The effect of BP lowering therapies on HR have been of concern in other studies. Robinson et al. [63] evaluated the effects of glucagon-like peptide-1 agonists in a meta-analysis of 32 RCTs with a minimum duration of 12 weeks. Compared to placebo, HR was increased significantly by 1.9 bpm, with a decrease in SBP of 1.8 mmHg. Based on a literature review, Toal et al. [64] reported in hypertensive populations small mean increases in HR (< 1 bpm) after at least one week of treatment with the calcium-channel blockers amlodipine and nifedipine. During the intervention with the calcium-channel blockers, increases in plasma norepinephrine, a marker of sympathetic nervous system activity, were observed [64]. These studies imply that BP reducing agents can adversely affect HR. Possibly, BP and HR are differently regulated by these agents than by potassium.

Subgroup analyses suggested an increased HR of 0.85 bpm of potassium supplementation in studies with an intervention duration of more than four weeks. Although, continuously no relationship was found between intervention duration and the potassium-induced effect on HR, it may be that potassium supplementation for a longer duration adversely affects HR. Studies with a longer intervention duration are needed to further explore this.

Resting HR may predict cardiovascular morbidity and mortality [1–3]. After a median follow up duration of 12 years, an increase in resting HR of 15 bpm was independently associated with a 24% and 32% higher cardiovascular mortality risk in 10 519 healthy men and 11 334 healthy women, respectively [1]. Another population-based study showed similar results: a 10 bpm increase in resting HR was associated with a 16% higher cardiovascular mortality risk in 6518 healthy subjects followed for 18 years [2]. Based on these epidemiological data, a persistent increase in HR of 1 bpm, which was the maximum effect observed in our meta-

analysis after stratification, is not expected to increase cardiovascular mortality risk by more than 3% over a period of 10 years.

From this meta-analysis, we conclude that increasing the intake of potassium by 2–3 g/day does not adversely affect HR in apparently healthy individuals. Potential adverse effects of potassium supplementation on HR in the long-term, albeit expected to be small, warrant further investigation.

Acknowlegdements
We thank Nancy Cook, Una Graham, Tom Sanders and Gloria Valdes for providing unpublished data for this meta-analysis.

Funding
The research is funded by TI Food and Nutrition, a public-private partnership on pre-competitive research in food and nutrition. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures
Authors declare that there are no conflicts of interest.

REFERENCES

1. Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham IM. Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J. 2010;159:612-9 e3.
2. Jensen MT, Marott JL, Allin KH, Nordestgaard BG, Jensen GB. Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. Eur J Prev Cardiol. 2012;19:102-8.
3. Khan H, Kunutsor S, Kalogeropoulos AP, Georgiopoulou VV, Newman AB, Harris TB, et al. Resting heart rate and risk of incident heart failure: three prospective cohort studies and a systematic meta-analysis. J Am Heart Assoc. 2015;4:e001364.
4. Ding F-H, Li Y, Li L-H, Wang J-G. Impact of heart rate on central hemodynamics and stroke: a meta-analysis of beta-blocker trials. Am J Hypertens. 2013;26:118-25.
5. McAlister FA, Wiebe N, Ezekowitz JA, Leung AA, Armstrong PW. Meta-analysis: beta-blocker dose, heart rate reduction, and death in patients with heart failure. Ann Intern Med. 2009;150:784-94.
6. Chen JM, Heran BS, Perez MI, Wright JM. Blood pressure lowering efficacy of beta-blockers as second-line therapy for primary hypertension. Cochrane Database Syst Rev. 2010;1.CD007185.
7. Drugs for cardiac arrhythmias. Treat Guidel Med Lett. 2007;5:51-8.
8. Stoelting RK. “Antihypertensive Drugs”, in Pharmacology and Physiology in Anesthetic Practice, Lippincott-Raven Publishers, 1999, 302-312.
9. Vrijkotte TG, van Doornen LJ, de Geus EJ. Effects of work stress on ambulatory blood pressure, heart rate, and heart rate variability. Hypertension. 2000;35:880-6.
10. Carter JB, Banister EW, Blaber AP. Effect of endurance exercise on autonomic control of heart rate. Sports Med. 2003;33:33-46.
11. Rennie KL, Hemingway H, Kumari M, Brunner E, Malik M, Marmot M. Effects of moderate and vigorous physical activity on heart rate variability in a British study of civil servants. Am J Epidemiol. 2003;158:135-43.
12. Wilmore JH, Stanforth PR, Gagnon J, Leon AS, Rao DC, Skinner JS, et al. Endurance exercise training has a minimal effect on resting heart rate: the HERITAGE Study. Med Sci Sports Exerc. 1996;28:829-35.
13. Persico AM. Persistent decrease in heart rate after smoking cessation: a 1-year follow-up study. Psychopharmacology (Berl). 1992;106:397-400.
14. Ohira T, Tanigawa T, Tabata M, Imano H, Kitamura A, Kiyama M, et al. Effects of habitual alcohol intake on ambulatory blood pressure, heart rate, and its variability among Japanese men. Hypertension. 2009;53:13-9.
15. Ryan JM, Howes LG. White coat effect of alcohol. Am J Hypertens. 2000;13:1135-8.
16. Garcia-Lopez M, Toledo E, Beunza JJ, Aros F, Estruch R, Salas-Salvado J, et al. Mediterranean diet and heart rate: the PREDIMED randomised trial. Int J Cardiol. 2014;171:299-301.
17. Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL, Katan MB. Effect of Fish Oil on Heart Rate in Humans: A Meta-Analysis of Randomized Controlled Trials. Circulation. 2005;112:1945-52.

18. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. Bmj. 2013;346:f1378.
19. Gijsbers L, Dower JI, Mensink M, Siebelink E, Bakker SJL, Geleijnse JM. Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens. 2015;29:592-8.
20. Matthesen SK, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest. 2012;72:78-86.
21. Khaw KT, Thom S. Randomised double-blind cross-over trial of potassium on blood-pressure in normal subjects. Lancet. 1982;2:1127-9.
22. Kaplan NM, Carnegie A, Raskin P, Heller JA, Simmons M. Potassium supplementation in hypertensive patients with diuretic-induced hypokalemia. N Engl J Med. 1985;312:746-9.
23. Sacks FM, Willett WC, Smith A, Brown LE, Rosner B, Moore TJ. Effect on blood pressure of potassium, calcium, and magnesium in women with low habitual intake. Hypertension. 1998;31:131-8.
24. Richards AM, Nicholls MG, Espiner EA, Ikram H, Maslowski AH, Hamilton EJ, et al. Blood-pressure response to moderate sodium restriction and to potassium supplementation in mild essential hypertension. Lancet. 1984;1:757-61.
25. Chalmers J, Morgan T, Doyle A, Dickson B, Hopper J, Mathews J, et al. Australian National Health and Medical Research Council dietary salt study in mild hypertension. J Hypertens Suppl. 1986;4:S629-37.
26. Brancati FL, Appel LJ, Seidler AJ, Whelton PK. Effect of potassium supplementation on blood pressure in African Americans on a low-potassium diet. A randomized, double-blind, placebo-controlled trial. Arch Intern Med. 1996;156:61-7.
27. Gu D, He J, Wu X, Duan X, Whelton PK. Effect of potassium supplementation on blood pressure in Chinese: a randomized, placebo-controlled trial. J Hypertens. 2001;19:1325-31.
28. Frassetto L, Morris RC, Jr., Sebastian A. Long-term persistence of the urine calcium-lowering effect of potassium bicarbonate in postmenopausal women. J Clin Endocrinol Metab. 2005;90:831-4.
29. Grimm RH, Kofron PM, Neaton JD, Svendsen KH, Elmer PJ, Holland L, et al. Effect of potassium supplementation combined with dietary sodium reduction on blood pressure in men taking antihypertensive medication. J Hypertens Suppl. 1988;6:S591-3.
30. Obel AO. Placebo-controlled trial of potassium supplements in black patients with mild essential hypertension. J Cardiovasc Pharmacol. 1989;14:294-6.
31. Patki PS, Singh J, Gokhale SV, Bulakh PM, Shrotri DS, Patwardhan B. Efficacy of potassium and magnesium in essential hypertension: a double-blind, placebo controlled, crossover study. Bmj. 1990;301:521-3.
32. Braschi A, Naismith DJ. The effect of a dietary supplement of potassium chloride or potassium citrate on blood pressure in predominantly normotensive volunteers. Br J Nutr. 2008;99:1284-92.
33. MacGregor GA, Smith SJ, Markandu ND, Banks RA, Sagnella GA. Moderate potassium supplementation in essential hypertension. Lancet. 1982;2:567-70.

34. Smith SJ, Markandu ND, Sagnella GA, MacGregor GA. Moderate potassium chloride supplementation in essential hypertension: is it additive to moderate sodium restriction? Br Med J (Clin Res Ed). 1985;290:110-3.
35. Sundar S, Sachdev KK, Vaish SK, Bhattacharya SK, Singh VP, Agarwal SK. Potassium supplementation in essential hypertension--a double blind placebo controlled study. J Assoc Physicians India. 1985;33:776-7.
36. Zoccali C, Cumming AM, Hutcheson MJ, Barnett P, Semple PF. Effects of potassium on sodium balance, renin, noradrenaline and arterial pressure. J Hypertens. 1985;3:67-72.
37. Barden AE, Vandongen R, Beilin LJ, Margetts B, Rogers P. Potassium supplementation does not lower blood pressure in normotensive women. J Hypertens. 1986;4:339-43.
38. Matlou SM, Isles CG, Higgs A, Milne FJ, Murray GD, Schultz E, et al. Potassium supplementation in blacks with mild to moderate essential hypertension. J Hypertens. 1986;4:61-4.
39. Poulter NR, Sever PS. Moderate potassium supplementation: ineffective in black normotensives. East Afr Med J. 1986;63:798-802.
40. Grobbee DE, Hofman A, Roelandt JT, Boomsma F, Schalekamp MA, Valkenburg HA. Sodium restriction and potassium supplementation in young people with mildly elevated blood pressure. J Hypertens. 1987;5:115-9.
41. Siani A, Strazzullo P, Russo L, Guglielmi S, Iacoviello L, Ferrara LA, et al. Controlled trial of long term oral potassium supplements in patients with mild hypertension. Br Med J (Clin Res Ed). 1987;294:1453-6.
42. Svetkey LP, Yarger WE, Feussner JR, DeLong E, Klotman PE. Double-blind, placebo-controlled trial of potassium chloride in the treatment of mild hypertension. Hypertension. 1987;9:444-50.
43. Mullen JT, O'Connor DT. Potassium effects on blood pressure: is the conjugate anion important? J Hum Hypertens. 1990;4:589-96.
44. Overlack A, Conrad H, Stumpe KO. The influence of oral potassium citrate/bicarbonate on blood pressure in essential hypertension during unrestricted salt intake. Klin Wochenschr. 1991;69 Suppl 25:79-83.
45. Valdes G, Vio CP, Montero J, Avendano R. Potassium supplementation lowers blood pressure and increases urinary kallikrein in essential hypertensives. J Hum Hypertens. 1991;5:91-6.
46. Fotherby MD, Potter JF. Potassium supplementation reduces clinic and ambulatory blood pressure in elderly hypertensive patients. J Hypertens. 1992;10:1403-8.
47. Overlack A, Maus B, Ruppert M, Lennarz M, Kolloch R, Stumpe KO. [Potassium citrate versus potassium chloride in essential hypertension. Effects on hemodynamic, hormonal and metabolic parameters]. Dtsch Med Wochenschr. 1995;120:631-5.
48. Whelton PK, Buring J, Borhani NO, Cohen JD, Cook N, Cutler JA, et al. The effect of potassium supplementation in persons with a high-normal blood pressure. Results from phase I of the Trials of Hypertension Prevention (TOHP). Trials of Hypertension Prevention (TOHP) Collaborative Research Group. Ann Epidemiol. 1995;5:85-95.
49. Naismith DJ, Braschi A. The effect of low-dose potassium supplementation on blood pressure in apparently healthy volunteers. Br J Nutr. 2003;90:53-60.

50. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr. 2010;104:1839-47.
51. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD, et al. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension. 2010;55:681-8.
52. Graham UM, McCance DR, Young IS, Mullan KR. A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens. 2014;28:333-9.
53. Higgins JPT, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj. 2011;343:d5928.
54. Streppel MT, Arends LR, van 't Veer P, Grobbee DE, Geleijnse JM. Dietary fiber and blood pressure: A meta-analysis of randomized placebo-controlled trials. Arch Intern Med. 2005;165:150-6.
55. Follmann D, Elliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. Journal of Clinical Epidemiology. 1992;45:769-73.
56. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88.
57. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statist Med. 2002;21:1539-58.
58. Egger M, Smith GD, Phillips AN. Meta-analysis: Principles and procedures. 1997;315:1533-7.
59. Kovesdy CP. Management of Hyperkalemia: An Update for the Internist. Am J Med. 2015;128:1281-7.
60. Almukdad H. Hyperkalemia revisited. Saudi J Kidney Dis Transpl. 2007;18:577-84.
61. Lohmeier TE, Iliescu R. The baroreflex as a long-term controller of arterial pressure. Physiology (Bethesda). 2015;30:148-58.
62. Mancia G, Parati G, Pomidossi G, Casadei R, Di Rienzo M, Zanchetti A. Arterial baroreflexes and blood pressure and heart rate variabilities in humans. Hypertension. 1986;8:147-53.
63. Robinson LE, Holt TA, Rees K, Randeva HS, O'Hare JP. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. BMJ Open. 2013;3.
64. Toal CB, Meredith PA, Elliott HL. Long-acting dihydropyridine calcium-channel blockers and sympathetic nervous system activity in hypertension: A literature review comparing amlodipine and nifedipine GITS. Blood Press. 2012;21:3-10.

SUPPLEMENTAL METHODS

Imputing SE of the Treatment Effect for Crossover Trials

When SE of HR after intervention and placebo period are given, the following formula is used to obtain the SE of the HR treatment effect in crossover trials:

$$SE(\bar{d}) = \sqrt{(SE\ \bar{x}^{\text{post-T2}}) + (SE\ \bar{x}^{\text{post-P2}}) - (2x\rho x(SE\ \bar{x}^{\text{post-T}}) \times (SE\ \bar{x}^{\text{post-P}}))}$$

$SE(x^{\text{post-T}})$ = standard error at the end of the treatment period
$SE(x^{\text{post-P}})$ = standard error at the end of the control period
ρ = correlation coefficient, according to Follmann et al. [1] we assumed a correlation of 0.50 between HR after intervention and control period
$SE(d)$ = standard error of the treatment effect

When SD of HR after intervention and placebo period are given, the following formula is used to obtain the SE of the HR treatment effect in crossover trials:

$$SE(\bar{d}) = \sqrt{\frac{(SD\ \bar{x}^{\text{post-T2}}) + (SD\ \bar{x}^{\text{post-P2}}) - (2x\rho x(SD\bar{x}^{\text{post-T}}) \times (SD\ \bar{x}^{\text{post-P}}))}{\sqrt{N\ \text{treatment}}}}$$

$SD(x^{\text{post-T}})$ = standard deviation at the end of the treatment period
$SD(x^{\text{post-P}})$ = standard deviation at the end of the control period
ρ = correlation coefficient, according to Follmann et al. [1] we assumed a correlation of 0.50 between HR after intervention and control period
N treatment = number of subjects in treatment period
$SE(d)$ = standard error of the treatment effect

Imputing SE of the Treatment Effect for Parallel Trials

1. Calculation of the SE of the difference in HR in both the treatment and control group

When SE of baseline and end measurements are given, the following formula is used to obtain the SE of HR change in both the treatment and control group:

$$SE(\bar{x}_i) = \sqrt{(SE\ \bar{x}_i^{\text{base}})^2 + (SE\ \bar{x}_i^{\text{end}})^2 - (2x\rho x(SE\ \bar{x}_i^{\text{base}}) \times (SE\ \bar{x}_i^{\text{end}}))}$$

$SE(x^{\text{base}})$ = standard error at the baseline
$SE(x^{\text{end}})$ = standard error at the end
ρ = correlation coefficient, according to Follmann et al. [1] we assumed a correlation of 0.50 between baseline and end HR
$SE(x_i)$ = standard error of the difference

When SD of baseline and end measurements are given, the following formula is used to obtain the SE of HR in both the treatment and control group:

$$SE(\bar{x}_i) = \sqrt{\frac{(SD\ \bar{x}_{i\ base})^2 + (SD\ \bar{x}_{i\ end})^2 - (2x\rho x(SD\ \bar{x}_{i\ base}) \times (SD\ \bar{x}_{i\ end}))}{\sqrt{N_i}}}$$

$SD(x^{base})$ = standard error at the baseline
$SD(x^{end})$ = standard error at the end
ρ = correlation coefficient, according to Follmann et al. [1] we assumed a correlation of 0.50 between baseline and end HR
N_i = number of subjects in the group
$SE(x_i)$ = standard error of the difference

2. Calculation of the SE of the HR treatment effect for parallel trials

When the SE of HR change in both the treatment and control group are reported or estimated (see formulae at point 1), the following formula is used to obtain the SE of the HR treatment effect:

$$SE(\bar{d}) = \sqrt{(SE\ \bar{x}^{T})^2 + (SE\ \bar{x}^{P})^2}$$

$SE(x^T)$ = standard error of the difference in the treatment group
$SD(x^P)$ = standard error at the difference in the control group
SE(d) = standard error of the treatment effect

SUPPLEMENTAL RESULTS

SUPPLEMENTAL TABLE 1. Search terms and number of citations retrieved via PubMed

Step	Search terms	# Citations[a]
1	Potassium[MeSH] OR Potassium[tiab] OR potassium*[tiab]	169.746
2	Diet[MeSH] OR Dietary Supplements[MeSH] OR diet*[tiab] OR supplement* [tiab] OR tablet[tiab] OR capsule[tiab] OR intake*[tiab] OR consumption[tiab]	913.385
3	Blood Pressure[MeSH] OR Hypertension[MeSH] OR Heart Rate[MeSH] OR pressure*[tiab] OR Hypertension[tiab] OR heart rate[tiab] OR pulse[tiab]	1.143.429
4	Randomized controlled trial[pt] OR clinical trial[pt] OR random*[tiab] OR trial[tiab] OR placebo[tiab] OR intervention[tiab] OR group[tiab]	2.989.201
5	#1 AND #2 AND #3 AND #4	1.592

[a] Search conducted at 31 October 2014.

SUPPLEMENTAL TABLE 2. Descriptives of the 22 randomized controlled trials evaluating the effect of an increased potassium intake on heart rate[a]

	Mean ± SD[b]
Design, no. of RCTs	
Parallel	5
Crossover	17
Total no. of subjects	1086
Men	698
Women	399
Age (years)	44.6 ± 12.5
Pre-study HR (bpm)[c]	70.2 ± 5.4
Pre-study SBP (mmHg)	142.7 ± 19.3
Pre-study DBP (mmHg)	88.5 ± 12.3
Intervention duration (weeks)[d]	4 (2 - 24)
Potassium dose (grams/day)[d]	2.5 (0.9 - 4.7)
Potassium type, no. of RCTs	
Potassium chloride	17
Potassium citrate	1
Mix of potassium chloride and bicarbonate	1
Mix of potassium citrate and bicarbonate	1
Unspecified	2
HR measurement, no. of RCTs	
Office supine HR	14
Office seated HR	7
24-hr ambulatory HR	1
Antihypertensive medication use during study, no. of RCTs	
No	18
Yes, treated during study when necessary	1
Not reported	3

Abbreviations: DBP, diastolic blood pressure; HR, heart rate; no, number; RCTs, randomized controlled trials; SBP, systolic blood pressure. [a] Descriptives are based on the study data included in the primary meta-analysis. [b] Unless indicated otherwise. [c] Data on pre-study HR is missing for Smith et al. [2]. [d] Value is median (range).

SUPPPLEMENTAL TABLE 3. Risk of bias assessment[a] of the 22 randomized controlled trials evaluating the effect of an increased potassium intake on heart rate

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants or personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)
Barden et al. [3]	?	?	?	?	+
Berry et al. [4]	+	+	+	+	+
Fotherby and Potter [5]	?	?	+	+	+
Gijsbers et al. [6]	+	+	+	+	+
Graham et al. [7]	+	+	+	+	+
Grobbee et al. [8]	?	?	+	?	?
He et al. [9]	+	+	+	+	+
MacGregor et al. [10]	?	?	+	?	+
Matlou et al. [11]	?	?	-	+	+
Matthesen et al. [12]	+	+	?	?	-
Mullen and O'Connor [13]	?	?	+	+	-
Naismith and Braschi [14]	+	+	+	+	+
Overlack et al. [15]	?	?	-	+	+
Overlack et al. [16]	?	?	-	+	?
Poulter and Sever [17]	?	?	?	?	-
Siani et al. [18]	?	+	+	+	?
Smith et al. [2]	?	?	+	+	+
Sundar et al. [19]	?	?	?	?	?
Svetkey et al. [20]	+	+	+	+	+
Valdés et al. [21]	?	?	+	?	?
Whelton et al. [22]	?	?	+	+	+
Zoccali et al. [23]	?	?	-	?	+

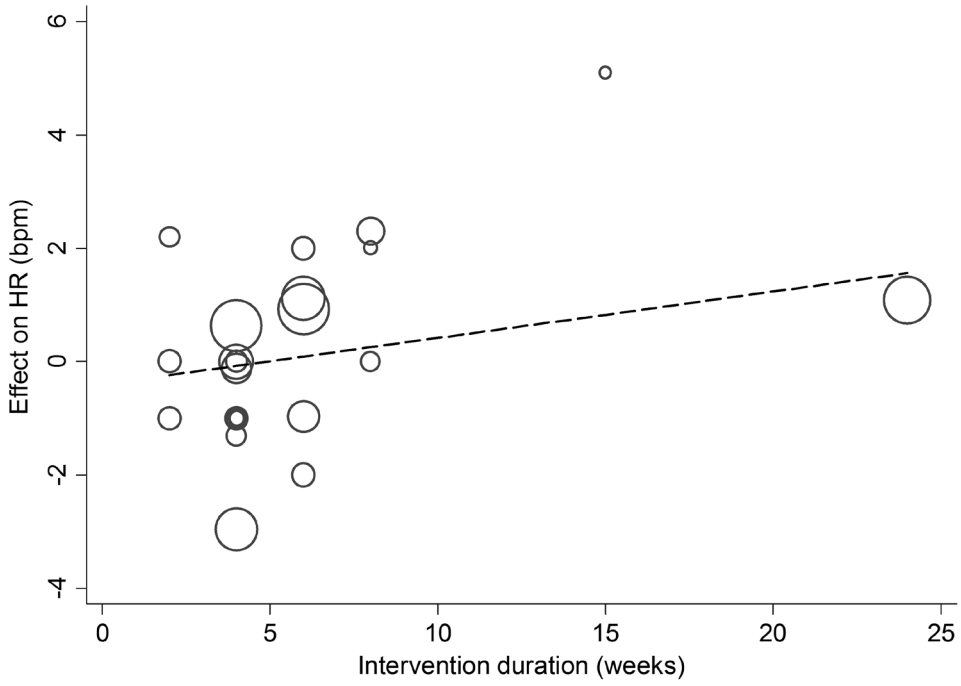
[a] Studies are low (+), unclear (?) or high (-) at risk of bias.

SUPPLEMENTAL TABLE 4. Descriptives[a] of the subgroups evaluating the effect of an increased potassium intake on heart rate

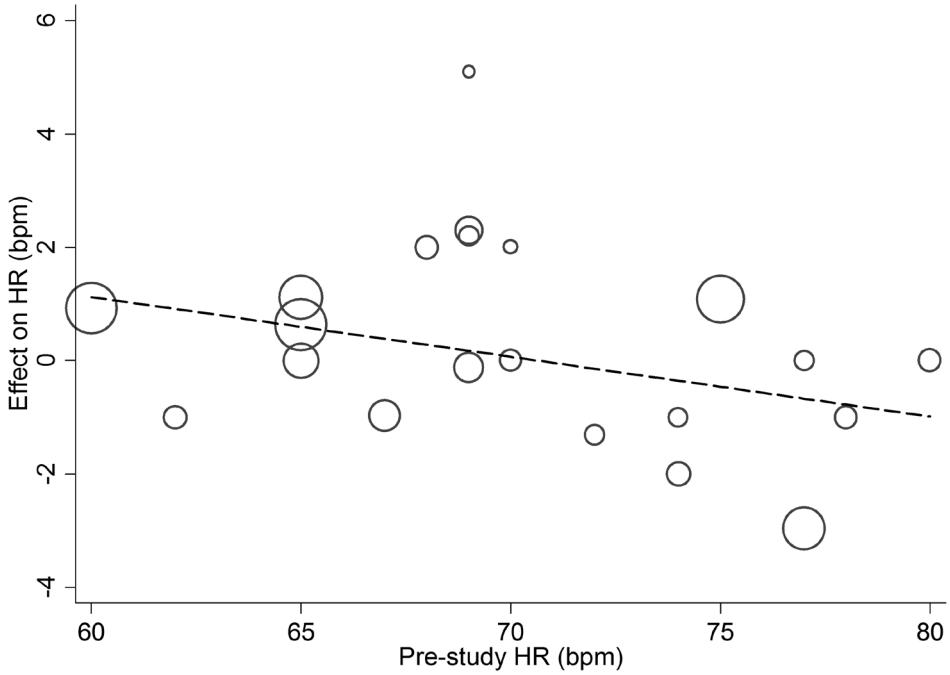
	Number of studies	Age	Intervention duration	Potassium dose	Pre-study HR	Potassium excretion before potassium intervention	Potassium excretion during potassium intervention	Change in potassium excretion	Sodium excretion during potassium intervention	Reduction in SBP
Overall	22	44.6 ± 12.5	6.1 ± 4.9	2.8 ± 1.0	70.2 ± 5.4[d]	67.2 ± 13.5[e,f,g]	118.7 ± 31.4[h,i]	54.8 ± 26.5[k,l]	145.7 ± 42.5[m]	4.2 ± 4.1
Study design										
Parallel	5	45.6 ± 3.3	11.4 ± 8.2	2.4 ± 1.4	73.0 ± 4.7	64.8 ± 12.9[g]	88.3 ± 8.0[h,i]	32.8 ± 8.4[k,l]	148.9 ± 39.5[m]	7.7 ± 5.3
Crossover	17	44.4 ± 14.2	4.6 ± 1.8	3.0 ± 0.8	69.4 ± 5.4[d]	67.8 ± 14.1[e,f]	124.0 ± 30.9	58.7 ± 26.8	144.9 ± 44.3	3.2 ± 3.1
Gender										
Men	2	29.0 ± 5.7	2.0 ± 0.0	2.7 ± 0.3	65.5 ± 4.9	58.0 ± 26.9	89.6 ± 14.7	30.4 ± 10.5	127.4 ± 19.3	0.6 ± 0.8
Women	2	41.5 ± 13.4	5.0 ± 1.4	2.8 ± 0.4	73.0 ± 1.4	56.5 ± 7.8	117.3 ± 4.6	64.5 ± 3.5	152.5 ± 17.7	4.2 ± 4.0
Mixed[e]	18	46.7 ± 12.1	6.7 ± 5.2	2.8 ± 1.1	70.5 ± 5.5[d]	69.8 ± 12.0[e,f,g]	122.5 ± 33.2[h,i]	56.6 ± 28.1[k,l]	147.0 ± 46.5[m]	4.6 ± 4.2
Age[b]										
≤ 45 years	12	36.3 ± 8.2	6.9 ± 6.5	2.8 ± 1.1	70.0 ± 5.8	63.9 ± 12.9[e]	117.6 ± 31.1[h]	56.2 ± 27.5[k]	149.6 ± 38.2	2.8 ± 4.4
> 45 years	10	54.6 ± 9.0	5.2 ± 1.7	2.9 ± 0.9	70.6 ± 5.1[d]	71.6 ± 13.9[f,g]	120.0 ± 33.5[i]	53.1 ± 26.8[l]	140.4 ± 49.5[m]	5.8 ± 3.0
Hypertensive population										
No	5	31.8 ± 7.2	7.2 ± 9.4	3.0 ± 0.6	69.6 ± 4.8	60.4 ± 16.3	112.9 ± 34.1	52.4 ± 27.2	147.5 ± 31.3	0.5 ± 0.7
Mixed	6	50.5 ± 8.6	5.7 ± 1.5	2.5 ± 1.3	67.2 ± 5.7	79.0 ± 14.0[e]	118.4 ± 48.9[h]	48.2 ± 37.9[k]	143.7 ± 56.6	5.5 ± 3.4
Yes[c]	11	47.3 ± 12.6	5.9 ± 3.5	3.0 ± 1.0	72.4 ± 4.9[d]	64.3 ± 7.4[f,g]	121.7 ± 21.7[i]	59.3 ± 21.6[l]	145.9 ± 42.4[m]	5.2 ± 4.4
Intervention duration[b]										
≤ 4 weeks	12	45.0 ± 15.3	3.5 ± 0.9	2.8 ± 0.6	71.0 ± 5.8[d]	64.3 ± 13.6[f]	115.4 ± 24.2	54.0 ± 21.2	138.9 ± 38.3	3.8 ± 3.8
> 4 weeks	10	44.2 ± 8.7	9.3 ± 5.9	2.9 ± 1.4	69.4 ± 5.0	71.1 ± 13.2[e,g]	123.6 ± 41.3[h,i]	56.0 ± 34.6[k,l]	154.7 ± 48.4[m]	4.6 ± 4.5
Potassium dose[b]										
≤ 2.5 grams/day	12	48.7 ± 10.1	6.9 ± 6.3	2.2 ± 0.5	69.8 ± 5.6[d]	64.5 ± 13.6[e,f]	101.3 ± 16.6[h]	39.8 ± 14.8[k]	137.7 ± 32.4	5.7 ± 4.4
> 2.5 grams/day	10	39.8 ± 13.9	5.2 ± 2.3	3.6 ± 0.9	70.7 ± 5.4	70.1 ± 13.6[g]	139.9 ± 32.7[i]	73.1 ± 26.7[l]	156.2 ± 53.4[m]	2.4 ± 2.9
Potassium salt type										
Potassium chloride	17	46.2 ± 13.1	5.6 ± 5.1	2.9 ± 0.9	70.7 ± 5.3[d]	68.6 ± 14.7[g,f]	121.4 ± 30.1[h,i]	56.7 ± 24.8[k,l]	152.2 ± 40.1[m]	3.8 ± 3.1
Potassium citrate	3	39.3 ± 12.5	5.3 ± 3.1	3.1 ± 1.6	63.7 ± 4.7	85.5 ± 12.0[e]	140.9 ± 73.6	64.0 ± 58.1	163.4 ± 66.9	3.5 ± 3.0
Potassium bicarbonate	1	51.0	4.0	2.5	65.0	80.0	125.0	48.0	129.0	1.0

HR measurement										
Office supine	14	45.4 ± 14.3	5.2 ± 3.2	2.7 ± 0.8	69.9 ± 6.1[d]	65.2 ± 10.1[e,f]	113.7 ± 23.1	50.6 ± 25.3	135.2 ± 39.3	4.5 ± 4.6
Office standing	7	44.3 ± 16.0	5.0 ± 4.5	2.7 ± 0.7	80.9 ± 4.1	62.0 ± 8.4	112.4 ± 17.8	52.0 ± 21.7	162.3 ± 24.1	4.8 ± 4.2
Office seated HR	7	45.7 ± 6.7	8.3 ± 7.3	2.9 ± 1.4	70.9 ± 4.5	69.7 ± 20.1[g]	122.8 ± 47.2[h,i]	59.0 ± 45.2[k,l]	161.1 ± 45.2[m]	4.2 ± 3.0
24-hr ambulatory HR	4	53.0 ± 22.0	4.5 ± 1.0	2.7 ± 1.0	71.8 ± 5.3	73.7 ± 9.7[e]	118.0 ± 35.7	55.2 ± 28.7	136.1 ± 44.9	2.1 ± 3.6
Pre-study HR[d]										
≤ 70 bpm	13	42.2 ± 12.6	5.8 ± 3.4	2.8 ± 1.1	66.8 ± 3.2	71.6 ± 15.0[e]	124.0 ± 37.7[h]	56.0 ± 31.9[k]	148.7 ± 46.4	3.6 ± 4.1
> 70 bpm	8	47.6 ± 12.8	7.0 ± 7.1	3.0 ± 0.9	75.9 ± 2.6	59.6 ± 5.4[g]	109.8 ± 19.0[i]	53.4 ± 18.7[l]	149.3 ± 31.1[m]	5.5 ± 4.2
Potassium excretion before potassium intervention[e,f,g]										
≤ 65 mmol/24h	10	45.0 ± 12.4	7.3 ± 7.0	2.8 ± 0.9	72.9 ± 3.8	56.5 ± 7.0	110.6 ± 27.8	55.8 ± 25.1	150.1 ± 30.9	5.0 ± 5.4
> 65 mmol/24h	9	42.6 ± 14.7	4.9 ± 1.8	2.8 ± 1.0	67.7 ± 4.6	79.0 ± 7.6	132.9 ± 34.8[h]	57.6 ± 31.2[k]	151.6 ± 52.0	3.6 ± 2.7
Potassium excretion during potassium intervention[h,i]										
≤ 120 mmol/24h	12	48.5 ± 13.3	6.8 ± 6.4	2.4 ± 0.4	69.8 ± 6.2[d]	64.5 ± 12.8[e,f]	100.1 ± 14.4	39.4 ± 15.6	132.4 ± 33.1	5.1 ± 4.6
> 120 mmol/24h	8	38.3 ± 10.6	5.0 ± 2.1	3.5 ± 0.9	70.4 ± 4.4	68.4 ± 14.5	146.5 ± 29.4	77.9 ± 22.6	162.9 ± 52.3	2.1 ± 2.6
Change in potassium excretion[k,l]										
≤ 50 mmol/24h	10	47.1 ± 13.4	7.1 ± 7.0	2.3 ± 0.4	68.4 ± 6.0[d]	64.1 ± 14.0[e,f]	97.3 ± 14.4	33.7 ± 10.7	129.3 ± 30.3	4.8 ± 5.0
> 50 mmol/24h	10	41.7 ± 12.8	5.0 ± 1.9	3.3 ± 0.9	71.5 ± 4.6	67.9 ± 13.1	140.0 ± 29.3	75.9 ± 19.5	160.0 ± 50.2	3.1 ± 3.1
Sodium excretion during potassium intervention[m,n]										
≤ 140 mmol/24h	9	47.2 ± 16.4	4.2 ± 1.2	2.5 ± 0.4	68.8 ± 5.5[d]	63.1 ± 15.7[e,f]	106.1 ± 19.7	45.8 ± 14.7	108.8 ± 25.3	4.0 ± 3.8
> 140 mmol/24h	12	42.2 ± 9.3	7.4 ± 6.3	2.9 ± 1.1	70.7 ± 5.2	69.5 ± 12.2	128.9 ± 36.1[h]	62.2 ± 32.1[k]	173.3 ± 29.5	4.2 ± 4.5
Reduction in SBP[b]										
≤ 3.0 mmHg	12	39.4 ± 12.8	5.8 ± 6.0	3.0 ± 0.8	68.7 ± 5.7[d]	65.3 ± 14.1[e,f]	120.5 ± 28.1	57.5 ± 25.3	131.8 ± 40.4	1.1 ± 1.5
> 3.0 mmHg	10	50.9 ± 9.2	6.5 ± 3.4	2.7 ± 1.2	71.9 ± 4.7	69.2 ± 13.4[g]	116.0 ± 37.7[h,i]	50.7 ± 29.5[k,l]	164.2 ± 39.9[m]	7.9 ± 2.9

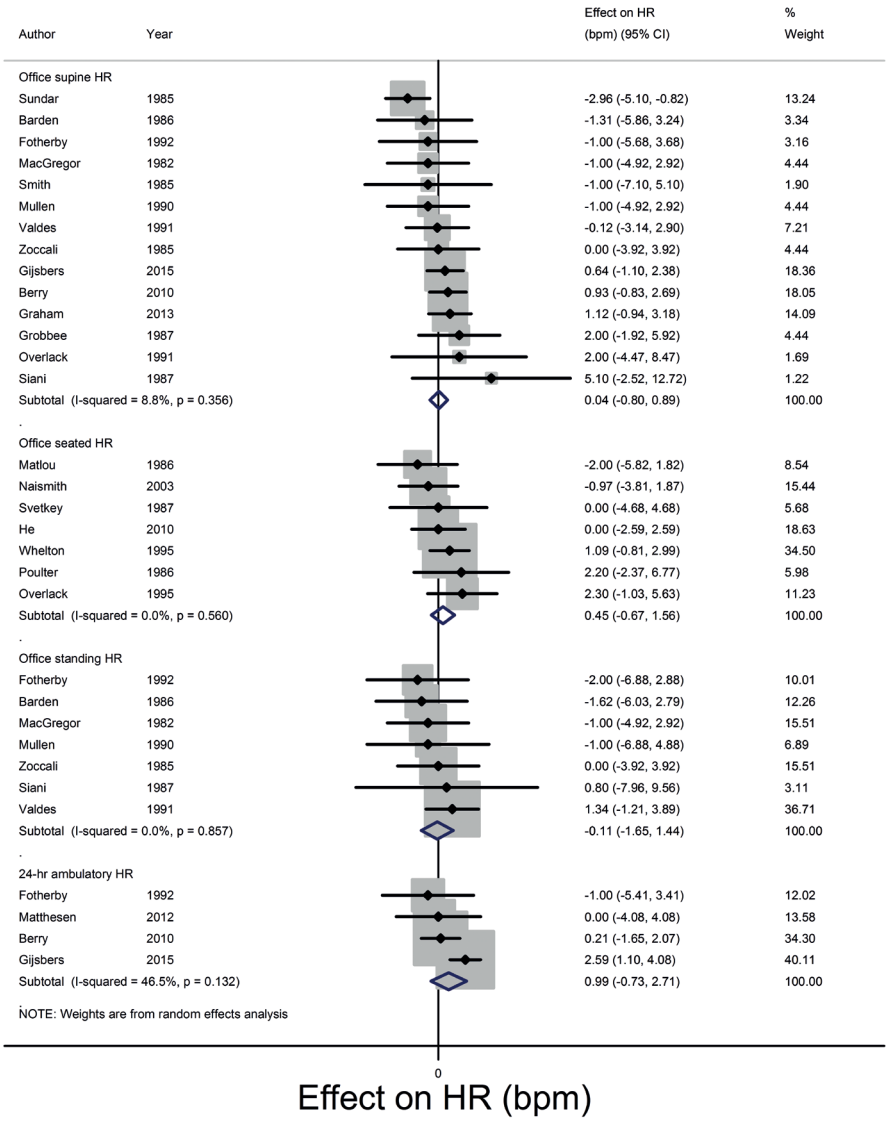
Abbreviations: HR, heart rate; SBP, systolic blood pressure. [a] Values are mean ± SD unless stated otherwise. [b] Cut-off points based on median. [c] SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg. [d] Data on pre-study HR is missing for Smith et al. [2]. [e] Data on potassium excretion before the intervention is missing for Berry et al. [20]. [f] Data on potassium excretion before the intervention is missing for Smith et al. [2]. [g] Data on potassium excretion before the intervention is missing for Svetkey et al. [20]. [h] Data on potassium excretion during potassium intervention is missing for Naismith and Braschi [14]. [i] Data on potassium excretion during potassium intervention is missing for Svetkey et al. [20]. [j] Multiply by 1.3 to account for non-urinary losses. [k] Data on the change in potassium excretion is missing for Naismith and Braschi [14]. [l] Data on the change in potassium excretion is missing for Svetkey et al. [20]. [m] Data on sodium excretion during potassium intervention is missing for Svetkey et al. [20]. [n] Multiply by 1.1 to account for non-urinary losses.



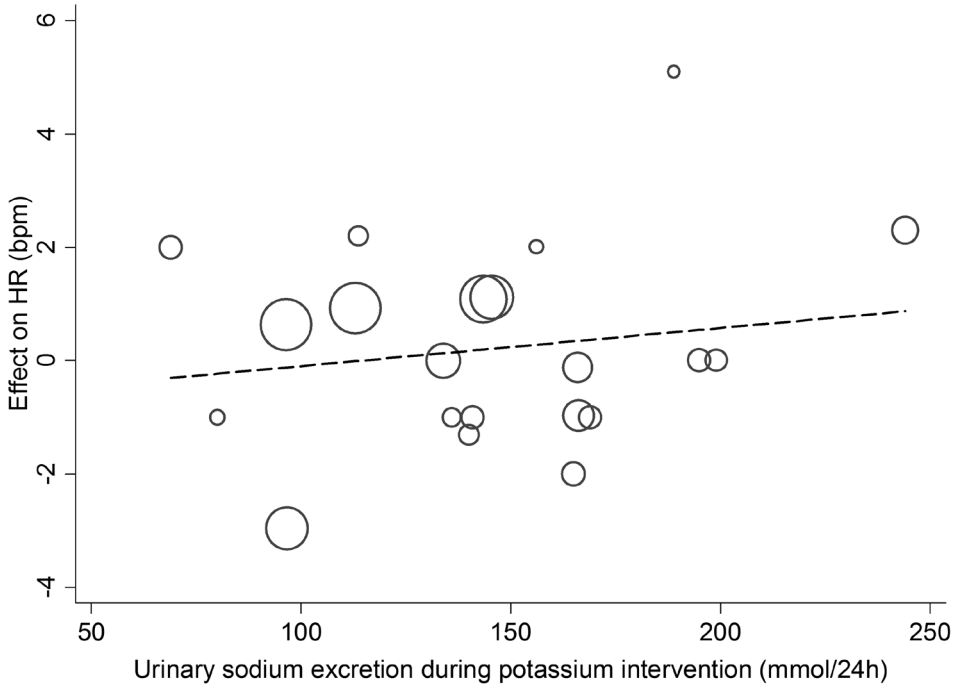
SUPPLEMENTAL FIGURE 1. Meta-regression of 22 randomized controlled trials exploring the relationship of potassium intervention duration with the effect on heart rate. Every circle indicates the point estimate for each trial. The size of the circle is proportional to the weight of the study, using a random-effects model. The dotted line is the linear regression line based on the weighted estimates of all included studies. Abbreviation: HR, heart rate.



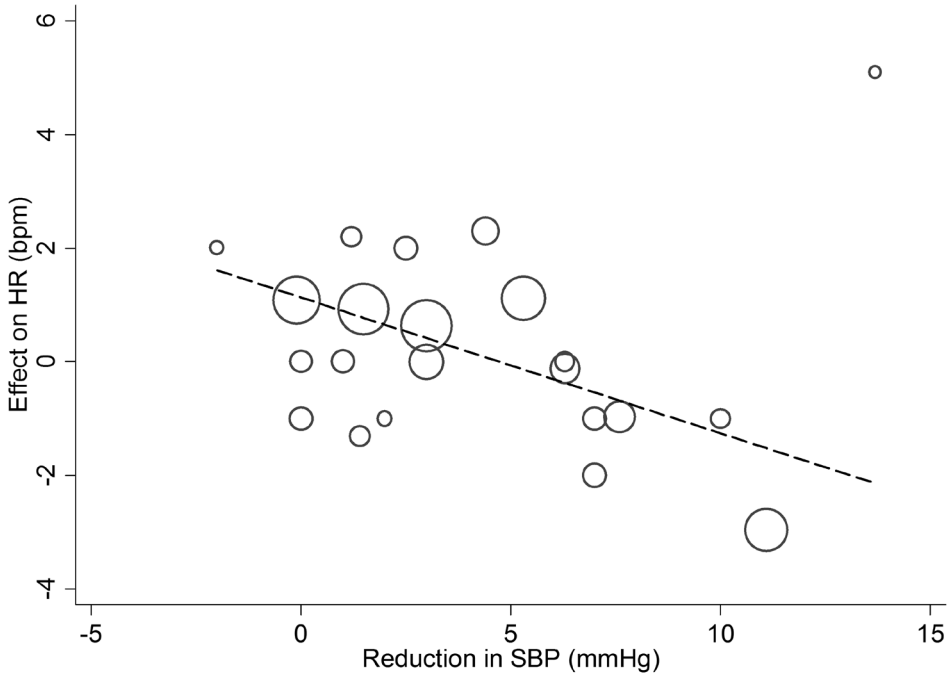
SUPPLEMENTAL FIGURE 2. Meta-regression of 21[a] randomized controlled trials exploring the relationship of pre-study heart rate with the effect on heart rate. Every circle indicates the point estimate for each trial. The size of the circle is proportional to the weight of the study, using a random-effects model. The dotted line is the linear regression line based on the weighted estimates of all included studies. [a] Data on pre-study heart rate is missing for Smith et al. [2]. Abbreviation: HR, heart rate.



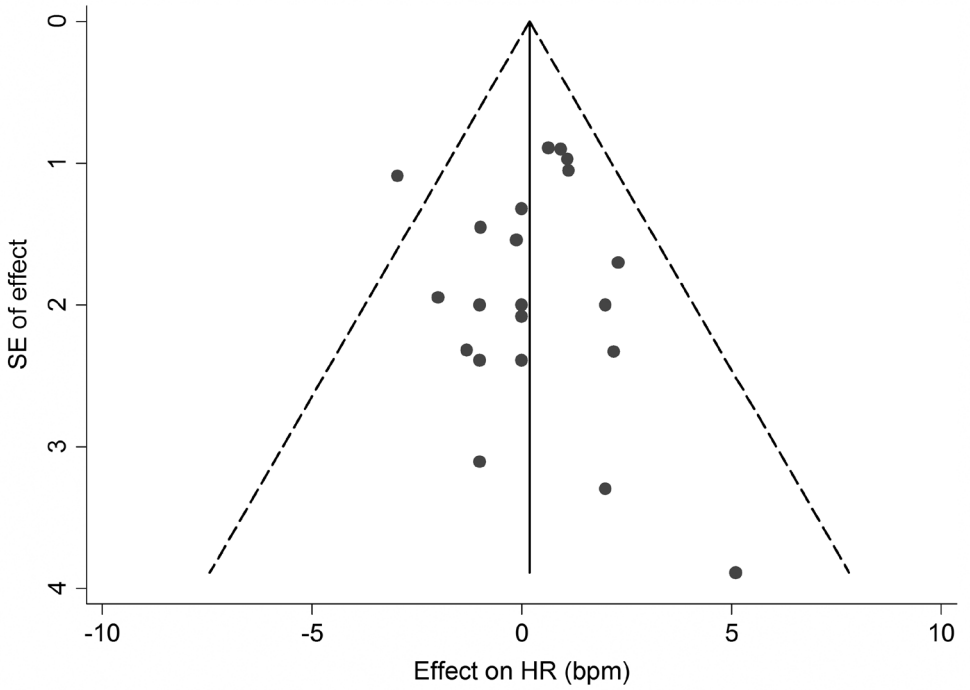
SUPPLEMENTAL FIGURE 3. Forest plot of 22 randomized controlled trials evaluating the effect of potassium supplementation on heart rate, stratified by heart rate measurement. Number of observations exceeds 22, because in some trials heart rate was measured in multiple positions. Black bullets indicate the point estimate for each trial, the horizontal lines represent the 95% CIs. The size of the square is proportional to the weight of the study, using a random-effects model. The stratum-specific treatment effect is indicated by the diamond, and its width corresponds with the 95% CI. Abbreviation: HR, heart rate.



SUPPLEMENTAL FIGURE 4. Meta-regression of 21[a] randomized controlled trials exploring the relationship of urinary sodium excretion during potassium intervention with the effect on heart rate. Every circle indicates the point estimate for each trial. The size of the circle is proportional to the weight of the study, using a random-effects model. The dotted line is the linear regression line based on the weighted estimates of all included studies. [a] Data on sodium excretion during potassium intervention is missing for Svetkey et al. [20]. Abbreviation: HR, heart rate.



SUPPLEMENTAL FIGURE 5. Meta-regression of 22 randomized controlled trials exploring the relationship of the reduction in systolic blood pressure with the effect on heart rate. Every circle indicates the point estimate for each trial. The size of the circle is proportional to the weight of the study, using a random-effects model. The dotted line is the linear regression line based on the weighted estimates of all included studies. Abbreviation: HR, heart rate; SBP, systolic blood pressure.



SUPPLEMENTAL FIGURE 6. Funnel plot of 22 randomized controlled trials evaluating the effect of potassium supplementation on heart rate. Every bullet indicates the effect on heart rate and SE for each trial. The overall effect of potassium on heart rate is indicated with the vertical line and the dotted lines represent the pseudo-95% confidence limits. Abbreviation: HR, heart rate; SE, standard error

SUPPLEMENTAL REFERENCES

1. Follmann D, Elliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. Journal of Clinical Epidemiology. 1992;45:769-73.
2. Smith SJ, Markandu ND, Sagnella GA, MacGregor GA. Moderate potassium chloride supplementation in essential hypertension: is it additive to moderate sodium restriction? Br Med J (Clin Res Ed). 1985;290:110-3.
3. Barden AE, Vandongen R, Beilin LJ, Margetts B, Rogers P. Potassium supplementation does not lower blood pressure in normotensive women. J Hypertens. 1986;4:339-43.
4. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr. 2010;104:1839-47.
5. Fotherby MD, Potter JF. Potassium supplementation reduces clinic and ambulatory blood pressure in elderly hypertensive patients. J Hypertens. 1992;10:1403-8.
6. Gijsbers L, Dower JI, Mensink M, Siebelink E, Bakker SJL, Geleijnse JM. Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens. 2015;29:592-8.
7. Graham UM, McCance DR, Young IS, Mullan KR. A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens. 2014;28:333-9.
8. Grobbee DE, Hofman A, Roelandt JT, Boomsma F, Schalekamp MA, Valkenburg HA. Sodium restriction and potassium supplementation in young people with mildly elevated blood pressure. J Hypertens. 1987;5:115-9.
9. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD, et al. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension. 2010;55:681-8.
10. MacGregor GA, Smith SJ, Markandu ND, Banks RA, Sagnella GA. Moderate potassium supplementation in essential hypertension. Lancet. 1982;2:567-70.
11. Matlou SM, Isles CG, Higgs A, Milne FJ, Murray GD, Schultz E, et al. Potassium supplementation in blacks with mild to moderate essential hypertension. J Hypertens. 1986;4:61-4.
12. Matthesen SK, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest. 2012;72:78-86.
13. Mullen JT, O'Connor DT. Potassium effects on blood pressure: is the conjugate anion important? J Hum Hypertens. 1990;4:589-96.
14. Naismith DJ, Braschi A. The effect of low-dose potassium supplementation on blood pressure in apparently healthy volunteers. Br J Nutr. 2003;90:53-60.
15. Overlack A, Conrad H, Stumpe KO. The influence of oral potassium citrate/bicarbonate on blood pressure in essential hypertension during unrestricted salt intake. Klin Wochenschr. 1991;69 Suppl 25:79-83.

16. Overlack A, Maus B, Ruppert M, Lennarz M, Kolloch R, Stumpe KO. [Potassium citrate versus potassium chloride in essential hypertension. Effects on hemodynamic, hormonal and metabolic parameters]. Dtsch Med Wochenschr. 1995;120:631-5.
17. Poulter NR, Sever PS. Moderate potassium supplementation: ineffective in black normotensives. East Afr Med J. 1986;63:798-802.
18. Siani A, Strazzullo P, Russo L, Guglielmi S, Iacoviello L, Ferrara LA, et al. Controlled trial of long term oral potassium supplements in patients with mild hypertension. Br Med J (Clin Res Ed). 1987;294:1453-6.
19. Sundar S, Sachdev KK, Vaish SK, Bhattacharya SK, Singh VP, Agarwal SK. Potassium supplementation in essential hypertension--a double blind placebo controlled study. J Assoc Physicians India. 1985;33:776-7.
20. Svetkey LP, Yarger WE, Feussner JR, DeLong E, Klotman PE. Double-blind, placebo-controlled trial of potassium chloride in the treatment of mild hypertension. Hypertension. 1987;9:444-50.
21. Valdes G, Vio CP, Montero J, Avendano R. Potassium supplementation lowers blood pressure and increases urinary kallikrein in essential hypertensives. J Hum Hypertens. 1991;5:91-6.
22. Whelton PK, Buring J, Borhani NO, Cohen JD, Cook N, Cutler JA, et al. The effect of potassium supplementation in persons with a high-normal blood pressure. Results from phase I of the Trials of Hypertension Prevention (TOHP). Trials of Hypertension Prevention (TOHP) Collaborative Research Group. Ann Epidemiol. 1995;5:85-95.
23. Zoccali C, Cumming AM, Hutcheson MJ, Barnett P, Semple PF. Effects of potassium on sodium balance, renin, noradrenaline and arterial pressure. J Hypertens. 1985;3:67-72.

CHAPTER 6

Impact of dietary assessment methods on the associations between sodium, potassium and blood pressure

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Submitted

ABSTRACT

Sodium intake raises blood pressure (BP), while potassium intake lowers it, as has repeatedly been demonstrated in randomized controlled trials. In observational studies these BP associations may be less clear because of inaccurate assessment of habitual dietary intake. In a cross-sectional study (NQplus) of 993 Dutch individuals, not on antihypertensive medication, we obtained BP associations for sodium and potassium intake based on data from different dietary assessment methods. Sodium and potassium intakes were estimated from two non-consecutive 24-h urinary samples, two non-consecutive web-based 24-h recalls, and a validated 180-item food frequency questionnaire (FFQ). The average daily sodium and potassium intakes in our cohort were 4.0 and 3.9 grams, respectively, based on urinary excretions after correction for non-urinary losses. BP was on average 125/74 mmHg and 16% had a systolic BP ≥ 140 mmHg. In multivariable linear regression models, sodium intake was not associated with systolic or diastolic BP for any of the dietary assessment methods including urinary sodium excretion (all $P > 0.08$). Potassium intake based on two 24-h urinary excretions was associated with a 1.6 mmHg (95% CI 0.3, 2.9; $P = 0.016$) lower systolic BP per gram per day after adjusting for age, sex, body mass index, highest completed education, smoking status, physical activity, alcohol intake and urinary creatinine. A similar, though non-significant, association was observed for potassium intake estimated by FFQ (-1.4 mmHg; 95% CI -2.9, 0.0; $P = 0.057$). Potassium intake estimated from web-based 24-h recalls was not associated with BP. We conclude that dietary assessment methods, including two 24-h urinary samples, are inadequate for studying the association of sodium intake with BP in cross-sectional studies. For potassium, however, two 24-h urinary collections and a 180-item FFQ seem appropriate, with associations being in the order of magnitude as observed in randomized controlled trials.

INTRODUCTION

Accurate assessment of habitual dietary intake in large populations is a challenging task in nutritional epidemiology, because invalid or imprecise information on dietary intake may alter the direction of diet-disease associations or yield false null results [1]. This may be especially the case for sodium, a nutrient that is added in variable amounts to foods and for which intake can vary largely from day to day within one individual. From randomized controlled trials we know that blood pressure (BP) increases after increasing sodium intake and decreases after increasing potassium intake [2-4]. Recent meta-analyses of randomized controlled trials with a duration of at least four weeks have shown a ~2.0 mmHg higher systolic BP for each 1 g/d increase in sodium intake [2], and a ~1.6 mmHg lower systolic BP for each 1 g/d increase in potassium intake [4].

Inconsistent associations with BP have been reported in cross-sectional studies, especially for sodium [5-7], which may be related to the method for measuring habitual sodium and potassium intake. Dietary assessment methods that are based on self-report, including the 24-h dietary recall and the food-frequency questionnaire (FFQ), are prone to misreporting and recall bias [8]. Furthermore, inaccurate or incomplete data in food composition tables may hamper the valid estimation of specific nutrient intakes [9]. Also, sodium added during cooking or at the table (i.e. discretionary salt) is difficult to measure. The purpose of this study was to evaluate whether dietary assessment methods would influence the associations of sodium and potassium intake with BP in observational studies. For this, we compared different methods in a single Dutch cohort, including two non-consecutive 24-h urinary excretions (considered as the most objective measure), two non-consecutive web-based 24-h recalls, and a 180-item FFQ.

METHODS

Study Design and Participants

The present analysis was carried out in the Nutrition Questionnaires plus (NQplus) study, an observational study that was set up to validate different dietary assessment methods and to assess associations of diet with intermediate health outcomes. Eligible for participation were men and women aged 20 to 70 years from Wageningen and its surroundings, The Netherlands, who were able to speak and write Dutch. The NQplus study was approved by the Medical Ethics Committee of Wageningen University and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. Between May 2011 and February 2013, 2048 persons entered the study. Baseline measurements included physical examination, 24-h urine collection, questionnaires on demographic and lifestyle factors, and a 180-item FFQ. Measurements were repeated one

year later. In the two years after baseline, participants were repeatedly invited to fill out web-based 24-h dietary recalls. For the present study on sodium, potassium and BP, we selected 1233 participants with BP data at baseline and year 1, reliable FFQ data (energy intakes men 800-4200 kcal, women 500-3500 kcal), two urine collections, and at least two web-based 24-h recalls. We excluded 12 pregnant women and 228 participants on antihypertensive medication (including Anatomical Therapeutic Chemical codes: C02, C03, C07, C08 and C09) at baseline or year 1, resulting in 993 participants for analysis.

24–h Urinary Sodium and Potassium

Participants were provided with instructions for 24-h urine collections. They received two 3-liter bottles, each containing 25 g of the preservative lithium dihydrogenphosphate, and three 80-mg *para*-aminobenzoid acid (PABA) tablets to ingest at mealtimes during the course of the urine collection. The 24-h urine collection started after discarding the first voiding on the morning of the collection day and included the first voiding on the morning of the following day. At the study center, the urine collections were mixed, weighted, aliquoted and stored at -80°C until further analysis.

Urinary sodium and potassium concentrations were measured with an ion-selective electrode module on a Roche 917 analyser, and were multiplied by collected urine volume to obtain 24-h excretion values. Intakes of sodium and potassium were calculated taking into account extra-renal and faecal losses of 14% for sodium [10] and 19% for potassium [11]. Urinary creatinine concentrations were measured at 520 nm on the Synchron LX20 by the modified Jaffé procedure using a commercial kit. PABA was used to check the completeness of the urine collections and was measured using the HPLC method [12].

Web–Based 24–h Recalls

Unannounced email invitations were sent to the participants to self-administer a recall over the previous day in the web-based program Compl-eat (www.compleat.nl). Invitations were valid for 24 hours, and if denied, the recall was randomly rescheduled within 3-10 days. Compl-eat is based on the five-step multiple pass method, which is a validated technique to increase the accuracy of recalls [13-16]. Portion sizes were reported in commonly used household measures, natural portions, and weight in grams or volume in liters. Discretionary salt use was not estimated. Average daily intakes of sodium, potassium, alcohol and total energy were calculated using the Dutch food composition database of 2011 [17].

FFQ

A 180-item semi-quantitative FFQ, which was self-administered and filled out online using the open-source survey tool LimesurveyTM (LimeSurvey Project Team / Carsten Schmitz. Hamburg, Germany), was used to assess habitual dietary intake. The FFQ was validated for the intake of energy, fats, dietary fiber and selected vitamins. The estimated mean energy

intake by the FFQ appeared to be accurate [18], and in comparison with the mean of three 24-h recalls, the FFQ showed an acceptable to good ranking for most nutrients [19]. The reference period for the FFQ was one month, and portion sizes were estimated using commonly used household measures. The FFQ was not developed to estimate salt intake, and hence discretionary salt use was not estimated. The Dutch food composition table of 2011 was used to compute the intake of sodium, potassium, alcohol and total energy [17].

BP

Brachial BP measurements were performed by trained research assistants according to a standard protocol. BP was measured in lying position after at least 10 minutes of rest with 2-minute intervals using an automated oscillometric device (IntelliSense HEM-907, Omron Health Care, USA) with an appropriate cuff size on the left upper arm. The first measurement was discarded and the five subsequent measurements were averaged.

Covariates

Height was measured to the nearest 0.5 centimeter using a stadiometer (SECA, Germany) and weight was measured to the nearest 0.1 kg on a digital scale (SECA, Germany or Tanita Corporation, The Netherlands) in light indoor clothing without shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). General and lifestyle questionnaires at baseline included information on age (years), sex, education (low/intermediate/high), smoking (never/former/current), diabetes (yes/no), and history of cardiovascular disease (yes/no). Physical activity was assessed using the Short QUestionnaire to Assess Health enhancing physical activity (SQUASH) [20], and was categorized according to the average time spent per week doing leisure-time physical activities with a moderate to vigorous intensity (≥ 4 metabolic equivalents [METs] if aged 18 to 55 years and ≥ 3 METs if aged > 55 years).

Data Analysis

In order to estimate habitual intake, we averaged baseline and year 1 urinary excretions, and averaged data from two web-based 24-h recalls. Median (interquartile range) number of days between two urine collections was 393 (363-430); between two web-based 24-h recalls 112 (52-191); and between baseline urinary collection and first web-based 24-h recall 132 (29-223). BP values obtained at baseline and year 1 were also averaged.

Continuous variables are presented as mean ± SD, or median with interquartile ranges (Q1-Q3) if not normally distributed. Categorical variables are presented as numbers and percentages. Partial Spearman correlation coefficients for the sodium and potassium intake estimates between the different methods were calculated, adjusting for sex. The associations of sodium and potassium intake with systolic and diastolic BP were estimated by means of multivariable linear regression models, and findings are reported as adjusted beta regression

coefficients per gram sodium or potassium per day with 95% confidence intervals. In model 1, estimates were adjusted for age and sex. In model 2, additional adjustments were made for body mass index (kg/m^2), highest completed education (low (e.g. primary education); intermediate (e.g. higher secondary education) and high (e.g. higher vocational education and university)), smoking status (never, former, current), leisure-time physical activity with moderate to vigorous intensity (0-200, >200-≤400, >400 min/week), and alcohol intake (for men: 0, >0-≤20, >20 g/day; for women: 0, >0-≤10, >10 g/day). Alcohol intake was estimated from FFQ or from two web-based 24-h recalls, depending on the dietary assessment method under study. In models of urinary sodium and potassium, we adjusted using alcohol data estimated by FFQ. Missing indicators were used for smoking status (2.7% missing) and physical activity (5.6% missing). In model 3, we further adjusted for potassium intake in the analysis of sodium intake, and vice versa; for urinary creatinine in the analyses of urinary sodium and potassium; and for total energy intake estimated from web-based 24-h recalls or FFQ, depending on the method under study. In analysis of sodium and potassium intakes based on repeated measurements (i.e. two urinary excretions and two web-based 24-h recalls), betas from linear regression analyses were multiplied by the inverse of the intra-class correlation (ICC) coefficient (ICC) to correct for random error [21]. ICC for two repeated measurements was calculated according to the following formula:

$$\text{ICC} = \frac{\text{between-person variance}}{\text{between-person variance} + (\text{within-person variance}/2))}$$

We performed sensitivity analyses by excluding participants with incomplete 24-h urines, as indicated by a urinary PABA recovery < 78% [12], leaving 694 participants. All analyses were done with SAS statistical software version 9.3 (SAS Institute Inc.). A two-sided P-value below 0.05 was considered statistically significant.

RESULTS

Participants

Baseline characteristics of the 495 men and 498 women are listed in Table 1. Participants were on average 53 years and their body mass index was 25.4 kg/m^2. Furthermore, 54% of the participants were classified as highly educated. Mean systolic/diastolic BP was 125/74 mmHg, with women having lower values (119/72 mmHg) than men (131/76 mmHg). Of all participants, 14 (1.4%) had a history of cardiovascular disease and 162 (16%) had a systolic BP ≥ 140 mmHg.

TABLE 1. Characteristics of 993 Dutch participants from the NQplus study, by sex[a]

	Men	Women
Participants, n	495	498
Age, y	55 ± 10	50 ± 11
Body mass index, kg/m^2	26.0 ± 3.3	24.8 ± 4.0
Systolic BP, mmHg	131 ± 13	119 ± 14
Diastolic BP, mmHg	76 ± 10	72 ± 10
Smoking status, n (%)		
Never	229 (47.3)	299 (62.0)
Former	212 (43.8)	152 (31.5)
Current	43 (8.9)	31 (6.4)
Education, n (%)		
Low	71 (14.3)	80 (16.1)
Middle	146 (29.5)	158 (31.7)
High	278 (56.2)	260 (52.2)
Physical activity, min/week	366 (210-600)	290 (150-510)
Energy intake based on 24-h recalls, kcal/d	2196 ± 631	1754 ± 488
Energy intake based on FFQ, kcal/d	2277 ± 586	1823 ± 459
Urinary creatinine, mmol/24h	14.8 ± 2.6	10.1 ± 2.2
History of cardiovascular disease, n (%)	11 (2.2)	3 (0.6)
Diabetes mellitus, n (%)	10 (2.0)	6 (1.2)

Abbreviations: BP, blood pressure; FFQ, food-frequency questionnaire. [a] Data are presented as n (%), mean ± SD, or median (interquartile range).

Sodium and Potassium Intake

Average sodium intake was 4.0 ± 1.3 g/d based on two 24-h urinary excretions after accounting for non-urinary losses. Based on self-reports, which do not take salt added during cooking or at the table into account, sodium intake was 2.3 ± 0.9 g/d when estimated from two web-based 24-h recalls, and 2.1 ± 0.7 g/d when estimated from FFQ data (Table 2). 24-h urinary sodium correlated weakly with sodium intake estimated from web-based 24-h recalls (sex-adjusted r = 0.15) or FFQ (r = 0.17). A higher correlation for sodium was observed between the web-based 24-h recall and FFQ (r = 0.40) (Table 3).

Average potassium intake was 3.9 ± 1.0 g/d based on two 24-h urinary excretions after accounting for non-urinary losses; 3.2 ± 0.9 g/d based on two web-based 24-h recalls; and 3.3 ± 0.8 g/d based on FFQ data (Table 2). Twenty-four-hour urinary potassium showed a correlation of 0.35 with potassium from web-based 24-h recalls, and 0.37 with potassium from FFQ. The correlation between web-based 24-h recalls and FFQ was 0.47 (Table 3).

TABLE 2. Mean intake for sodium and potassium based on two 24–h urinary samples, two web–based 24–h recalls, and food–frequency questionnaire in 993 Dutch participants

	Two 24-h urinary excretions[a]	Two web-based 24-h recalls[b]	FFQ[b]
Total			
Sodium intake, g/d	4.0 ± 1.3	2.3 ± 0.9	2.1 ± 0.7
Potassium intake, g/d	3.9 ± 1.0	3.2 ± 0.9	3.3 ± 0.8
Men			
Sodium intake, g/d	4.5 ± 1.2	2.6 ± 1.0	2.4 ± 0.7
Potassium intake, g/d	4.2 ± 1.0	3.4 ± 1.0	3.6 ± 0.9
Women			
Sodium intake, g/d	3.4 ± 1.0	2.1 ± 0.8	1.9 ± 0.6
Potassium intake, g/d	3.6 ± 1.0	2.9 ± 0.8	3.1 ± 0.7

Abbreviations: FFQ, food-frequency questionnaire. Values are means ± standard deviations. [a] Corrected for non-urinary losses of 14% for sodium [10] and 19% for potassium [11]. [b] Salt added during cooking or at the table were not taken into account.

TABLE 3. Sex–adjusted Spearman correlation coefficients (r) between two 24–h urinary excretions, two web–based 24–h recalls, and food–frequency questionnaire for estimated sodium and potassium intake in 993 Dutch participants

	r (95% CI)	P
Sodium intake		
Two 24-h urinary excretions[a] and two web-based 24-h recalls	0.15 (0.09, 0.21)	<0.001
Two 24-h urinary excretions[a] and FFQ[b]	0.17 (0.11, 0.23)	<0.001
Two web-based 24-h recalls[b] and FFQ[b]	0.40 (0.34, 0.45)	<0.001
Potassium intake		
Two 24-h urinary excretions[c] and two web-based 24-h recalls	0.35 (0.29, 0.40)	<0.001
Two 24-h urinary excretions[c] and FFQ	0.37 (0.21, 0.42)	<0.001
Two web-based 24-h recalls and FFQ	0.47 (0.42, 0.52)	<0.001

Abbreviation: FFQ, food-frequency questionnaire. [a] Corrected for non-urinary losses of 14% [10]. [b] Salt added during cooking or at the table were not taken into account. [c] Corrected for non-urinary losses of 19% [11].

Association of Sodium and Potassium Intake with BP

Sodium intake, based on urinary excretions, web-based 24-h recalls or FFQ, was not associated with systolic and diastolic BP after adjusting for covariates (Table 4). Systolic BP estimates ranged from -1.3 to +0.5 mmHg (all P > 0.3) and diastolic BP estimates from -1.8 to -0.5 mmHg (all P > 0.08) per gram per day of sodium, depending on the method of assessment. Similar results were found in those with complete urine collection (Supplemental Table 1).

TABLE 4. Association between different assessments of sodium intake and blood pressure in 993 Dutch participants

	Two 24-h urine collections[a]		Two web-based 24-h recalls[a]		FFQ	
	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P
Systolic BP, mmHg						
Model 1[b]	2.0 (0.9, 3.2)	<0.001	-1.7 (-4.0, 0.7)	0.16	-1.0 (-2.1, 0.2)	0.10
Model 2[c]	0.5 (-0.7, 1.7)	0.39	-1.7 (-4.0, 0.6)	0.14	-0.8 (-1.9, 0.3)	0.17
Model 3[d]	0.5 (-0.8, 1.8)	0.46	-1.3 (-4.3, 1.7)	0.38	0.4 (-1.7, 2.4)	0.73
Diastolic BP, mmHg						
Model 1[b]	0.6 (-0.2, 1.5)	0.15	-1.8 (-3.5, -0.1)	0.042	-1.1 (-2.0, -0.3)	0.009
Model 2[c]	-0.8 (-1.6, 0.1)	0.068	-2.1 (-3.7, -0.4)	0.014	-1.0 (-1.8, -0.2)	0.014
Model 3[d]	-0.5 (-1.4, 0.5)	0.32	-1.8 (-3.9, 0.4)	0.11	-1.3 (-2.7, 0.2)	0.086

Abbreviations: BP, blood pressure; FFQ, food-frequency questionnaire. [a] Estimates have been corrected for random error as explained in the text. [b] Adjusted for age and sex. [c] Model 1 covariates plus body mass index (kg/m^2), highest completed education (low, intermediate, high), smoking status (never, former, current), physical activity (0-200, >200-≤400, >400 min/week), and alcohol intake (for men: 0, >0-≤20, >20 g/d; for women: 0, >0-≤10, >10 g/d). [d] Model 2 covariates plus potassium intake based on the method by which sodium intake was assessed, and urinary creatinine for estimates based on two 24-h urine collections and total energy intake for estimates based on web-based 24-h recalls and food-frequency questionnaire.

Twenty-four-hour urinary potassium was inversely associated with BP, with each 1 g/d higher potassium intake associated with a 1.6 mmHg (95% CI 0.3, 2.9; P = 0.016) lower systolic BP and 1.5 mmHg (95% CI 0.5, 2.4; P = 0.002) lower diastolic BP after adjusting for covariates. Potassium intake estimated by FFQ showed a similar, though borderline significant, association with systolic BP (-1.4 mmHg per 1 g/d increment; 95% CI -2.9, 0.0; P = 0.057), and a significant association with diastolic BP (-1.1 mmHg per 1 g/d increment; 95% CI -2.1, -0.1; P = 0.033) after adjusting for covariates. Potassium intake estimated from two web-based 24-h recalls was not associated with BP (Table 5). Similar results for urinary potassium were found in those with complete urine collection. However, the association between potassium intake estimated from FFQ data and systolic BP became stronger (-2.6 mmHg per 1 g/d; 95% CI -4.3, -0.8; P = 0.004 (Supplemental Table 2).

DISCUSSION

In this cross-sectional analysis of 993 untreated Dutch individuals, BP was unrelated to sodium intake estimated from different dietary assessment methods. For potassium intake estimated from two 24-h urine collections or FFQ, however, an inverse association was found. BP was not associated with potassium intake estimated from web-based 24-h recalls.

TABLE 5. Association between different assessments of potassium intake and blood pressure in 993 Dutch participants

	Two 24-h urine collections[a]		Two web-based 24-h recalls[a]		FFQ	
	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P
Systolic BP, mmHg						
Model 1[b]	-0.5 (-1.8, 0.7)	0.38	-1.5 (-3.3, 0.2)	0.091	-1.4 (-2.3, -0.5)	0.003
Model 2[c]	-1.0 (-2.1, 0.2)	0.11	-0.9 (-2.7, 0.8)	0.31	-1.2 (-2.1, -0.3)	0.009
Model 3[d]	-1.6 (-2.9, -0.3)	0.016	-0.3 (-2.7, 2.1)	0.81	-1.4 (-2.9, 0.0)	0.057
Diastolic BP, mmHg						
Model 1[b]	-1.2 (-2.1, -0.3)	0.009	-1.3 (-2.7, 0.0)	0.045	-1.1 (-1.7, -0.4)	0.002
Model 2[c]	-1.5 (-2.4, -0.7)	<0.001	-0.8 (-2.1, 0.4)	0.20	-0.9 (-1.6, -0.3)	0.006
Model 3[d]	-1.5 (-2.4, -0.5)	0.002	0.0 (-1.8, 1.7)	0.98	-1.1 (-2.2, -0.1)	0.033

Abbreviations: BP, blood pressure; FFQ, food-frequency questionnaire. [a] Estimates have been corrected for random error as explained in the text. [b] Adjusted for age and sex. [c] Model 1 covariates plus body mass index (kg/m^2), highest completed education (low, intermediate, high), smoking status (never, former, current), physical activity (0-200, >200-≤400, >400 min/week), and alcohol intake (for men: 0, >0-≤20, >20 g/d; for women: 0, >0-≤10, >10 g/d). [d] Model 2 covariates plus sodium intake based on the method by which potassium intake was assessed, and urinary creatinine for estimates based on two 24-h urine collections and total energy intake for estimates based on web-based 24-h recalls and food-frequency questionnaire.

The causal relations of sodium and potassium with BP have repeatedly been demonstrated in randomized controlled trials. Meta-analyses have shown a ~2 mmHg increase in systolic BP per gram of sodium intake [2], and ~1.6 mmHg decrease in systolic BP per gram of potassium intake [4]. In a recent trial in 36 untreated (pre)hypertensive adults from the same target population as the NQplus cohort, 24-h systolic BP increased by ~2.5 mmHg per gram of sodium and decreased by ~1.3 mmHg per gram of potassium, respectively [22]. The ~1.5 mmHg lower systolic BP in the present cross-sectional study per gram of potassium estimated from 24-h urine or FFQ is in line with these findings [4, 22]. Potassium intake based on two 24-h recalls, which was modestly correlated with urinary (r = 0.35) and FFQ potassium (r = 0.47), was not associated with BP. Participants filled in the 24-h recalls using a web-based tool. This tool is based on the five-step multiple pass method, a proven technique to increase the accuracy of recalls [13-16]. The web-based method as such, however, has not yet been validated.

In our cross-sectional study, we could not confirm the BP effect of sodium as established in randomized controlled trials [2, 3]. This discrepancy is likely caused by the difficulty in accurately assessing habitual sodium intake [1, 9]. Two non-consecutive 24-h urine collections or recalls could be insufficient to characterize an individual for habitual sodium intake. Averaging multiple timed 24-h urinary excretions with checks for completeness of collections is considered as the most accurate method to assess habitual sodium intake,

as it includes discretionary salt and it takes into account the high day-to-day variation of sodium intake [9]. The web-based 24-h recalls and FFQ in our study were not designed to estimate sodium intake and included no question about discretionary salt. Furthermore, assessment of habitual sodium intake by self-reports is prone to error due to reliance on food composition tables, which are often incomplete or do not accurately capture the highly variable content of sodium across similar foods of different brands [23].

Underreporting is also a problem in self-report methods [1]. These methodological issues in assessing sodium intake by self-reports are reflected in our study by the large underestimation of sodium intake by the web-based recalls and the FFQ (1.5-2.1 g/d) compared to the urinary excretions and the low correlations observed between sodium intake based on self-reports and urinary excretions (r = 0.15-0.17). For potassium intake, the underestimation was less (0.5-0.8 g/d) and the correlations were higher (r = 0.35-0.37). In line with our findings, Freedman et al. [24] showed in their pooled analysis of five US validation studies that 24-h recalls and FFQ capture sodium intake less well than potassium intake. Dietary potassium has a lower day-to-day variability compared to sodium [25], likely because the food content of potassium is less variable. Potassium is naturally present in higher amounts in foods such as fruits, vegetables, legumes and dairy products, and less often added during food processing [26]. Estimating habitual intake based on only two urinary samples may therefore be more accurate for potassium than for sodium.

A major strength of this study is the extensive dietary information obtained through various methods. This includes 24-h urine collections, and the use of PABA to exclude incomplete urine collections in sensitivity analyses. We were able to correct our estimates for random error because of repeated 24-h urine collections and 24-h recalls. A limitation of our study is the cross-sectional design, which could give rise to reverse causality bias when studying sodium and potassium intake in relation to BP. However, we excluded individuals on antihypertensive treatment who could have changed their diets (e.g. reduction in salt use) because of their increased cardiovascular risk. Also, our study was carried out in volunteers who were on average highly educated, which limits the generalizability of our results. Our results for potassium intake based on self-reports may be less accurate in populations with lower level of education and/or more obesity.

In conclusion, in this cross-sectional analysis we observed no association of sodium intake with BP for any of the dietary assessment methods used, including 24-h urines. BP associations for potassium intake estimated from 24-h urine and FFQ, however, were in line with known effects from randomized controlled trials. Thus, observational studies may yield reliable results for potassium intake in relation to BP, whereas the results for sodium intake will likely be biased towards the null.

Acknowlegdements

The authors thank Anne van de Wiel, MSc, for data collection and data preparation, and Diewertje Sluik, PhD, for her assistance in data preparation.

Funding

The NQplus study was funded by ZonMw (grant no. 91110030), Wageningen University and TI Food and Nutrition, a public-private partnership on pre-competitive research in food and nutrition. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosures

Authors declare that there are no conflicts of interest.

REFERENCES

1. Cobb LK, Anderson CAM, Elliott P, Hu FB, Liu K, Neaton JD, et al. Methodological issues in cohort studies that relate sodium intake to cardiovascular disease outcomes: a science advisory from the american heart association. Circulation. 2014; 129:1173-86.
2. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ (Clinical research ed). 2013; 346:f1325.
3. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ (Clinical research ed). 2013; 346:f1326.
4. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ (Clinical research ed). 2013; 346:f1378.
5. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. BMJ (Clinical research ed). 1988; 297:319-28.
6. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. N Engl J Med. 2014; 371:601-11.
7. Sharma S, McFann K, Chonchol M, Kendrick J. Dietary sodium and potassium intake is not associated with elevated blood pressure in US adults with no prior history of hypertension. Journal of Clinical Hypertension. 2014; 16:418-23.
8. Willett W. Nutritional Epidemiology. New York: Oxford University Press; 1998.
9. Rhee M-Y. High sodium intake: review of recent issues on its association with cardiovascular events and measurement methods. Korean Circ J. 2015; 45:175-83.
10. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr. 1984; 40:786-93.
11. Freisling H, van Bakel MME, Biessy C, May AM, Byrnes G, Norat T, et al. Dietary reporting errors on 24 h recalls and dietary questionnaires are associated with BMI across six European countries as evaluated with recovery biomarkers for protein and potassium intake. Br J Nutr. 2012; 107:910-20.
12. Jakobsen J, Ovesen L, Fagt S, Pedersen AN. Para-aminobenzoic acid used as a marker for completeness of 24 hour urine: assessment of control limits for a specific HPLC method. Eur J Clin Nutr. 1997; 51:514-9.
13. Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. J Nutr. 2006; 136 :2594-9.
14. Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. J Am Diet Assoc. 2004; 104:595-603.
15. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. Am J Clin Nutr. 2003; 77:1171-8.

16. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. Am J Clin Nutr. 2008; 88:324-32.
17. NEVO-tabel 2011, RIVM/Voedingscentrum, Den Haag, 2011.
18. Siebelink E, Geelen A, de Vries JHM. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr. 2011; 106:274-81.
19. Streppel MT, de Vries JHM, Meijboom S, Beekman M, de Craen AJM, Slagboom PE, et al. Relative validity of the food frequency questionnaire used to assess dietary intake in the Leiden Longevity Study. Nutrition journal. 2013; 12:75.
20. Wendel-Vos GCW, Schuit AJ, Saris WHM, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003; 56:1163-9.
21. Berglund L. Regression dilution bias: tools for correction methods and sample size calculation. Ups J Med Sci. 2012; 117:279-83.
22. Gijsbers L, Dower JI, Mensink M, Siebelink E, Bakker SJL, Geleijnse JM. Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens. 2015; 29:592-8.
23. Champagne CM, Cash KC. Assessment of salt intake: how accurate is it? Proc Nutr Soc. 2013; 72:342-7.
24. Freedman LS, Commins JM, Moler JE, Willett W, Tinker LF, Subar AF, et al. Pooled results from 5 validation studies of dietary self-report instruments using recovery biomarkers for potassium and sodium intake. American journal of epidemiology. 2015; 181:473-87.
25. Caggiula AW, Wing RR, Nowalk MP, Milas NC, Lee S, Langford H. The measurement of sodium and potassium intake. Am J Clin Nutr. 1985; 42:391-8.
26. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28. Version Current: September 2015.

SUPPLEMENTAL RESULTS

SUPPLEMENTAL TABLE 1. Association between different assessments of sodium intake and blood pressure in 694 Dutch participants with complete 24–h urine collections

	Two 24-h urine collections[a]		Two web-based 24-h recalls[a]		FFQ	
	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P
Systolic BP, mmHg						
Model 1[b]	1.8 (0.5, 3.1)	0.008	-2.1 (-5.1, 0.8)	0.16	-1.3 (-2.7, 0.1)	0.068
Model 2[c]	0.3 (-1.1, 1.6)	0.71	-2.3 (-5.2, 0.5)	0.11	-1.1 (-2.4, 0.3)	0.12
Model 3[d]	0.1 (-1.4, 1.5)	0.93	-2.4 (-6.2, 1.3)	0.21	0.1 (-2.3, 2.5)	0.93
Diastolic BP, mmHg						
Model 1[b]	0.8 (-0.2, 1.7)	0.11	-1.7 (-3.8, 0.5)	0.12	-1.5 (-2.5, -0.5)	0.004
Model 2[c]	-0.6 (-1.6, 0.3)	0.20	-2.1 (-4.2, 0.0)	0.047	-1.4 (-2.3, -0.4)	0.006
Model 3[d]	-0.4 (-1.5, 0.6)	0.42	-1.7 (-4.4, 1.0)	0.21	-1.0 (-2.6, 0.7)	0.27

Abbreviations: BP, blood pressure; FFQ, food-frequency questionnaire. [a] Estimates have been corrected for random error as explained in the text. [b] Adjusted for age and sex. [c] Model 1 covariates plus body mass index (kg/m^2), highest completed education (low, intermediate, high), smoking status (never, former, current), physical activity (0-200, >200-≤400, >400 min/week), and alcohol intake (for men: 0, >0-≤20, >20 g/d; for women: 0, >0-≤10, >10 g/d). [d] Model 2 covariates plus potassium intake based on the method by which sodium intake was assessed, and urinary creatinine for estimates based on two 24-h urine collections and total energy intake for estimates based on web-based 24-h recalls and food-frequency questionnaire.

SUPPLEMENTAL TABLE 2. Association between different assessments of potassium intake and blood pressure in 694 Dutch participants with complete 24–h urine collections

	Two 24-h urine collections[a]		Two web-based 24-h recalls[a]		FFQ	
	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P	β per 1 g/d increment (95% CI)	P
Systolic BP, mmHg						
Model 1[b]	-0.8 (-2.1, 0.5)	0.24	-1.2 (-3.2, 0.9)	0.28	-2.1 (-3.3, -1.0)	<0.001
Model 2[c]	-1.1 (-2.4, 0.2)	0.10	-0.7 (-2.8, 1.3)	0.49	-1.8 (-2.9, -0.7)	0.001
Model 3[d]	-1.6 (-3.0, -0.2)	0.028	-0.2 (-3.0, 2.6)	0.89	-2.6 (-4.3, -0.8)	0.004
Diastolic BP, mmHg						
Model 1[b]	-1.4 (-2.3, -0.4)	0.006	-1.2 (-2.7, 0.3)	0.12	-1.7 (-2.5, -0.9)	<0.001
Model 2[c]	-1.6 (-2.5, -0.7)	<0.001	-0.9 (-2.3, 0.6)	0.23	-1.5 (-2.2, -0.7)	<0.001
Model 3[d]	-1.7 (-2.7, -0.7)	0.001	-0.2 (-2.2, 1.8)	0.82	-1.7 (-2.9, -0.4)	0.010

Abbreviations: BP, blood pressure; FFQ, food-frequency questionnaire. [a] Estimates have been corrected for random error as explained in the text. [b] Adjusted for age and sex. [c] Model 1 covariates plus body mass index (kg/m^2), highest completed education (low, intermediate, high), smoking status (never, former, current), physical activity (0-200, >200-≤400, >400 min/week), and alcohol intake (for men: 0, >0-≤20, >20 g/d; for women: 0, >0-≤10, >10 g/d). [d] Model 2 covariates plus sodium intake based on the method by which potassium intake was assessed, and urinary creatinine for estimates based on two 24-h urine collections and total energy intake for estimates based on web-based 24-h recalls and food-frequency questionnaire.

CHAPTER 7

General discussion

High sodium intake and low potassium intake are known to elevate blood pressure (BP), a major risk factor for cardiovascular diseases (CVD). Little is known about the effects of these minerals on other vascular outcomes. In this thesis the BP effects of sodium and potassium intake were investigated in the broader context of cardiovascular health, focusing on various BP measures, endothelial (dys)function, arterial stiffness, body fluid balance and heart rate.

Methods used in this thesis include a human randomized controlled trial (RCT) with a cross-over design in which diet was fully controlled, a meta-analysis of RCTs and an epidemiological analysis of a cross-sectional study. A major strength of our trial is that the effects of supplemental sodium and potassium were examined simultaneously, in the same setting and in the same individuals. Effects can therefore be directly compared. Another strength is the fully controlled diet, which strongly reduces the intra-individual variability resulting from dietary influences thereby increasing statistical power to demonstrate effects that are exclusively attributable to sodium and potassium intake. The power is furthermore strengthened by its design as a cross-over study, allowing analysis of paired samples.

The experimental part of the thesis is based on apparently healthy Dutch men and women, aged 40–80 years, with a fasting systolic BP between 130 and 159 mmHg. These individuals represent a large segment of the middle-aged and older population. In the Netherlands, 58% of men and 40% of women aged 40–49 years, and ~90% of men and women aged 70 years or older have systolic BP values over 130 mmHg [1].

Table 1 gives an overview of the findings of this thesis. In this final Chapter the main findings for sodium and potassium are described and put in context. After that, the implications for public health are discussed.

TABLE 1. Overview of the main findings on the vascular effects of sodium and potassium intake in this thesis

Study (Chapter)	Population	Exposure	Outcome	Main findings
RCT (Chapter 2)	36 Dutch individuals with an untreated SBP of 130–159 mmHg	Supplemental sodium of 3 g/d and supplemental potassium of 3 g/d, each for 4 weeks, on top of a relatively low sodium, low potassium diet	• Serum sodium and potassium • Office BP and heart rate • 24-h BP and heart rate • Measures of arterial stiffness	During sodium supplementation: • Non-significant effect on serum sodium (0.39 mmol/L) • Decrease in serum potassium of 0.10 mmol/L • Increase in office BP of 7.5/3.3 mmHg • Increase in 24-h BP of 7.5/2.7 mmHg • No effect on office and 24-h heart rate • No effect on pulse wave velocity and augmentation index During potassium supplementation: • Decrease in serum sodium of 0.68 mmol/L • Increase in serum potassium of 0.13 mmol/L • Decrease in 24-h BP of 3.9/1.6 mmHg • Increase in 24-h heart rate of 2.6 bpm • Non-significant effect on pulse wave velocity (-0.35 m/s) • No effect on augmentation index
RCT (Chapter 3)	Idem	Idem	• Flow-mediated dilation • Circulating endothelial and inflammatory markers	During sodium supplementation: • No effect on flow-mediated dilation • Increase in plasma endothelin-1 of 0.24 pg/ml • No effect on other endothelial or inflammatory biomarkers During potassium supplementation: • Increase in flow-mediated dilation of 1.16% • No effect on endothelial and inflammatory biomarkers
RCT (Chapter 4)	Idem	Idem	• Plasma parameters of body fluid balance	During sodium supplementation: • Reduction in plasma renin and aldosterone • Increase in plasma copeptin, NT-proBNP and MR-proANP During potassium supplementation: • Reduction in plasma MR-proANP • Increase in plasma copeptin, renin, aldosterone and serum urea
Meta-analysis of 22 randomized, placebo-controlled trials (Chapter 5)	Healthy adults	Potassium supplementation	Heart rate	• No overall effect of increased potassium intake on heart rate (0.19 bpm; P=0.56) • No evidence for dose-response relationship • No significant effects in subgroups
Cross-sectional study (Chapter 6)	993 Dutch adults not treated with antihypertensive medication	Dietary sodium and potassium intake assessed from 2 non-consecutive 24-h urine samples, validated 180-item FFQ and 2 non-consecutive web-based 24-h recalls	Office BP	For sodium intake: • No association with BP For potassium intake: • Estimated from urinary excretions: inverse significant association (-1.6 mmHg per g/d increment) with systolic BP • Estimated from FFQ: inverse, borderline significant association (-1.4 mmHg per g/d increment; P=0.057) • Estimated from web-based 24-h recalls: no association

Abbreviations: bpm, beats per minute; BP, blood pressure; FFQ, food-frequency questionnaire; MR-proANP, midregional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro b-type natriuretic peptide; RCT, randomized-controlled trial.

VASCULAR EFFECTS OF SODIUM INTAKE

Sodium Intake and BP

The causal relationship of sodium with BP has repeatedly been demonstrated in RCTs. We performed a randomized double-blind cross-over study of 36 untreated (pre)hypertensive individuals on a recommended sodium level (~2 g/d). We showed that increasing sodium intake by ~3.0 g/d increased 24-h urinary sodium by 98 mmol (equals 2.2 g), and increased office BP by 7.5/3.3 mmHg and 24-h BP by 7.5/2.7 mmHg, compared to placebo. Assuming a linear relation, this is equivalent to an effect in systolic BP of 0.7–0.8 mmHg per 10 mmol change in 24-h urinary sodium, which is comparable to the result of a meta-analysis of randomized trials with a minimum duration of 4 weeks, in which a 75 mmol reduction in 24-h urinary excretion decreased systolic BP by 5.4 mmHg (0.7 mmHg per 10 mmol/24h) in untreated hypertensives [2]. Meta-analyses [2-4] and RCTs [5, 6] have shown that sodium reduction affects BP in a dose-response fashion, meaning that each reduction in sodium intake will benefit BP.

The well-established relation between sodium intake and BP was not confirmed in our cross-sectional analysis of 993 Dutch individuals from the Nutritional Questionnaire plus study. In this study, sodium intake was estimated from 2 non-consecutive 24-h urine samples, 2 non-consecutive web-based 24-h recalls, and a validated 180-item food-frequency questionnaire (FFQ). In observational studies, accurate assessment of habitual dietary intake is challenging. Averaging multiple timed 24-h urinary excretions with checks for completeness of collections is considered as the most accurate method to assess habitual sodium intake, as it includes discretionary salt and it takes into account the high day-to-day variation of sodium intake [7]. In our study, we had to rely on only 2 24-h urine samples (approximately 1 year apart), which may have been too imprecise for estimating an individual's habitual intake. Moreover, the web-based 24-h recalls and FFQ used to estimate sodium intake did not include a question about discretionary salt, and these self-report methods are prone to error due the underreporting and reliance on food composition tables that may be incomplete, not up to date, or not accurately capturing the highly variable content of sodium in similar foods of different brands [8, 9].

Besides inaccurate assessment of sodium intake, other methodological constraints, including limited range of sodium intake, small study population and issues related to the cross-sectional design (e.g. reverse causality and residual confounding), may explain the lack of finding in our study. Methodological errors and biases have also been reported to be responsible for the inconsistent findings in observational studies relating sodium intake to cardiovascular disease [8, 9]. When evaluating the effect of sodium intake on cardiovascular health, results obtained from observational studies should thus be interpreted with caution.

Sodium Intake and Endothelial Function

We found no effect of 4 weeks of increased sodium intake on brachial artery flow-mediated dilation (FMD), which suggests that supplemental sodium can affect BP without altering endothelial function. Other randomized cross-over studies with intervention durations of 2–6 weeks reported improvements in FMD of 1.5–2.4% after sodium restriction [10-12]. In an RCT with 17 middle-aged and older subjects with pre-hypertension (5 on antihypertensive medication), FMD improved by 2.4% after 4 weeks of sodium restriction [12]. In an RCT in 29 overweight and obese subjects with systolic BP between 95 and 138 mmHg, a 2-week low-sodium diet improved FMD by 1.5% compared to the normal-sodium diet [10]. The same researchers showed in 25 overweight and obese subjects with a systolic BP < 139 mmHg that FMD improved by 2.1% after a modest sodium restriction for 6 weeks [11]. In RCTs that showed an FMD response for sodium, 'background' urinary sodium ranged from 1.5–2.6 g/24h and sodium doses from 1.4–2.3 g/d. The 'background' urinary sodium of 2.5 g/24h in our study is in the range of other studies and our dose of 3.0 g/d is even higher, and hence both cannot explain why we had a null finding for sodium and FMD. It should be noted, however, that RCTs of nutritional factors and FMD are burdensome and that drop-out rates may be as high as 50% [6], possibly giving biased results. Even in the case of significant effects on FMD, findings from these RCTs should be interpreted with caution.

In our study, potassium intake was reduced and therefore 'background' urinary potassium (~2 g/24h) was lower than in studies showing an FMD response for sodium (~3 g/24h) [5-7], but whether this difference in potassium intake can explain the discrepancy in results is not clear. Also, subjects in our study had on average a higher baseline brachial artery diameter and a lower FMD than subjects in studies showing effects on FMD. Important to note is that although FMD is considered the best available measure of endothelial function, it is a sensitive measurement which can be influenced by minor changes in methodology such as placement of the cuff/probe and duration of the cuff inflation, or by external factors (e.g. exercise and caffeine intake) [13]. In our study, FMD measurements were done in fasting subjects according to a strict protocol, adhering to 2 commonly used guidelines for FMD measurements [14, 15]. We were able to detect significant changes in FMD after increased potassium intake (discussed below). Therefore, we consider it unlikely that measurement flaws account for our null findings for sodium intake.

Besides FMD, we measured blood biomarkers involved in cellular adhesion, coagulation and low-grade inflammation. We also observed no effects of increased sodium intake on these biomarkers, except for an increase of 0.24 pg/ml in vasoconstrictor endothelin-1, considered to be a biomarker of endothelial function. In 2 studies by other research groups that showed an effect on FMD also the effects of sodium restriction on endothelial blood biomarkers were assessed [11, 12]. One study showed a decrease of 0.20 pg/ml in endothelin-1 and no changes in adhesion molecules after moderate sodium restriction for 6 weeks [11], in line

with our findings. The other study showed, in contrast, higher endothelin-1 levels after 4 weeks of sodium restriction, but this effect was not significant [12].

Sodium Intake and Other Outcomes

Arterial stiffness is an indicator of vascular health and closely linked to BP [16, 17]. In our RCT, 4 weeks of supplemental sodium had no effect on arterial stiffness, which was non-invasively assessed by measuring augmentation index and pulse wave velocity using applanation tonometry. The 4-week duration may have been too short to induce changes in arterial stiffness. Nevertheless, in other trials with a 4–6 week duration, dietary sodium did affect pulse wave velocity, a direct measure of arterial stiffness, in (pre)hypertensive subjects [18-20], but not in normotensive subjects [11, 21]. It has been hypothesized that short-term interventions may affect arterial stiffness by influencing functional mechanisms, such as vascular tone and endothelial function rather than vascular structure [22].

In a *post-hoc* analysis we examined the effects of sodium intake on fluid balance, a key factor in BP regulation. For increased sodium intake, we found increases in estimated glomerular filtration rate (eGFR), plasma natriuretic peptides, and decreases in plasma renin and aldosterone, in line with previous findings [23, 24]. The increase in eGFR likely reflects induced hyperfiltration, which is associated with increased intraglomerular pressure [25, 26]. The stimulation of release of plasma natriuretic peptides allows for increased natriuresis, and the suppression of renin-angiotensin-aldosterone system (RAAS) results in a decreased tendency for sodium reabsorption. These physiological changes indicate that compensatory responses are stimulated upon deranged fluid status. These compensatory responses are known to be more pronounced with sudden and large changes in sodium intake and much smaller or minimal with a longer term modest change in sodium intake [2]. Hence these effects seen in our trial after a 4-week increase in sodium intake are likely to attenuate over time, but were not examined in our study. We also investigated the effects of sodium intake on plasma copeptin, which is part of the precursor of arginine vasopressin [27]. Copeptin was found to be a reliable surrogate for arginine vasopressin that is more stable *ex vivo* and easier to measure [28, 29]. Secretion of vasopressin is stimulated by hypovolemia and increased osmolarity [30]. We found that supplemental sodium for 4 weeks increased plasma copeptin concentrations. This was also observed in another RCT and based on this finding the authors suggested that the sodium-induced increase of osmolarity is a more potent stimulus for vasopressin secretion than sodium-induced increase of blood volume, as the latter would inhibit vasopressin release [30].

VASCULAR EFFECTS OF POTASSIUM INTAKE

Potassium Intake and BP

We found that supplemental potassium of ~3 g/d on top of the relatively low-sodium diet increased 24-h urinary potassium by 63 mmol (equals 2.5 g) and lowered 24-h BP by 3.9/1.6 mmHg. In a meta-analysis of 21 RCTs of at least 4 weeks duration, increased potassium reduced systolic BP by 3.5 mmHg. When stratified by hypertension status, systolic BP was reduced by ~5 mmHg in people with hypertension, but unaffected in people with normal BP (with the latter based on 3 studies) [4]. Subgroup analysis in our trial showed, however, that supplemental potassium reduced 24-h systolic BP by 6 mmHg in subjects with a normal BP (see Table 2). We established a high contrast in potassium intake and other dietary factors were fully controlled. In the meta-analysis of Aburto et al. [4], largest improvements in BP were found for achieved potassium intakes of 3.5 to 4.7 g/d, without a clear dose-response association. However, in a meta-analysis of 15 RCTs in which subjects were not on antihypertensive treatment, a dose-response association was found between increased urinary potassium excretion and the reduction in BP [31], indicating that each increment in potassium intake would improve BP. The meta-analysis of Aburto et al. [4] also suggested that potassium may be more effective in reducing BP at higher levels of sodium consumption: systolic BP was reduced by 7 mmHg for sodium intake of > 4 g/d and by 2 mmHg for sodium intake of 2–4 g/d, although these estimates were not significantly different. In our trial, subjects consumed a relatively low-sodium diet (2.2 g/d). It is possible that the effect of increased potassium intake on BP in individuals with Western, high-salt diets are greater than observed in our study in which the 'background' sodium intake was reduced.

TABLE 2. Effects of potassium supplementation on BP and heart rate by pre–treatment systolic BP

	Values after 4 weeks of intervention[a]		Treatment effect[b]	
	Potassium	Placebo	Potassium vs placebo	P
SBP < 140 mmHg (n=11)				
24-h SBP, mmHg	118.2 ± 6.5	124.5 ± 11.2	-6.0 (-11.4, -0.5)	0.033
24-h DBP, mmHg	73.4 ± 4.9	77.3 ± 5.4	-3.8 (-6.6, -1.1)	0.008
24-h HR, bpm	70.6 ± 8.6	68.1 ± 9.4	2.5 (-0.5, 5.6)	0.10
SBP ≥ 140 mmHg (n=25)				
24-h SBP, mmHg	128.9 ± 14.3	131.6 ± 14.9	-2.7 (-6.3, 0.8)	0.13
24-h DBP, mmHg	75.6 ± 8.8	76.2 ± 9.3	-0.6 (-2.4, 1.3)	0.54
24-h HR, bpm	67.5 ± 8.6	64.9 ± 9.4	2.6 (0.8, 4.4)	0.005

Abbreviation: BP, blood pressure; bpm, beats per minute; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure. [a] Unadjusted mean ± SD. [b] Mean differences (95% CI) obtained from linear mixed-effects model for repeated measurements using the compound symmetry covariance structure.

The inverse relation between potassium intake and BP was also observed in our cross-sectional analysis of 993 apparently healthy Dutch individuals not on antihypertensive medication from the Nutritional Questionnaire plus study. Each 1 g/d increase in potassium intake, based on 2 24-h urine samples, was associated with a 1.6/1.5 mmHg lower systolic/diastolic BP. Potassium intake estimated by FFQ showed a similar, though borderline significant, association with systolic BP (-1.4 mmHg per 1 g/d increment), and a significant association with diastolic BP (-1.1 mmHg per 1 g/d increment). These BP estimates for potassium are in the order of magnitude as observed in our RCT (i.e. 1.3 mmHg decrease in 24-h systolic BP per gram of potassium) and other RCTs [32]. However, no association was observed for potassium intake estimated from 2 web-based 24-h recalls. The tool is based on the 5-step multiple pass method, a proven technique to increase the accuracy of recalls [33-36]. The web-based method as such, however, has not yet been validated. Based on these results, it seems that 2 24-h urinary collections and a FFQ are appropriate for studying the association between potassium intake and BP in cross-sectional studies.

Potassium Intake and Endothelial Function

In our trial, potassium supplementation improved FMD by 1.2% and tended to lower IL-8 (a biomarker of inflammation), without affecting other blood biomarkers of endothelial function and inflammation. Another RCT in untreated hypertensives investigating the effects of potassium chloride supplements and potassium bicarbonate supplements, each for 4 weeks, showed for ~45 mmol/24h higher urinary potassium increases in FMD of 2.7% and 1.5%, respectively [37]. In contrast, increasing potassium intake for 6 weeks through potassium citrate supplements and fruit and vegetables with a maximum increase in urinary potassium excretion of 27 mmol/24h caused no change in FMD in early hypertensives [38]. Possibly, a minimum potassium dose is required to affect FMD. We examined the effect of potassium supplementation on FMD while dietary sodium was reduced, i.e. urinary sodium excretion of 2.2 g/d. In the other FMD studies 'background' urinary sodium ranged from 2.7 to 3.1 g/d. Since the difference in sodium are only small, we cannot draw conclusions about the relevance of the 'background' sodium diet.

Although not as comprehensively assessed as in our trial, other RCTs demonstrated no effect of potassium on endothelial biomarkers [38-40], or the inflammatory marker C-reactive protein [38, 40]. To our knowledge, other studies have not addressed the effects on other inflammatory markers than C-reactive protein. Since in our study the effect on IL-8 was no longer significant after the exclusion of intervention periods in which subjects were non-compliant, and other cytokines were not affected, we consider increased potassium intake unlikely to have a major impact on low-grade inflammation in the short-term.

Potassium Intake and Other Outcomes

In our RCT, augmentation index, the indirect measure of arterial stiffness, was unaffected by supplemental potassium. The effect of potassium on pulse wave velocity (-0.35 m/s), the direct measure of arterial stiffness, was not statistically significant. A recent meta-analysis of 6 RCTs including ours showed also no effect of potassium on augmentation index [41]. Pulse wave velocity in the meta-analysis was unlikely to be affected by potassium (pooled estimate: -0.34 m/s, P=0.39), but the authors noted that the small number and heterogeneity of studies made it difficult to draw a definite conclusion [41]. Four weeks of supplemental potassium in an RCT in 42 untreated hypertensives [37] and 6 weeks of supplemental potassium in 40 subjects at increased CVD risk [40] significantly reduced pulse wave velocity by 0.8 and 0.4 m/s, respectively. Both studies used a potassium dose of 2.5 g/d, which is comparable to the dose in our study. A trial in 48 early hypertensives with lower doses of potassium, found no effect [38]. Our study showed a reduction in pulse wave velocity, but may have been underpowered to detect a statistically significant effect. More studies are needed to determine whether potassium supplementation affects pulse wave velocity.

Potassium is thought to exert a BP lowering effect, at least in part, through stimulation of natriuresis [42, 43]. To gain more insight in the effects on fluid balance, we investigated the effect of potassium intake on body fluid balance parameters. We found that 4 weeks of potassium supplementation decreased plasma MR-pro-ANP and increased plasma copeptin, renin, aldosterone and 24-h heart rate. Our findings suggest that increased potassium intake decreases effective circulating volume and cardiac output to such an extent that counter regulatory mechanisms (i.e., increased secretion of vasopressin, stimulation of RAAS, and increased heart rate) are activated to maintain volume homeostasis. Limited studies have addressed the effect of potassium intake on fluid balance. The stimulation of the RAAS was also found in an RCT of subjects at moderate cardiovascular risk after 6 weeks of 2.5 g/d potassium supplementation [40], but not in an RCT of untreated hypertensives with a similar dose for 4 weeks [37]. To our knowledge, no other study has investigated the effects of potassium intake on copeptin. Most likely, vasopressin was increased to raise extracellular volume towards normal by stimulation of water reabsorption. Changes in body weight in our trial were not a good indicator of fluid retention because of the fully controlled dietary setting in which we adjusted individuals' energy intake to keep body weight constant.

We found a 2.6-bpm increase in ambulatory heart rate during potassium, but no effect on office heart rate. To further investigate whether heart rate was affected by potassium intake we performed a meta-analysis of 22 randomized, placebo-controlled trials in healthy adults. Results indicated that potassium supplementation of 2–3 g/d for a minimum duration of 2 weeks is not expected to affect heart rate in apparently healthy adults. Besides our RCT, only 3 other RCTs studied the effect of potassium on 24-h heart rate [38, 39, 44]. Subgroup analyses suggested a pooled estimated for 24-h ambulatory heart rate of 0.99 beats/min,

but results were not significant (P=0.26) and the analysis relied heavily on our trial. Other stratified analyses (e.g. by duration and potassium dose) yielded also no significant effects of potassium intake on heart rate in subgroups, and there was no evidence for a dose-response relationship in meta-regression analyses. Overall, we conclude that potassium supplementation in the normal range of dietary intakes is unlikely to have a persistent effect on heart rate in apparently healthy individuals.

Conclusion Based of Main Findings and Directions for Future Research

From the findings presented in this thesis, we can conclude that increasing sodium intake from the recommended maximum target to a level that is common in Western societies strongly raises BP in individuals with an untreated mildly elevated BP. We cannot draw a definite conclusion about the effects of sodium on endothelial function. Where previous studies indicated that sodium intake affects endothelial function, our study indicated based on a comprehensive assessment no effect. Because the number of studies is still limited and results are inconsistent, more RCTs are needed to determine whether sodium intake affects endothelial function, and whether the effect could depend on 'background' potassium intake. A high-sodium diet likely deranges fluid status and elicits RAAS suppression and other compensatory responses to maintain volume homeostasis. Longer-term RCTs are required to investigate whether these effects in fluid parameters attenuate over time, reaching a new steady-state.

Increasing potassium intake lowers BP, even when people are on a relatively low-sodium diet. The BP response is likely accompanied by an improvement in endothelial function as assessed by FMD, but without changes in other indicators of endothelial function or low-grade inflammation. Since we found an effect on FMD, one may argue whether we measured the best biomarkers of endothelial function. Endothelial function is a complex process involving a number of factors and it needs to be determined which factors are directly related to potassium intake-induced changes in FMD. We cannot conclude whether the improvement in endothelial function preceded or followed BP reduction, or whether these changes are independent. Dose-response studies are required to explore whether there may be a threshold minimum dose above it is unlikely to detect an effect, because an RCT with a lower dose of potassium did not find an effect on FMD.

Supplemental potassium during a relative low-sodium diet is likely to decrease effective circulating volume to such an extent that several mechanisms (i.e. increased secretion of vasopressin, stimulation of RAAS, and increased heart rate) are activated to maintain volume homeostasis. Since the available evidence is scarce and inconclusive, more studies are warranted. Although in our RCT ambulatory heart rate was increased after potassium supplementation, our meta-analysis showed that increasing potassium intake by 2–3 g/d for at least 2 weeks is unlikely to affect heart rate in apparently healthy adults. Based on the work

presented in this thesis, we cannot conclude whether sodium and potassium affect arterial stiffness in relative healthy individuals with untreated elevated BP. Larger and longer-term RCTs in healthy and patient populations are needed to investigate whether these minerals could be meaningful for improving vascular compliance.

In the present thesis a number of physiological pathways have been described, but there may be more mechanisms that could explain effects of sodium and potassium on BP and vascular health. These could involve the sympathetic nervous system, glucose metabolism and the immune system [45, 46]. Since these outcomes have been implicated in the pathogenesis of hypertension it would be worthwhile investigating whether they are modulated by sodium and potassium intake. Potential outcome measurements in future studies include plasma and/or urinary catecholamines, serum cortisol, serum insulin, glucose and HbA1c, and T helper cells [45].

PUBLIC HEALTH IMPLICATIONS

Impact on Cardiovascular Disease

As much as 18% of global deaths can be attributed to elevated BP [47]. In a meta-analysis of individual data, each 20-mmHg increment in systolic BP was associated with 51% greater risk of stroke mortality and 43% greater risk of ischemic heart disease mortality in European individuals aged 40–89 years [48]. These estimates highlight the importance of interventions to reduce BP.

We demonstrated that a 7.5-mmHg lower systolic BP can be achieved by reducing salt intake from the level common in Western societies (12–13 g/d) to the recommended level (5–6 g/d). Furthermore, when subjects adhere to the recommended level for salt intake, increasing potassium intake can lower BP even further by 3–4 mmHg. Thus, lowering salt intake and increasing potassium intake can lower systolic BP by 11 mmHg in untreated individuals. If we extrapolate the findings of the meta-analysis to our findings, a reduction of 11 mmHg in office systolic BP would be associated with a 32% lower risk of stroke mortality and 27% lower risk of ischemic heart disease mortality [48]. This estimated impact on mortality risk is based on office BP. Since ambulatory BP, a measure used in our study, is suggested to be prognostically superior to office BP, risk reductions may be even greater [49]. For comparison, the systolic BP reduction that we observed for a low sodium, high potassium intake is of the same magnitude as may be achieved with antihypertensive medication in individuals with a systolic BP of 120–140 mmHg [50]. Systolic BP reductions ranging from 2 to 9 mmHg were achieved when angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers and/or calcium channel blockers were used as antihypertensive medication [50].

Apart from BP, changes in FMD may be predictive for CVD. In meta-analyses each 1% increase in FMD was associated with an 8–13% lower risk of cardiovascular events [51, 52]. The improvement in FMD by 1.2% found for an increased potassium intake could theoretically lead to a CVD risk reduction of 10–16%. A comparable impact may be expected from potassium-induced changes in systolic BP of 4 mmHg, i.e. 13% lower stroke mortality risk and 11% lower ischemic heart disease mortality risk [48].

Strategies for Lowering Sodium and Increasing Potassium Intake

In the present thesis, the effects of increasing the intake of salt from 5–6 to 12–13 g/d and potassium from 2–3 to 5–6 g/d were studied. These intakes are within the range of intake commonly consumed, and hence the doses used in our study are achievable in real-life settings. Around the world people consume on average 9–12 g of salt on a daily basis, which is far beyond the recommended maximum intake of 5–6 g per day. Main strategies to reduce sodium intake include 1) the consumers' selection of low-sodium foods instead of high-sodium foods by reading food labels and avoiding processed foods, 2) use of less salt in cooking and at the table, and 3) lowering the sodium content by reformulation of industrially produced foods. Since in Western societies 80% of daily salt intake comes from salt in processed foods rather than from table salt [53] and consumers may not be able to change their dietary behavior, reformulation of foods may be the most effective means to reduce sodium intake [54]. Hendriksen et al. [55] estimated that reducing the level of sodium intake in processed foods to a technologically feasible minimal level may cut down median sodium intake by 38% in the general Dutch population (e.g. from 3.0 g/d to 1.9 g/d in adult men). Governments worldwide, with the help of advocacy groups, put pressure on the food industry to add less salt to foods [54]. A few countries have set mandatory limits for specific food products (e.g. bread) [54].

In most populations potassium intakes are below recommendations [56]. Potassium is abundantly available in unrefined foods such as fruits and vegetables, legumes, whole grains and dairy products and promoting the intake of these foods will increase potassium intake. To illustrate, the intake of a glass of milk, a kiwi and a banana provides 1 gram of potassium [57]. Potassium-rich unrefined food products are included in guidelines for healthy diets. Adherence to the DASH eating plan will result in a potassium intake of 4.7 g/d [58]. The latest versions of the DASH diet as advised by the Dietary Guidelines for Americans are also low in sodium (menus available for sodium levels of 1.5 and 2.3 g/d) [58]. This diet is based on the DASH-sodium trial [6] and is similar to our trial with regard to the targets for sodium and potassium intake. A difference is that the changes in sodium and potassium intake in the DASH-sodium trial are established by means of a food-based approach and in our trial by the use of supplements. Since potassium-rich foods, like fruits and vegetables, may have additional beneficial effects on health besides the potassium-induced effects, increasing potassium intake by the consumption of foods instead of supplements is preferred. Examples

of other nutrients that contribute to the health effects of fruits, vegetables and other plant foods are fibers, polyphenols and magnesium [59].

Under normal physiological conditions the body is able to excrete excess amounts of potassium, mainly through urine [60], and hence the high potassium intake (5 g/d) as achieved in our trial does not pose the general population at risk for adverse health effects. However, in clinical practice, some individuals may be more vulnerable to undesirable effects of high potassium intake. These include patients with impaired kidney function, especially those with a glomerular filtration rate of less than 20 ml/min/1.73 m^2, and patients using potassium-sparing diuretics or medication that affect the RAAS (e.g. angiotensin-converting enzyme inhibitors or angiotensin receptor blockers). In these patients, changes in serum potassium after high potassium intake may pose them at risk for hyperkalemia and cardiac arrhythmias [61]. The value of increasing potassium intake, either through healthy diet, salt substitutes, or supplements, in patients who use cardiovascular medication and in patients with impaired kidney function warrants more research. As common in medical practice, medication is the primary choice of antihypertensive treatment, after which dietary advice may follow. Clinical studies are needed in which diet is changed first, and medication adopted accordingly, to find out how BP and vascular health could benefit from changes in potassium (and sodium) intake in vulnerable patient groups.

OVERALL CONCLUSIONS

In this thesis we examined the BP effects of sodium and potassium intake in the broader context of cardiovascular health, focusing also on endothelial (dys)function, arterial stiffness, body fluid balance and heart rate. Based on the results of this thesis we conclude that increasing sodium intake from a recommended level to a level that is common in Western societies strongly raises BP. The results for endothelial function and arterial stiffness are inconclusive, and more (longer-term) studies are warranted. Increasing the intake of potassium lowers BP and may improve endothelial function, also in people already adhering to the dietary guideline for sodium. Both sodium and potassium intake affected fluid parameters, likely indicating that compensatory responses are stimulated to maintain body fluid balance. Evidence for the effects of potassium on endothelial function and fluid balance parameters, however, is limited and further research is needed. Based on the work presented in this thesis, it is unlikely that increasing potassium intake in the normal range of dietary intakes affects heart rate in apparently healthy adults. When evaluating the effect of sodium and potassium interventions on cardiovascular health, results obtained from observational studies should be interpreted with caution, particularly for sodium intake. Around the world people consume on average 9–12 g of salt and 2–4 g of potassium on a daily basis. A more optimal intake of sodium and potassium can be achieved through adherence to dietary

guidelines and product reformulation by food industry. This could reduce BP by more than 10 mmHg and lower the number of cardiovascular deaths by at least one-quarter in Western populations.

REFERENCES

1. Risicofactoren En Gezondheidsevaluatie Nederlandse Bevolking, een Onderzoek Op GGD'en (REGENBOOG-project); National Institute for Public Health and Environment (RIVM)/Statistics Netherlands (CBS). 2004. Report NO: RIVM Report 260854005.
2. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ. 2013; 346:f1325.
3. He FJ, MacGregor GA. How far should salt intake be reduced? Hypertension. 2003; 42:1093-9.
4. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ. 2013; 346:f1326.
5. MacGregor GA, Markandu ND, Sagnella GA, Singer DR, Cappuccio FP. Double-blind study of three sodium intakes and long-term effects of sodium restriction in essential hypertension. Lancet. 1989; 2:1244-7.
6. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. N Engl J Med. 2001; 344:3-10.
7. Rhee M-Y. High sodium intake: review of recent issues on its association with cardiovascular events and measurement methods. Korean Circ J. 2015; 45:175-83.
8. Cogswell ME, Wang CY, Chen TC, Pfeiffer CM, Elliott P, Gillespie CD, et al. Validity of predictive equations for 24-H urinary sodium excretion in adults aged 18-39 y1-5. American Journal of Clinical Nutrition. 2013; 98:1502-13.
9. Cobb LK, Anderson CAM, Elliott P, Hu FB, Liu K, Neaton JD, et al. Methodological issues in cohort studies that relate sodium intake to cardiovascular disease outcomes: a science advisory from the american heart association. Circulation. 2014; 129:1173-86.
10. Dickinson KM, Keogh JB, Clifton PM. Effects of a low-salt diet on flow-mediated dilatation in humans. Am J Clin Nutr. 2009; 89:485-90.
11. Dickinson KM, Clifton PM, Keogh JB. A reduction of 3 g/day from a usual 9 g/day salt diet improves endothelial function and decreases endothelin-1 in a randomised cross_over study in normotensive overweight and obese subjects. Atherosclerosis. 2014; 233:32-8.
12. Jablonski KL, Racine ML, Geolfos CJ, Gates PE, Chonchol M, McQueen MB, et al. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J Am Coll Cardiol. 2013; 61:335-43.
13. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007; 115:1285-95.
14. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002; 39:257-65.

15. Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. American journal of physiology Heart and circulatory physiology. 2011; 300:H2-12.
16. Franklin SS. Arterial stiffness and hypertension: a two-way street? Hypertension (Dallas, Tex : 1979). 2005; 45:349-51.
17. Gokce N, Holbrook M, Duffy SJ, Demissie S, Cupples LA, Biegelsen E, et al. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. Hypertension (Dallas, Tex : 1979). 2001; 38:1349-54.
18. He FJ, Marciniak M, Visagie E, Markandu ND, Anand V, Dalton RN, et al. Effect of modest salt reduction on blood pressure, urinary albumin, and pulse wave velocity in white, black, and Asian mild hypertensives. Hypertension. 2009; 54:482-8.
19. Jablonski KL, Fedorova OV, Racine ML, Geolfos CJ, Gates PE, Chonchol M, et al. Dietary sodium restriction and association with urinary marinobufagenin, blood pressure, and aortic stiffness. Clin J Am Soc Nephrol. 2013; 8:1952-9.
20. Todd AS, Macginley RJ, Schollum JB, Johnson RJ, Williams SM, Sutherland WH, et al. Dietary salt loading impairs arterial vascular reactivity. Am J Clin Nutr. 2010; 91:557-64.
21. Todd AS, Macginley RJ, Schollum JBW, Williams SM, Sutherland WHF, Mann JI, et al. Dietary sodium loading in normotensive healthy volunteers does not increase arterial vascular reactivity or blood pressure. Nephrology. 2012; 17:249-56.
22. Pase MP, Grima NA, Sarris J. The effects of dietary and nutrient interventions on arterial stiffness: a systematic review. Am J Clin Nutr. 2011; 93:446-54.
23. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. The Cochrane database of systematic reviews. 2011;:CD004022.
24. Pimenta E, Gaddam KK, Oparil S, Aban I, Husain S, Dell'Italia LJ, et al. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. Hypertension. 2009; 54:475-81.
25. Mallamaci F, Leonardis D, Bellizzi V, Zoccali C. Does high salt intake cause hyperfiltration in patients with essential hypertension? J Hum Hypertens. 1996;(3):157-61.
26. Campbell KL, Johnson DW, Bauer JD, Hawley CM, Isbel NM, Stowasser M, et al. A randomized trial of sodium-restriction on kidney function, fluid volume and adipokines in CKD patients. Acta Veterinaria Scandinavica. 2014:57.
27. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem. 2006; 52:112-9.
28. Heida JE, Boesten LSM, Ettema EM, Muller Kobold AC, Franssen CFM, Gansevoort RT, et al. Comparison of ex vivo stability of copeptin and vasopressin. Clinical chemistry and laboratory medicine. 2016.
29. Morgenthaler NG, Struck J, Jochberger S, Dunser MW. Copeptin: clinical use of a new biomarker. Trends Endocrinol Metab. 2008; 19:43-9.

30. Tasevska I, Enhorning S, Burri P, Melander O. High salt intake increases copeptin but salt sensitivity is associated with fluid induced reduction of copeptin in women. Int J Hypertens. 2014; 2014:641587.
31. Binia A, Jaeger J, Hu Y, Singh A, Zimmermann D. Daily potassium intake and sodium-to-potassium ratio in the reduction of blood pressure: a meta-analysis of randomized controlled trials. J Hypertens. 2015; 33:1509-20.
32. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ (Clinical research ed). 2013; 346:f1378.
33. Blanton CA, Moshfegh AJ, Baer DJ, Kretsch MJ. The USDA Automated Multiple-Pass Method accurately estimates group total energy and nutrient intake. J Nutr. 2006; 136:2594-9.
34. Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. J Am Diet Assoc. 2004; 104:595-603.
35. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. Am J Clin Nutr. 2003; 77:1171-8.
36. Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. Am J Clin Nutr. 2008; 88:324-32.
37. He FJ, Marciniak M, Carney C, Markandu ND, Anand V, Fraser WD, et al. Effects of potassium chloride and potassium bicarbonate on endothelial function, cardiovascular risk factors, and bone turnover in mild hypertensives. Hypertension. 2010; 55:681-8.
38. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB. Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr. 2010; 104:1839-47.
39. Matthesen SK, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. Scand J Clin Lab Invest. 2012; 72:78-86.
40. Graham UM, McCance DR, Young IS, Mullan KR. A randomised controlled trial evaluating the effect of potassium supplementation on vascular function and the renin-angiotensin-aldosterone system. J Hum Hypertens. 2014; 28:333-9.
41. Tang X, Wu B, Luo Y, Peng L, Chen Y, Zhu J, et al. Effect of potassium supplementation on vascular function: A meta-analysis of randomized controlled trials. Int J Cardiol. 2017; 228:225-32.
42. Smith SR, Klotman PE, Svetkey LP. Potassium chloride lowers blood pressure and causes natriuresis in older patients with hypertension. Journal of the American Society of Nephrology: JASN. 1992; 2:1302-9.
43. Fujita T, Ando K. Hemodynamic and endocrine changes associated with potassium supplementation in sodium-loaded hypertensives. Hypertension. 1984; 6:184-92.
44. Fotherby MD, Potter JF. Potassium supplementation reduces clinic and ambulatory blood pressure in elderly hypertensive patients. J Hypertens. 1992; 10:1403-8.

45. Kong YW, Baqar S, Jerums G, Ekinci EI. Sodium and Its Role in Cardiovascular Disease - The Debate Continues. Frontiers in endocrinology. 2016; 7:164.
46. Ekmekcioglu C, Elmadfa I, Meyer AL, Moeslinger T. The role of dietary potassium in hypertension and diabetes. J Physiol Biochem. 2015.
47. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380:2224-60.
48. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002; 360:1903-13.
49. Niiranen TJ, Maki J, Puukka P, Karanko H, Jula AM. Office, Home, and Ambulatory Blood Pressures as Predictors of Cardiovascular Risk. Hypertension. 2014.
50. Sipahi I, Swaminathan A, Natesan V, Debanne SM, Simon DI, Fang JC. Effect of antihypertensive therapy on incident stroke in cohorts with prehypertensive blood pressure levels: a meta-analysis of randomized controlled trials. Stroke; a journal of cerebral circulation. 2012; 43:432-40.
51. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010; 26:631-40.
52. Ras RT, Streppel MT, Draijer R, Zock PL. Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. Int J Cardiol. 2013; 168:344-51.
53. van Rossum CTM, Buursma-Rethans EJM, Fransen HP, Verkaik-Kloosterman J, Hendriksen MAH. Zoutconsumptie van kinderen en volwassenen in Nederland: Resultaten uit de Voedselconsumptiepeiling 2007-2010 (Salt consumption of children and adults in the Netherlands: Results from the Dutch National Food Consumption Survey 2007-2010). Bilthoven, the Netherlands: National Institute for Public Health and Environment (RIVM). 2012.
54. Webster J, Trieu K, Dunford E, Hawkes C. Target salt 2025: a global overview of national programs to encourage the food industry to reduce salt in foods. Nutrients. 2014; 6:3274-87.
55. Hendriksen MAH, Verkaik-Kloosterman J, Noort MW, van Raaij JMA. Nutritional impact of sodium reduction strategies on sodium intake from processed foods. Eur J Clin Nutr. 2015; 69:805-10.
56. van Mierlo LAJ, Greyling A, Zock PL, Kok FJ, Geleijnse JM. Suboptimal potassium intake and potential impact on population blood pressure. Arch Intern Med. 2010; 170:1501-2.
57. NEVO-online versie 2016/5.0, RIVM, Bilthoven.
58. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. December 2015.
59. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. 2013; 4:384S-92S.
60. National Research Council. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, DC: The National Academies Press, 2005.
61. He FJ, MacGregor GA. Beneficial effects of potassium on human health. Physiol Plant. 2008; 133:725-35.

ENGLISH SUMMARY

ENGLISH SUMMARY

Cardiovascular diseases (CVD) are the main cause of death worldwide. Annually, about 17.5 million people die from CVD, accounting for ~30% of deaths worldwide. Elevated blood pressure (BP) is a major risk factor for CVD and the largest single contributor to global mortality. BP is a modifiable risk factor that is largely determined by lifestyle factors, including diet. Dietary minerals, in particular sodium and potassium, play an important role in BP regulation. While adverse effects of sodium and beneficial effects of potassium on BP have repeatedly been shown in human intervention studies, evidence on other vascular effects of these dietary minerals is still scarce. Therefore, we investigated the BP effects of sodium and potassium intake in healthy humans in a broader (patho)physiological context, focusing also on endothelial function, arterial stiffness, fluid regulation and heart rate.

In Chapter 2, the effects of sodium and potassium supplementation on BP and arterial stiffness were examined by means of a randomized placebo-controlled crossover trial. Thirty-six untreated Dutch individuals with mildly elevated BP on a fully controlled diet that was relatively low in sodium (2–3 g/d) and potassium (2–3 g/d) received capsules with sodium (3 g/d), potassium (3 g/d) or placebo, for 4 weeks each, in random order. After each intervention, fasting office BP, 24-h ambulatory BP and measures of arterial stiffness were assessed. The results of this study showed that increased sodium intake strongly raised office and ambulatory systolic BP (7–8 mmHg) whereas increased potassium intake lowered systolic BP (3–4 mmHg). Potassium supplementation increased ambulatory heart rate, but office heart rate was not affected. Measures of arterial stiffness were not materially affected by increased sodium or potassium intake, possibly due to the relatively short intervention period.

In the same study we investigated the effects of increased sodium and potassium intake on the functional measure of endothelial function (flow-mediated dilation), and on a comprehensive set of biomarkers of endothelial dysfunction and low-grade inflammation (Chapter 3). Four weeks of supplemental sodium had no effect on brachial flow-mediated dilation, or on the blood biomarkers of endothelial dysfunction and low-grade inflammation, except for an increase in serum endothelin-1 (a biomarker of endothelial dysfunction). Potassium supplementation improved flow-mediated dilation by 1.2% and tended to lower the low-grade inflammation marker interleukin-8. This suggests that potassium may beneficially influence vascular health by improving endothelial function.

In a *post-hoc* analysis of the same study in 35 untreated individuals, the humoral effects of supplemental sodium and potassium were assessed using a panel of markers that are involved in osmoregulation and volume regulation (Chapter 4). Results showed that supplemental sodium increased plasma natriuretic peptides and plasma copeptin, and suppressed the renin-angiotensin system. Supplemental potassium decreased plasma MR-pro-ANP, increased plasma copeptin, and stimulated the renin-angiotensin system. These

findings suggest that the mineral-induced changes in BP elicit several counter regulatory mechanisms to maintain volume homeostasis.

In Chapter 5, the effect of potassium supplementation on heart rate was assessed in a meta-analysis of 22 randomized, placebo-controlled trials in healthy adults. Overall, increasing potassium intake by 2–3 g/d for at least two weeks did not affect resting heart rate. Twenty-four-hour ambulatory heart rate was not significantly affected in subgroup analysis of 4 RCTs, including ours. Other subgroup analyses for characteristics of the study and study population also showed no significant effects, and there was no evidence for a dose-response relationship. These results suggest that increasing potassium intake is not expected to adversely affect heart rate in apparently healthy adults.

In Chapter 6, BP associations for sodium and potassium intake using different dietary assessment methods were examined. Data of 993 healthy Dutch adults not on antihypertensive medication were analyzed using a cross-sectional approach. Sodium and potassium intake were estimated from two non-consecutive 24-h urinary samples (considered as the gold standard), two non-consecutive web-based 24-h recalls, and a validated 180-item food frequency questionnaire (FFQ). This study showed no significant associations of sodium intake with BP, regardless of the dietary assessment method used. Potassium intake estimated from 24-h urine and FFQ was inversely associated with BP (~1.5 mmHg reduction per 1 g/d increment). This suggests that dietary assessment methods in cross-sectional studies may be inadequate for estimating the association of sodium intake with BP, but may yield reliable results for potassium intake.

As discussed in Chapter 7, the studies presented in this thesis indicate that increasing sodium intake from a recommended level to a level that is common in Western societies for four weeks strongly raises BP in individuals with an untreated mildly elevated BP. The results for endothelial function and arterial stiffness are inconclusive, and hence more (longer-term) studies are warranted. Increasing the intake of potassium lowers BP and improves endothelial function, even in individuals on a relatively low-sodium diet. Both sodium and potassium intake affected fluid parameters, likely indicating that compensatory responses are stimulated to maintain body fluid balance. Although in our RCT ambulatory heart rate was increased after supplemental potassium, the meta-analysis showed that increasing potassium intake is unlikely to affect heart rate in apparently healthy adults. When evaluating the effectiveness of sodium and potassium intake on cardiovascular health, results obtained from observational studies should be interpreted with caution, particularly for sodium intake.

Around the world people consume on average 9–12 g of salt and 2–4 g of potassium on a daily basis. A more optimal intake of sodium and potassium can be achieved through adherence to dietary guidelines and product reformulation by food industry. This could reduce BP by more than 10 mmHg and lower the number of cardiovascular deaths by at least one-quarter in Western populations.

DANKWOORD | ACKNOWLEGDMENTS

Yes, mijn proefschrift is af! Dit had ik niet voor elkaar gekregen zonder de hulp van velen. Graag wil ik iedereen bedanken die heeft bijgedragen aan dit proefschrift.

Allereerst gaat mijn dank uit naar mijn promotor en dagelijkse begeleider Marianne Geleijnse. Marianne, hoe had ik dit volbracht zonder jou. Jouw deur stond altijd voor mij open, ook toen ik de Wageningen Universiteit al had verlaten. Ik heb de afgelopen jaren ontzettend veel van jou geleerd en ik bewonder jouw passie voor het zoutonderzoek. Bedankt voor de inspirerende overleggen, je betrokkenheid bij mijn onderzoek en je vertrouwen. Ik vond het fijn om met je samen te werken.

Daarnaast dank voor mijn promotor Pieter van 't Veer. Pieter, bedankt voor de mogelijkheid om bij jou te promoveren. Onze overleggen waren wat minder frequent, maar het meedenken en je methodologische inzichten heb ik erg gewaardeerd.

Prof. Daan Kromhout, Prof. Ronald Mensink, Dr Dick Thijssen en Prof. Edith Feskens bedankt voor het plaatsnemen in mijn promotiecommissie en het beoordelen van mijn proefschrift.

Een belangrijk en erg leuk onderdeel van mijn promotieonderzoek was de KaNa-trial. Dit onderzoek was niet mogelijk geweest zonder de inzet van de vele deelnemers en onderzoeksmedewerkers. Graag wil ik alle KaNa-trial deelnemers bedanken voor hun enthousiaste medewerking, eerlijkheid (iets met libido-verhogende werking van de supplementen) en bereidwilligheid. Petje af dat jullie 13 weken zonder (al te veel) te klagen hebben deelgenomen aan een gecontroleerde voedingsproef. Jullie hebben daarmee een belangrijke bijdrage geleverd aan dit proefschrift. Daarnaast wil ik Harrie, Sarah en Danielle bedanken voor alle hulp tijdens de KaNa-trial. Het was erg fijn dat ik op jullie kon rekenen en het was een plezier om met jullie samen te werken. Harrie, jij hebt alle fases van het onderzoek meegemaakt: van het werven van deelnemers op de markt tot het verwerken van de onderzoekresultaten in mooie figuren. Ook zorgde jij voor de nodige lol tijdens de uitvoering van het onderzoek. bedankt daarvoor. Ook speciale dank voor Els, Yvonne, Ingrid en alle andere dames van het diëtistenteam voor het samenstellen en bereiden van de maaltijden. De hoeveelheid werk die jullie hebben verzet tijdens de voedingsproef is bewonderingswaardig. Karin, fijn dat je de blindering hebt verzorgd voor de KaNa-trial. Jantien, Anita en Diana bedankt voor het bloedprikken en de organisatie daarvan, en Marlies, Nhien, Robert, Dini en Marjo voor het uitvoeren van de labanalyses. Dieuwerke en Ilse, leuk dat er sensorische metingen hebben plaatsgevonden in de KaNa-trial en dat we onze onderzoeken op deze manier hebben kunnen aanvullen.

Tijdens mijn promotieonderzoek heb ik epidemiologische analyses mogen uitvoeren in de NQplus studie. Edith, Anne en Diewertje bedankt voor het beschikbaar stellen van de

data en voor jullie hulp in het prepareren van de data. Anouk en Jeanne, bedankt voor de samenwerking en jullie bijdrage aan het artikel.

Collega's en partners van TIFN, bedankt voor de samenwerking, input en leerzame bijeenkomsten. Diederik, fijn dat je mij hebt wilt helpen met het voorbereiden van de KaNa-trial. Jos, Peter en Yvo bedankt voor de training in het uitvoeren van de FMD, PWV en PWA-metingen en het analyseren van de ultrasoundbeelden. Stephan en Ineke, bedankt voor het mooie artikel dat is voortgekomen uit onze fijne samenwerking. Leuk dat er nog meer onderweg is. Anouk, ik vond het erg fijn om collega's te zijn binnen het TIFN project en de afdeling Humane Voeding. Altijd weer gezellig als we kamergenootjes konden zijn tijdens een congres of ander event.

Ook wil ik graag (oud-)collega's van de afdeling Humane Voeding, die niet direct betrokken waren bij mijn promotieonderzoek, bedanken. Sabita, jij was die degene die mij tijdens mijn Master thesis klaarstoomde en motiveerde voor het promotieonderzoek. Onze fijne samenwerking heeft geresulteerd in drie mooie zuivel meta-analyses, dank daarvoor. Janette, ik kijk met veel plezier terug op onze samenwerking tijdens twee van deze meta-analyses. Leuk dat ik je mocht helpen bij het creëren van de Ding's spaghetti plots. Daan, bedankt voor je interesse in mijn project en in mij als persoon. Het dagje uit met Marianne en Susanne, waarbij we een deel van Route 66 hebben afgelegd, was erg gezellig. Hendriek, bij jou kon ik altijd terecht voor hulp bij de statistische analyses, dat heb ik erg gewaardeerd. Agnes, Annemarie, Anouk, Inge, Laura, Nikkie en Swetlana, dank voor de gezellige organisatie van de PhD-tour naar Australië. Het was een geslaagde trip. Andere mede PhD-ers het was leuk om jullie tijdens de PhD-tour, lunches en events beter te leren kennen.

Tijdens mijn promotieonderzoek heb ik een aantal studenten begeleid. Christine, Sibabalele en Famke, ik vond het erg leuk en leerzaam om jullie te begeleiden. Bedankt voor jullie bijdrage. Famke, jouw Master thesis is de basis geweest voor een mooi artikel, bedankt daarvoor. Ik wens je veel succes met jouw promotieonderzoek.

Lieve mensen van kamer 116, jullie mogen zeker niet ontbreken in dit dankwoord. Ik ben erg blij dat ik mijn promotieonderzoek met zulke gezellige kamergenoten heb mogen beleven. Wat hebben we veel afgekletst: de maandagochtend was grotendeels gereserveerd om de weekenden door te spreken en de overige onderwerpen werden besproken tijdens de steeds langer durende lunchpauzes. We hebben het enorm gezellig gehad en niet voor niets is kamer 116 inmiddels een welbekend begrip geworden. Laten we vooral blijven afspreken. Eveline, 4 jaar lang was jij mijn echte kamergenootje. Wij hebben vele gesprekken over van alles en nog wat gevoerd. Bedankt voor al je handige SAS-codes, gelukkig kan ik die inmiddels wat beter ontcijferen. James, leuk dat ik zowel je kamer 116, als TIFN-collega mocht zijn. Enorm bedankt voor het uitvoeren van de vele FMD-metingen tijdens de KaNa-trial. Jammer dat

we elkaar tegenwoordig niet meer kunnen opzoeken bij Nutricia. Femke, samen met James, zorgde jij voor de vele kamer 116-uitjes en het opzetten van de welbekende poules, welke voor veel plezier hebben gezorgd. Ik vind het erg leuk dat we tegenwoordig weer collega's zijn, dat we wederom een kamer delen en dat we lekker samen kunnen stoeien met SAS. Harrie, jij zorgde altijd voor veel hilariteit op kamer 116. Je uiteenzettingen over bitcoins, de te dure fietsenbanden en het 'per ongeluk' nog in het bezit hebben van de unieke reklabels zijn slechts enkele voorbeelden van onderwerpen die voor veel plezier hebben gezorgd.

Ook de tijdelijke bewoners van kamer 116, Lisette, Lisa, Claudia, Elly, Fabian, Jaike, Laura, Rianne, Sabine en Sifra, bedankt voor jullie gezelligheid. Jaike, geweldig dat we sinds een half jaar weer collega's zijn en dat we zelfs al hebben mogen samenwerken in een project.

En dan mijn twee paranimfen. Lieve Ellen en Susanne, ik ben ontzettend trots dat jullie vandaag naast mij willen staan. Ellen (zussie), mijn grote zus, ook jij mag zeker niet ontbreken in dit dankwoord. Je bent degene die mij te allen tijde van raad wilt voorzien en mij helpt bij het doorhakken van knopen. Onze hechte band met bijna dagelijks contact vind ik heel bijzonder en ik ben enorm trots dat ik jouw zus en de tante van Jurre mag zijn. Susanne, ik vond het een eer om bij jouw promotie aan jouw zijde te staan, en ben erg blij dat jij nu aan de mijne staat. Al snel nadat jij intrek nam in kamer 116, bleek dat we dat we eindeloos konden kletsen, veelal over niet-werkgerelateerde onderwerpen. Onze reizen naar Australië en San Francisco waren gaaf. Ik ben blij dat we elkaar nog regelmatig zien en ik hoop dat dat zo blijft. Wie weet worden we ooit weer collega's ;-), dat zou ik in ieder geval erg gezellig vinden.

Inmiddels ben ik alweer ruim 1.5 jaar werkzaam bij OCS Life Sciences. OCS, bedankt voor de mogelijkheid om mijn proefschrift af te ronden en voor de vele kansen die jullie mij bieden. OCS collega's, bedankt voor de gezelligheid en de fijne samenwerking.

Ook wil ik graag mijn lieve vriendinnen bedanken. Sanne, we zijn al vriendinnetjes sinds dat ik het me kan herinneren. Hoewel we elkaar nu wat minder vaak zien, onze vriendschap blijft vertrouwd en we hebben altijd genoeg om over bij te praten. Bedankt dat jij de omslag van dit proefschrift hebt ontworpen. Ik ben enorm blij met het resultaat. Joyce en Karen, afgelopen jaren hebben we vele heerlijke etentjes, wijntjes en gezellige avondjes gehad. Ik hoop dat er nog vele zullen volgen. Misschien kunnen we nu dan eindelijk eens naar Marrakech? Loes, wij raken echt nooit uitgepraat, onze dates zijn altijd te kort. Ik vind het fijn dat we over van alles en nog wat kunnen praten en ik ben erg blij met zo'n lief en oprecht vriendinnetje als jij. Yvonne, wij zijn samen gestart in Wageningen. Ook al ging mijn keuze uit naar een andere Master, wij zijn elkaar niet uit het oog verloren. Als we elkaar weer zien, is het altijd erg gezellig. Lotte, Nanette, Hanneke, Cora, Priscilla en Anke, bedankt voor de nodige ontspanning, afleiding en gezelligheid.

Lieve familie en schoonfamilie, ook al was het soms lastig te begrijpen wat ik nu precies deed, bedankt voor jullie interesse in mijn onderzoek. Nicky en Margreet, fijn dat jullie hebben meegedacht over de stellingen, het heeft leuke discussies teweeggebracht. Ellen en Jan, jullie hebben me altijd onwijs veel gesteund. Bedankt voor jullie adviezen, maar vooral voor de gezelligheid. Jan, jij ook bedankt voor je hulp bij de opmaak van mijn proefschrift. Lieve neefjes en nichtjes: Jorn, Sem, Loek, Jurre, Fame en Beau, wat kan ik van jullie genieten. Bedankt voor jullie vrolijkheid en de vele knuffels.

Allerliefste papa en mama, als er iemand dag en nacht voor je klaarstaat, dan zijn jullie het wel. Jullie steunen mij onvoorwaardelijk in alles wat ik doe en daar ben ik jullie ontzettend dankbaar voor. Mede dankzij jullie hulp (op het gebied van bouw, huishouden of wat dan ook) kon ik me focussen op de afronding van mijn proefschrift. Bedankt voor jullie liefde en voor het opgroeien in een heerlijk warm nest. Het is altijd weer fijn om 'thuis' te komen.

Lieve Noud, het hoofdstuk proefschrift is nu (eindelijk) afgerond. Bedankt voor je nuchterheid, geduld, zorgzaamheid (inclusief het brengen van de vele theetjes) en onvoorwaardelijke steun, hoe onbegrijpelijk de onderzoekswereld soms ook voor je is. Nu is het tijd om samen van onze heerlijke huis te gaan genieten. Wat ben ik trots op wat wij, maar voornamelijk jij, hebt neergezet.

ABOUT THE AUTHOR

Curriclum vitae
List of publications
Overview of completed activities

CURRICULUM VITAE

Lieke Gijsbers was born on 24th August 1987 in Nijmegen, the Netherlands and was raised in Afferden (Gelderland). After completing secondary school at Pax Christi College in Druten in 2005, she started studying Health and Society at Wageningen University. After obtaining her Bachelor's degree in 2008, she continued her education at Wageningen University in Nutrition and Health. In 2010, Lieke completed her MSc thesis entitled "The relationship between dairy intake and diabetes type 2: dose-response meta-analysis of observational studies". Thereafter, she did an internship at GGD (Municipal Health Services) Nijmegen where she investigated, in collaboration with TNO (Netherlands Organization for Applied Scientific Research) Leiden, the association between sleeping and overweight in children. In 2011, Lieke obtained her Master's degree with a specialization in Nutritional Epidemiology and Public Health. After graduation, she started as a PhD student at the Division of Human Nutrition of Wageningen University. Her PhD research focused on the vascular effects of sodium and potassium intake, and was conducted within the framework of TiFN. During the course of her PhD project, she was involved in teaching activities and attended several national and international courses and conferences. Moreover, she was selected as a participant in the 46th Ten-Day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention in Coorg, India. Furthermore, she was a member of the organizing committee of a study tour for PhD students of the Division of Human Nutrition to research institutes, universities and companies in Australia and a member of the PhD committee to represent all PhD students from the Division of Human Nutrition within the division. Since January 2016, Lieke works as a clinical programmer at OCS Life Sciences in 's-Hertogenbosch.

LIST OF PUBLICATIONS

Gijsbers L, Geelen A, de Vries JH, van 't Veer P, Geleijnse JM. Impact of dietary assessment methods on the associations between sodium, potassium and blood pressure. Submitted

Guo J, Astrup A, Lovegrove JA, **Gijsbers L**, Givens DI, Soedamah-Muthu SS. Milk and dairy consumption and risk of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. Eur J Epidemiol. 2017;32:269-287

Gijsbers L, Mölenberg FJ, Bakker SJ, Geleijnse JM. Potassium supplementation and heart rate: a meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis. 2016;26:674-82

de Goede J, Soedamah-Muthu SS, Pan A, **Gijsbers L**, Geleijnse JM. Dairy consumption and risk of stroke: a systematic review and updated dose-response meta-analysis of prospective cohort studies. J Am Heart Assoc. 2016;5

Gijsbers L, Ding EL, Malik VS, de Goede J, Geleijnse JM, Soedamah-Muthu SS. Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. Am J Clin Nutr. 2016;103:1111-24

Riphagen IJ*, **Gijsbers L***, van Gastel MD, Kema IP, Gansevoort RT, Navis G, Bakker SJ, Geleijnse JM. Effects of potassium supplementation on markers of osmoregulation and volume regulation: results of a fully controlled dietary intervention study. J Hypertens 2016;34:215-20. (*Contributed equally)

Gijsbers L, Dower JI, Schalkwijk CG, Kusters YHAM, Bakker SJL, Hollman PCH, Geleijnse JM. Effects of sodium and potassium supplementation on endothelial function: a fully controlled dietary intervention study. Br J Nutr 2015;114:1419-1426

Dower JI, Geleijnse JM, **Gijsbers L**, Schalkwijk C, Kromhout D, Hollman PC. Supplementation of the pure flavonoids epicatechin and quercetin affects some biomarkers of endothelial dysfunction and inflammation in (pre)hypertensive adults: a randomized double-blind, placebo-controlled, crossover trial. J Nutr 2015;145:1459-1463

Bolhuis DP, **Gijsbers L**, de Jager I, Geleijnse JM, de Graaf K. Encapsulated sodium supplementation of 4 weeks does not alter salt taste preferences in a controlled low sodium and low potassium diet. Food Quality and Preference 2015;46:58-65

Dower JI, Geleijnse JM, **Gijsbers L**, Zock PL, Kromhout D, Hollman PCH. Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomized, double-blind, placebo-controlled, crossover trial. Am J Clin Nutr 2015;101:914-921

Gijsbers L, Dower JI, Mensink M, Siebelink E, Bakker SJL, Geleijnse JM. Effects of sodium and potassium supplementation on blood pressure and arterial stiffness: a fully controlled dietary intervention study. J Hum Hypertens 2015;29:592-598

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline Specific Activities

- European Congress of Epidemiology 2015, The Netherlands Epidemiology Society (in collaboration with the International Epidemiological Association & European Epidemiology Federation), Maastricth, The Netherlands, 2015
- 25th European Meeting on Hypertension & Cardiovascular Protection, European Society of Hypertension, Milan, Italy, 2015
- NWO Nutritional Science Days, Nationale Academie van Voedingswetenschappen, Deurne, The Netherlands, 2014
- 46th Ten-Day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention, International Society of Cardiovascular Disease Epidemiology and Prevention, Coorg, India, 2014
- Epidemiology and Prevention | Nutrition, Physical Activity and Metabolism – Scientific Sessions, American Heart Association, San Francisco, USA, 2014
- NWO Nutritional Science Days, Nationale Academie van Voedingswetenschappen, Deurne, The Netherlands, 2013
- Master Class in Longitudinal Data Analysis (Mixed Models), Graduate School VLAG, Wageningen, The Netherlands, 2013
- Zoutcongres, Voeding Nu and Food Micro, Amsterdam, The Netherlands, 2012
- NWO Nutritional Science Days, Nationale Academie van Voedingswetenschappen, Deurne, The Netherlands, 2012
- Vascular Biology (PhD training course), Dutch Heart Foundation, Arnhem, The Netherlands, 2011
- NWO Nutritional Science Days, Nationale Academie van Voedingswetenschappen, Deurne, The Netherlands, 2011
- Regression Analysis; Erasmus Summer Programme, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2011
- Introduction to Data-Analysis; Erasmus Summer Programme, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2011
- Clinical Trials; Erasmus Summer Programme, Netherlands Institute for Health Sciences (NIHES), Rotterdam, The Netherlands, 2011
- Nutritional and Lifestyle Epidemiology, Graduate School VLAG, Wageningen, The Netherlands, 2011

General Courses

- Career Orientation, Wageningen Graduate School, Wageningen, The Netherlands, 2015
- Scientific Writing, Wageningen Graduate School, Wageningen, The Netherlands, 2013
- Techniques for Writing and Presenting a Scientific Paper, Wageningen Business School, Wageningen, The Netherlands, 2013
- Workshop Basic IP for TIFN researchers, TIFN, Wageningen, The Netherlands, 2012
- Working with EndNote X4, Wageningen University Library, Wageningen, The Netherlands, 2011
- PhD Introduction Week, Graduate School VLAG, Baarlo, The Netherlands, 2011

Optionals

- Epi-research meetings, Rothman lunches, methodology club meetings and staff seminars, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands, 2011 - 2015
- TIFN expert meetings, TIFN, Utrecht, The Netherlands, 2011 – 2015
- TIFN annual conference, TIFN, Vlaardingen, The Netherlands, 2015
- TIFN midterm review, TIFN, Wageningen, The Netherlands, 2014
- PhD study tour, Division of Human Nutrition, Melbourne/Sydney, Australia, 2013
- PhD study tour, Division of Human Nutrition, South West USA and Mexico, 2011
- Preparation of PhD research proposal, Wageningen University, Wageningen, The Netherlands, 2011
- TIFN team workshop, TIFN, Wageningen, The Netherlands, 2011

The studies described in this thesis were performed within the framework of TiFN.
Financial support from Wageningen University and TiFN for printing this thesis is gratefully acknowledged.

Cover design: Sanne Gijsbers, Nijmegen
Layout design: Proefschriftmaken, Vianen
Printed by: Proefschriftmaken, Vianen

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