Clinical impact of nonosmotic sodium storage

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CHAPTER 1

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GENERAL INTRODUCTION

Historically, the use of salt goes back only several thousand years. Hunter-gatherer diets, for example, contained less than 1 gram of salt per day\(^1\). With the advent of agriculture and civilization, dietary salt intake increased rapidly. Nowadays, the average dietary salt intake is about 10 grams per day. This greatly exceeds the advice of the World Health Organization (WHO) to limit daily salt intake to 5 grams, which is equal to 2 grams of sodium\(^2-4\). In the United States, 90% of adults exceeded these recommendations in 2009-2012\(^5\). The majority of consumed sodium comes from processed foods\(^2\). Many people are therefore unaware of the fact that they eat more sodium than advised by the WHO.

Sodium intake and blood pressure

The notion to limit sodium consumption originates from the strong correlation between sodium intake and blood pressure (BP) that has been demonstrated by many studies. The International Study of Salt and Blood Pressure (INTERSALT) was one of the first large epidemiological studies, including over 10,000 subjects, that demonstrated a positive association between BP and dietary sodium intake, both within a population as well as across populations\(^6\). At the population level, a 2 gram higher daily sodium intake was associated with a systolic and diastolic BP increase of 6 and 3 mmHg, respectively\(^6\). Interestingly, the INTERSALT study showed that the BP increase that is normally seen with increasing age was not observed in populations consuming low sodium diets, indicating that high sodium intake plays a crucial role in the development of hypertension (Figure 1).

The results of the INTERSALT study have been confirmed by many randomized controlled interventional trials that have shown that BP decreases in response to a reduction in dietary sodium intake\(^7-8\). The BP lowering effects were consistent in both normotensive and hypertensive individuals, men and women, subjects with and without concurrent antihypertensive therapy, and across all ethnicities\(^7-8\). In addition, population studies in Portugal, Japan and China that were able to actively reduce sodium intake in one city while sodium intake was not changed in a comparable city have shown similar beneficial effects of sodium reduction on BP\(^9-11\). A decrease of 0.4 to 0.8 grams of daily sodium intake resulted in a 3 to 5 mmHg decrease in systolic BP\(^9-11\). Baseline dietary sodium intake was above 5 grams per day in all of these cities\(^9-11\).

The magnitude of the BP-lowering effect of dietary sodium reduction has been shown to be dependent on the degree of sodium reduction, both in hypertensive and normotensive individuals\(^13, 14\). Well-controlled, interventional studies that have investigated a step-wise decrease in sodium intake have shown a clear dose-response relationship between sodium intake, ranging from 1.2 to 4.8 grams sodium per day, and BP levels\(^13, 14\). These data suggest that
the WHO advice to limit sodium intake to 2 grams per day, which has not been achieved in any civilized society yet, may even be not low enough. This view has been adapted by the British National Institute for Health and Care Excellence (NICE) that is aiming for a maximum daily sodium intake of 1.2 grams in 2025. The feasibility of this target, however, may be questioned as lowering sodium intake to 2 grams has been proven to be a major task.

With regard to kidney disease, high sodium intake has been shown to lead to similar detrimental effects. Besides a decrease in BP, a reduction in sodium intake has been shown to decrease albuminuria, prevent glomerular hyperfiltration and potentiate the response to renin-angiotensin-aldosterone system receptor blockers and diuretics\textsuperscript{15,16}.

In recent years, reduction of world-wide sodium intake has therefore been one of the main areas of focus of the WHO with the aim to achieve a target less than 2 grams a day by 2025.

\textbf{Figure 1.} The main results of the INTERSALT study.

The results of the INTERSALT study demonstrating that the age-related increase of BP is related to high sodium intake and not observed in populations consuming <1.4 grams of sodium. Adapted from the Intersalt Cooperative Research Group\textsuperscript{12}.

\textbf{Sodium homeostasis and BP regulation: The classic view}

The mechanism behind the sodium-induced increase in BP has been described by Guyton and Borst, who both demonstrated that long-term control of arterial pressure is closely related with body fluid homeostasis\textsuperscript{17,18}. Being the principal cation in the extracellular volume, sodium is responsible for preservation and regulation of the effective circulating and extracellular volume. In this concept, solely the kidney is responsible for matching sodium excretion with sodium
intake. The kidney has therefore been thought to be the most important regulator of total body sodium, and thereby extracellular volume.

Renal responses to changes in sodium intake are not instant. The kidney needs about 3 to 5 days to adapt to a new level of sodium intake. To control extracellular osmolality in response to an acute increase in body osmoles following sodium intake or infusion, water will shift from the intracellular to the extracellular compartment (Figure 2). In addition, within hours to days, the elevated plasma osmolality will stimulate water intake and antidiuretic hormone (ADH) production, which results in water retention and an increase in extracellular volume and BP. When a new steady state of sodium homeostasis is achieved, in which the kidney is able to match sodium intake and excretion again, this will be at the expense of an increased extracellular volume and BP. The increase in BP is the result of an increased cardiac output and total peripheral resistance, of which the latter is caused by autoregulatory vasoconstriction.

The effect of sodium intake on BP varies considerably among individuals, discriminating sodium sensitive individuals, who are characterized by a BP increase after an increase in sodium intake, from sodium resistant individuals, who do not develop a BP increase. In other words, sodium sensitive individuals have an impaired renal capacity for sodium excretion and need a higher BP to excrete a certain amount of sodium. The exact underlying mechanism has not yet been resolved but many risk factors for sodium sensitivity have been described such as aging, hypertension, kidney disease, African-American ancestry and a low nephron number. As the kidney is considered to be the key organ for regulation of total body sodium content, all risk factors are thought to limit renal capacity for sodium excretion, thereby increasing extracellular volume and BP. Consequently, polymorphisms of genes that are involved in renal sodium handling have a similar effect on sodium sensitivity.

In a study with 27 years of follow-up, Weinberger et al. have shown that the presence of sodium sensitivity has serious consequences for cardiovascular risk. Sodium sensitive individuals, even when normotensive, had a similar incidence of mortality as hypertensive individuals, while sodium resistant normotensive individuals showed better survival. These data demonstrate that a significant part of the cardiovascular risk attributable to high sodium consumption is mediated by hypertension.
Figure 2. The classic view of sodium homeostasis.

(A) Body water is divided over the intracellular (2/3rd) and extracellular compartment (1/3rd). Because cell membranes are permeable for water, the osmolality is equal in both compartments. Within the extracellular compartment, sodium is the principal cation. The principal cation in the intracellular compartment is potassium. (B) External sodium will be added to the extracellular volume. To control body water osmolality, water will shift from the intracellular compartment to the extracellular compartment resulting in a slight rise of body water osmolality and plasma sodium concentration. Adapted from23.

Blood pressure and cardiovascular risk
Hypertension, defined as a systolic BP higher than 140 mmHg or a diastolic BP above 90 mmHg, is the most common condition seen in primary care. Approximately 1 billion people worldwide suffer from hypertension, which has been estimated to increase to 1.5 billion in 202525. In the great majority (90-95%) of hypertensive subjects the exact etiology of hypertension is unknown26. This type of hypertension, called primary or essential hypertension, is often
multifactorial and associated with the presence of various risk factors including ageing, high dietary sodium intake, obesity, kidney disease, African-American descent, smoking and genetic predisposition. Of all risk factors, high sodium intake is considered to be one of the crucial environmental factors in primary hypertension as low sodium intake is able to abolish the effect of many other risk factors, such as age, as demonstrated by the INTERSALT study\textsuperscript{6, 26}.

Hypertension is in most cases an asymptomatic condition but may ultimately result in cardiovascular diseases such as myocardial infarction, stroke, heart failure, kidney failure and peripheral artery disease. Hypertension is therefore the leading risk factor for global disease burden, accounting for 9.4 million deaths each year\textsuperscript{27}. In 1967, the Veteran Administration (VA) Cooperative Study Group on Antihypertensive Agents performed one of the first randomized, placebo-controlled studies that demonstrated a clear benefit of BP lowering therapy on cardiovascular morbidity and mortality\textsuperscript{28}. From then, many clinical studies using various antihypertensive drugs have confirmed this finding\textsuperscript{29, 30}. Meta-analyses have found that a 10 mmHg decrease in systolic BP or a 5 mmHg decrease in diastolic BP in hypertensive patients is typically associated with a 30-40% lower risk of stroke death and a 25% lower risk of ischemic heart disease\textsuperscript{28, 30}. Even a small 2 mmHg reduction in BP was shown to decrease stroke mortality development and progression of kidney disease. A meta-analysis of randomized controlled trials demonstrated that BP values above 140 and 160 mmHg increased the risk for kidney failure or doubling of plasma creatinine levels twofold and threefold, respectively\textsuperscript{31}.

**Blood pressure control**

Despite the presence of many cheap and effective antihypertensive drugs, half of the patients with hypertension have a BP that remains uncontrolled (>140/90 mmHg)\textsuperscript{32, 33}, which increases cardiovascular risk considerably and results in major health care costs. If, for example, in the United States, all hypertensive patients would have their BP controlled according to the guidelines this would result in around 56,000 less cardiovascular events and 13,000 less deaths from cardiovascular causes annually\textsuperscript{34}. Moreover, this would save over a billion dollar of health care costs every year\textsuperscript{34}. These numbers stress the fact that antihypertensive therapies should be improved to control the cardiovascular burden of hypertension. This could be achieved by improvement of the application of current antihypertensive drugs or development of antihypertensive drugs with new working mechanisms. In this respect, drugs that target sodium sensitivity are of interest as this is one of the main contributing factors in patients with uncontrolled hypertension. In addition, the present measures that have been taken to limit sodium intake on a population level, which do not have the desired effects on sodium intake yet, may help to improve BP control.
Sodium and cardiovascular risk

Considering the causal relationship between dietary sodium intake and BP, and BP and cardiovascular risk, one would expect a strong relationship between sodium intake and cardiovascular risk. However, in recent years, large cohort studies have challenged this concept. As no adequately powered randomized controlled studies have assessed the effects of sodium reduction on cardiovascular endpoints, these inconsistent cohort studies have had great influence on the worldwide sodium debate in recent years.

So far, only one trial has been designed to investigate the effects of sodium intervention on cardiovascular events. However, in this study, sodium was replaced by potassium. Although this study reported a long-term benefit on cardiovascular mortality in the sodium intervention group, the BP lowering effects of potassium cannot be ignored. It is therefore not known to what extent these effects may be attributed to sodium reduction itself.

Other randomized interventional studies that have tested the effects of sodium reduction on cardiovascular outcome include the Trials of Hypertension Prevention (TOHP) I and II. Both trials were designed to test the feasibility and efficacy, in terms of BP control, of sodium reduction in patients with prehypertension. Eighteen months of lifestyle intervention on sodium reduction in TOHP I resulted in a 1-gram decrease in daily sodium intake and a 1.7/0.8 mmHg decrease in BP, whereas TOHP II investigated a 36-month sodium intervention that decreased daily sodium intake with 0.9 grams and BP with 1.2/0.7 mmHg. Although neither trial was designed or powered to study cardiovascular outcome, 77% of the participants were retrieved to analyse the incidence of cardiovascular events during a 10-15 year follow-up. Relative to controls, the sodium intervention group had a 25% lower risk for cardiovascular events. No effects on mortality were found.

Two considerably smaller studies (n = 681, follow-up 30 months; n = 67, follow-up 24 months) that evaluated the effects of sodium reduction in hypertensive patients did not identify a cardiovascular protective effect of sodium reduction, although one of these studies showed a non-significant 20% reduction of cardiovascular disease in the sodium intervention group. The fact that a trial designed to detect a 10% reduction in cardiovascular events as a result of sodium reduction would require approximately 28,000 participants with a 5-year follow-up underlines that all of these studies have serious power problems.

Data from prospective cohort studies and meta-analyses of these cohort studies show contrasting results regarding the optimal sodium intake for cardiovascular and renal protection. It is important to note that all cohort studies are subject to severe methodological issues such as residual confounding factors, reverse causality and inaccurate estimation of sodium intake. For estimation of individual sodium intake during a long follow-up period, for example, most studies relied on a single baseline measurement using a questionnaire, a morning urine sample or a 24-hour urine collection. It goes without saying that the average
individual sodium intake over multiple years may be classified incorrectly using these methods. Without entering the debate about the optimal sodium intake or whether low sodium intake (<2 grams/day) may be harmful, most studies seem to agree that high sodium intake (>5 grams/day) is worse than moderate sodium intake (2-3 grams/day). Considering the fact that most hypertensive patients have a high sodium intake, especially those who are not responding to antihypertensive treatment, advice on reduction of sodium intake seem the right choice.

As the BP increasing effects of sodium may be largely responsible for the detrimental effects of high sodium intake, the different BP responses to sodium intake among individuals (e.g. sodium sensitivity), may have a significant obscuring effect on the relation between sodium intake and cardiovascular risk. This is supported by the finding of Weinberger et al. that cardiovascular risk differs in sodium sensitive and sodium resistant individuals. Future research should therefore relate the cardiovascular protective effects of sodium reduction to the BP effects.

**New insights in sodium homeostasis**

Recent long-term sodium balance studies have shown that sodium homeostasis is more complicated than the classical two-compartment model. These studies, which accurately measured sodium intake and excretion during 200 consecutive days in an enclosed habitat, demonstrated that 24-hour sodium intake and 24-hour sodium excretion may differ up to 100 mmol during constant sodium intake. This view runs against the assumption of the two compartment model that sodium intake and excretion is perfectly matched by the kidney during stable sodium intake. As a result of these differences between sodium intake and sodium excretion total body sodium varied up to 400 mmol. Surprisingly, these large changes in total body sodium did not result in any change of extracellular volume, body weight or BP. This is not in line with the current physiological concept that changes in total body sodium are always accompanied by changes in extracellular volume and body weight. These data advocate the presence of a buffer where large amounts of sodium can be stored non-osmotically (e.g. without water retention). Experimental studies have identified such a buffer in the skin interstitium and muscle where negatively-charged polysaccharides, called glycosaminoglycans, are able to bind and osmotically inactivate sodium. As a result of sodium binding by glycosaminoglycans sodium retention is not accompanied by water retention.

Nonosmotic sodium storage may complicate diagnostics and therapies that are based on the two compartment model. For example, the use of 24-hour urine collection for estimation of sodium intake is based on the assumption that sodium excretion is similar to sodium intake during stable sodium intake. In addition, infusion strategies for hypo- or hypernatremic patients are based on the two compartment model. Storage and release of sodium from a third compartment therefore complicates estimation of sodium intake and treatment of hypo- and
hypernatremic patients. Skin sodium accumulation can be detected by $^{23}$Na-MRI and has been observed in patients who are prone to volume expansion such as patients with hypertension, heart failure, hyperaldosteronism and dialysis patients. This may therefore represent severe sodium excess\textsuperscript{59-62}. The effect of skin sodium accumulation on BP and cardiovascular risk remains to be determined. Altogether, these data show that sodium homeostasis cannot be fully explained by the current physiological views based on the two compartment model.

**OUTLINE OF THE THESIS**

In **CHAPTER 2** I introduce the main hypothesis of this thesis that nonosmotic sodium storage is clinically relevant and has a significant impact on volume and osmoregulation. In addition, I hypothesize that the endothelial surface layer (ESL) is able osmotically inactivate sodium and plays a crucial role in sodium and water homeostasis. The ESL is a dynamic layer of glycosaminoglycans, glycoproteins, proteoglycans and adsorbed plasma proteins covering the inner surface of all blood vessels. Considering the large systemic volume of the ESL of 1.5 liter and the sodium binding properties of glycosaminoglycans, non-osmotic sodium storage by endothelial glycosaminoglycans may help to prevent the detrimental effects of sodium excess such as volume overload and hypertension. ESL damage, on the other hand, which has been observed in diabetic and chronic kidney disease patients, may predispose patients to volume overload and hypertension. In this respect, ESL restoration may help to prevent sodium induced volume expansion and hypertension. To assess these hypotheses we have performed in-vitro experiments, animal experiments, clinical research and epidemiological studies.

In **CHAPTER 3**, we investigate the effects of dysfunctional glycosaminoglycans on sodium and water homeostasis. Heparan sulfate is the most abundant glycosaminoglycan in the ESL and is present in high concentrations in the dermo-epidermal junction of the skin. Exostosin glycosyltransferase 1 and 2 (EXT1 and EXT2) genes are responsible for polymerization of heparan sulfates and heterozygous loss of these genes has been shown to reduce heparan sulfate synthesis. We therefore hypothesize that heterozygous loss of EXT1 and EXT2 in mice (EXT1-2$^{-/-}$) would result in abnormal sodium and water homeostasis. We have analysed skin sodium, water and glycosaminoglycan content, hemodynamics, ESL thickness and endothelial function in EXT1-2$^{-/-}$ mice and wildtypes. In addition, we investigate the effect of an acute and chronic sodium load on sodium homeostasis and hemodynamics in both groups.

In **CHAPTER 4**, we describe the results of a short-term sodium balance study in healthy volunteers. We hypothesize that changes in plasma sodium concentration following hypertonic saline infusion cannot be predicted by the Adrogue-Madias and Nguyen-Kurtz formula\textsuperscript{63, 64}. These formulas are widely implemented to estimate the effects of intravenous infusion of fluids.
on plasma sodium concentration in daily clinical practice. However, these formulas are based on the two-compartment model and do not take into account the new concept of non-osmotic sodium storage. In addition, we assess whether urinary sodium and potassium excretion during a four-hour follow up is in line with the expected excretion according to the observed changes in plasma sodium and potassium concentration. We hypothesize that urinary sodium and potassium excretion could not be predicted by changes in plasma sodium and potassium concentration as significant amounts of sodium may be stored nonosmotically.

Non-osmotic sodium storage may have a significant influence on sodium excretion. 24-hour urine sodium excretion may therefore not reflect 24-hour sodium intake. In addition, sodium intake changes with diet, which is different every day, and may be relatively low or high at the day of measurement. Nevertheless, a single 24-hour urine collection is often used to estimate daily sodium intake in large cohort studies to determine the relationship between sodium intake and long-term outcome. We analyse, in CHAPTER 5, whether individual sodium intake as well as the relationship between sodium intake and long-term renal or cardiovascular outcome is affected by the number of 24-hour urine collections that were used to estimate the average individual sodium intake during long-term follow-up. As a single 24-hour urine sodium measurement may not reflect long-term sodium intake, we hypothesize that the use of a single baseline measurement versus multiple 24-hour follow-up measurements would result in different estimates of individual sodium intake. Also, we hypothesize that these different methods of estimating sodium intake may affect the observed relation between sodium intake and long-term outcome.

Patients with diabetes mellitus and chronic kidney disease are characterized by a decreased ESL volume and increased shedding of ESL components. These patients are also prone to develop sodium and water retention and hypertension. As non-osmotic sodium storage by ESL glycosaminoglycans may help to control the detrimental effects of sodium excess, the perturbed ESL of these patients may contribute to development of hypertension. In CHAPTER 6, we assess the effects of ESL restoration on BP. This chapter describes the results of a meta-analysis including 3,019 subjects in whom we investigated the effects of sulodexide on BP. Sulodexide is an orally available drug that consists of the glycosaminoglycans heparan sulfate and dermatan sulfate and is known to increases ESL thickness65. We therefore hypothesize that ESL restoration by sulodexide would lower BP.

In CHAPTER 7 we concentrate on two large, randomized, double-blinded, placebo-controlled trials including 1,900 patients that had previously investigated the effects of sulodexide on albuminuria in diabetic patients with micro- and macroalbuminuria. Previous studies have shown that a higher degree of albuminuria is related to a lower ESL volume. We hypothesize that the BP lowering effects of sulodexide are due to ESL restoration. In this post-hoc
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analysis, we therefore test whether the degree of albuminuria, reflecting ESL status, is a modifier of the BP response to sulodexide.

Besides the ability to store sodium without volume effects, the ESL has been shown to protect the vascular wall against development of atherosclerosis, inflammation and thrombosis. In CHAPTER 8 we assess whether ESL status was related to the presence of cardiovascular disease. We hypothesize that patients with a decreased ESL thickness would have an increased cardiovascular risk, as measured with the Framingham risk score. ESL thickness was measured with an experimental non-invasive technique, sidestream dark field imaging. We compare ESL thickness of healthy volunteers and individuals from the outpatient vascular medicine clinic with different cardiovascular risk profiles.

Besides development of new antihypertensive drugs, optimization of current antihypertensive strategies that affect sodium balance may help to control the cardiovascular burden of hypertension. In CHAPTER 9 we compare the cardiovascular protective effects of two subtypes of thiazide diuretics, one of the most prescribed class of antihypertensive drugs. Thiazide-like diuretics, such as chlorthalidone, have a longer elimination half-life and more efficient 24-hour BP lowering capacity than thiazide-type diuretics, such as hydrochlorothiazide, that are prescribed in 90-95% of all cases. We therefore hypothesize that thiazide-like diuretics would prevent cardiovascular events and mortality more efficiently compared to thiazide-type diuretics. So far, no randomized controlled trials have compared the effects of thiazide-type and thiazide-like diuretics on cardiovascular outcome. To test our hypothesis, we conduct a meta-analysis in which we indirectly compare the cardiovascular protective effects of thiazide-type and thiazide-like diuretics by using placebo and other antihypertensive drugs as a common comparator.

In CHAPTER 10 I discuss the potential impact of nonosmotic sodium storage on the treatment of dysnatremic patients and patients with hypertension. In addition, I discuss the role of the skin interstitium and the ESL in sodium homeostasis as well as the possible interaction between both compartments. Finally, I discuss the potential impact of optimization of thiazide diuretic therapy in daily practice and on a population level.

REFERENCES


