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INTERACTION BETWEEN LOW DIETARY POTASSIUM AND HIGH DIETARY SODIUM INTAKE ON BLOOD PRESSURE IN ADULT RATS

Lebogang Palesa Mokotedi

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree

of

Master of Science in Medicine.

Johannesburg, 2016

DECLARATION

I, Lebogang Palesa Mokotedi, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine, in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The work contained in this thesis, has not been submitted for any degree or examination in this university, or any other University.

Lebogang Palesa Mokotedi

Signed on 23rd day of MAY 2016

I certify that the studies contained in this dissertation have the approval by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics approval number is 2014/07/C

Lebogang Palesa Mokotedi

Mokotedi

Signed on 23rd day of MAY 2016

.....	.....
Aletta Millen (supervisor)	Frederic Michel (supervisor)
.....day of 2016	.....day of 2016

CONFERENCE PRESENTATIONS

Data presented in this dissertation has been presented at the 43rd Annual Conference of the Physiology Society of Southern Africa held at the Khaya Ibhubesi Conference venue in Parys, September 2015. The title of the presentation was "Interaction between high sodium and low potassium intake on blood pressure in adult rats." This presentation was awarded third prize for the Wyndham oral presentation competition.

STATEMENT OF CONTRIBUTION TO DATA COLLECTION

Although during the course of my MSc I learnt the techniques of echocardiography and vascular reactivity, these are specialized techniques which require a high degree of skill which only comes with experience. Hence in the interests of collecting accurate and hence meaningful data experts collected these data.

ABSTRACT

Although it is well known that an increase in sodium intake (Na^+) increases BP and is involved in the development of salt-sensitive hypertension (SS-HTN), the mechanism responsible for this increase in BP is uncertain. Further while low dietary potassium (K^+) is also associated with the development of SS-HTN it is uncertain to what extent dietary potassium (K^+) affects Na^+-induced increases in BP. The purpose of this study was to determine whether Na^+-induced increases in BP and target organ changes are altered by reductions in K^+ intake. Four-month-old male Sprague-Dawley (SD) rats were randomly assigned to three dietary intervention groups for six weeks: a normal Na^+ (0.3%), normal K^+ (1.6%) group (CON, n=12), a high Na^+ (6%), normal K^+ (1.6%) group (NK^+-HNa^+, n=12) and a high Na^+ (6%), low K^+ (0.01%) group (LK^+-HNa^+, n=12). Tail-cuff BP, body weight, food and water intake were measured weekly. At termination, urine parameters, right kidney weight as well as left ventricular dimensions and function were measured. Vascular reactivity of the mesenteric and renal arteries was also assessed using a wire-myograph. During the diet intervention, water intake was significantly higher in the NK^+-HNa^+ and LK^+-HNa^+ groups compared to the CON group ($P<0.0001$). Although food intake was significantly lower in the NK^+-HNa^+ and LK^+-HNa^+ groups compared to the CON group during the first week ($P=0.03$ and $P=0.05$ respectively), no significant differences in body weight were observed between the groups ($P>0.05$). The urinary Na^+/K^+ ratio was higher in the LK^+ HNa^+ compared to the CON and NK^+-HNa^+ groups ($P<0.001$). Following the 6 week dietary intervention, the systolic BP was significantly higher in the NK^+-HNa^+ and the LK^+-HNa^+ groups compared to the CON group ($P=0.05$ and $P=0.04$ respectively). The diastolic BP was significantly higher in the NK^+-HNa^+ and LK^+-

HNa^+ groups compared to the CON group (P=0.05 and P=0.02, respectively). The increase in BP was not different between the NK^+-HNa^+ and LK^+-HNa^+ groups (P>0.05). In the mesenteric arteries, there was a significant increase in vascular responsiveness to phenylephrine in the NK^+-HNa^+ group compared to the CON group (P=0.02). However the vascular responsiveness to phenylephrine in the mesenteric arteries was similar between the NK^+-HNa^+ and LK^+-HNa^+ groups (P=0.82). No significant differences in vascular reactivity were observed in the renal arteries between the three groups. No significant differences were observed in the left ventricular dimensions and function between the different diet groups (P>0.05). In conclusion, 6 weeks of high Na^+ intake increases BP, induces greater phenylephrine-induced contractions in mesenteric arteries but does not affect heart dimensions and function. The greater phenylephrine-induced contractions with a high Na^+ intake may be responsible for the increase in BP. However a reduction in dietary K^+ intake does not have any effect on the high Na^+-induced changes in BP or mesenteric artery reactivity.

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LIST OF ABBREVIATIONS

α	alpha
µM	micro molar
ACh	acetylcholine
ADH	antidiuretic hormone
ANOVA	analysis of variance
BHT	border-line hypertensive
BP	blood pressure
BW	body weight
Ca^{2+}	calcium
$CaCl_2$	calcium chloride
CVD	cardiovascular disease
CO_2	carbon dioxide
CON	control
cGMP	cyclic guanosine monophosphate
DASH	Dietary Approach to Stop Hypertension
DBP	diastolic blood pressure
DOCA	deoxycorticosterone acetate
DSR	dahl salt-resistant
DSS	dahl salt sensitive
EC_{50}	half maximum contraction response
ECF	extracellular fluid
EDHF	endothelial derived hyperpolarizing
EDRF	endothelial derived relaxing factor

Emax	maximum contraction response
FI	food intake
FS_{end}	endocardial fractional shortening
FS_{mid}	midwall fractional shortening
g/day	gram per day
GFR	glomerular filtration rate
LK^+-HNa^+	low potassium-high sodium
NK^+-HNa^+	normal potassium- high sodium
HW	heart weight
IP3	inositol triphosphate
K^+	potassium
KCl	potassium chloride
kg	kilogram
KH_2PO_4	potassium phosphate
INTERSALT	International Study of Salt and Blood Pressure
LV	left ventricle
LVW	left ventricular weight
LVEDD	left ventricular end diastolic volume
LVESD	left ventricular end systolic diameter
MAP	mean arterial pressure
$MgSO_4$	magnesium sulphate
mg	milligram
mL	millilitres
ml/min	millilitres per minute

mm Hg	millimetres of mercury
mN	milli Netwon
mmol/l	milli mole per litre
Na^+	sodium
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
Na^+/K^+	sodium-to-potassium ratio
NHANES	National Health and Nutrition Examination Survey
NO	nitric oxide
NT	normotensive
O_2	oxygen
Phe	phenylephrine
PRA	plasma renin activity
PVW	pulse wave velocity
PWED	left ventricular end diastolic posterior wall thickness
PWES	left ventricular end systolic posterior wall thickness
RAAS	renin angiotensin aldosterone system
RAS	renin angiotensin system
RKW	right kidney weight
RCTs	randomized control trials
rpm	revolutions per minute
RVW	right ventricular weight
SBP	systolic blood pressure
SEM	standard error of mean

SD	Sprague Dawley
SHR	Spontaneously hypertensive rats
SS-HTN	salt-sensitive hypertension
SNP	sodium nitroprusside
TPR	total peripheral resistance
UK^+	urinary potassium excretion
UNa^+	urinary sodium excretion
U.S	United States
VPR	Volume Pressure Recording
VSM	vascular smooth muscle
WHO	World Health Organisation
WI	water intake
WKY	Wistar Kyoto

CHAPTER 1

INTRODUCTION

Hypertension, or high blood pressure (BP), is a serious clinical and public health challenge in both developed and developing countries and South Africa is no exception (Bertram *et al.*, 2012). Hypertension is a major risk factor for cardiovascular disease including stroke (Connor *et al.*, 2009), congestive heart failure (Stewart *et al.*, 2008), and peripheral vascular disease (Nguyen *et al.*, 2013) and is recognized as one of the leading causes of morbidity and mortality worldwide (WHO, 2012). According to the WHO, hypertension affects approximately 1 billion individuals worldwide and its occurrence is estimated to increase by 60% to a total of 1.54 billion individuals by 2025 (WHO, 2013). In South Africa, approximately 21% of the population suffers from hypertension which accounts for almost 10 million individuals (Kowalski, 2007). In South Africa, hypertension-related mortality was estimated at 9% of all deaths in the year 2000 (Norman *et al.*, 2007). Moreover 50% of strokes and 42% of ischaemic heart disease were attributed to primary hypertension (Norman *et al.*, 2007), indicating the considerable burden of hypertension in South Africa (Bradshaw *et al.*, 2003; Norman *et al.*, 2007).

Furthermore the burden of hypertension in black South Africans has risen substantially in the past couple of decades (Sliwa *et al.*, 2008; Tibazarwa *et al.*, 2009) and may be explained by the transition from traditional African lifestyles to a more westernized lifestyle (Hamer *et al.*, 2011). Considering the substantial burden of hypertension in South Africa, focusing on the fundamental causes and understanding the mechanisms that contribute to the development of hypertension is important in order to improve management and treatment strategies.

Although the causes for the development of hypertension have been studied widely, the underlying mechanisms are still not well understood. Numerous

genetic and environmental factors are believed to contribute to the development of hypertension (Carretero and Oparil 2000; Oparil *et al.*, 2003).

Although a number of environmental factors are associated with hypertension, dietary factors have substantial effects on BP homeostasis (Carretero and Oparil 2000; Oparil *et al.,* 2003). In this regard a substantial body of evidence from both human (Appel *et al.*, 2006; Danaei et al., 2009; Cook *et al.*, 2007; Cook *et al.*, 2009; Umesawa *et al.*, 2008) and animal studies (Giardina *et al.*, 2001; Koga *et al.*, 2008; Siegel *et al.*, 2004; Sofala *et al.*, 2002; Wu *et al.*, 2000) suggest that high dietary sodium (Na^+) intake is an important dietary factor in the development of hypertension.

Furthermore evidence suggests that groups of African descent tend to increase BP to a greater degree in response to a Na^+ load than other ethnic groups (Gibbs *et al.*, 1999; Luft and Weinberger, 1997) and hence have a higher prevalence of "salt-sensitive" hypertension (SS-HTN) (He *et al.*, 1998; Sowers *et al.*, 1988; Tiffin *et al.*, 2010; Vollmer *et al.*, 2001; Weinberger *et al.*, 1986; Wright *et al.*, 2003). Although Na^+ has until recently been considered the most influential dietary factor in the development of hypertension, numerous studies have reported that a dietary deficit in potassium (K^+) also has a critical role in the development of SS-HTN (Adrogué and Madias, 2007; Cohen and Townsend, 2008; Hedayati *et al.*, 2012). Evidence indicates that groups of African descent have a lower dietary K^+ intake which has been associated with an elevation in BP and a higher risk for SS-HTN (Houston, 2011; Turban et al., 2008).

In this regard, studies suggest that although Na^+ and K^+ relate to cardiovascular outcomes, the ratio of Na^+ and K^+ (Na^+/K^+) is believed to be a better determinant

of SS-HTN than either Na^+ or K^+ alone (Perez and Chan, 2014; Sacks *et al.*, 2001).

Despite the evidence that not only increased Na^+, but also decreased K^+ may contribute to the pathogenesis of SS-HTN, public health initiatives to date have focused exclusively on Na^+ reduction. Moreover, most of the studies on SS-HTN in humans have been cross-sectional. Our laboratory has demonstrated in black South Africans that Na^+ intake (indexed as urinary Na^+/K^+) is more closely associated with central (aortic) than with peripheral (brachial) BP (Redelinghuys *et al.*, 2010) and that associations between urinary Na^+/K^+ and BP may in part be explained by insulin resistance (Millen *et al.*, 2013), circulating angiotensinogen (Michel *et al.*, 2012) and aldosterone-to-renin ratio (Scott *et al.*, 2011). However, due to the cross-sectional nature of these studies, direct assessments of end-organ damage and mechanisms of these relationships cannot be drawn.

Moreover as Na^+ supplementation and K^+ restriction in humans are ethically limited by the extent to which Na^+ intake may be increased and K^+ intake may be decreased, animal studies are required to appropriately assess the impact of variations in Na^+ and K^+ intake on BP.

Furthermore, the mechanisms responsible for SS-HTN in a high Na^+ and a low K^+ environment are still relatively unknown as very little evidence is available regarding SS-HTN in rats with a low K^+ diet. In order to have effective treatment strategies, a detailed and systematic investigation is needed to further elucidate possible mechanisms of SS-HTN.

In the present dissertation, chapter 2 begins with a literature review that summarises the current knowledge and incongruities in the field, which will highlight the reasons for conducting the study presented in this thesis. In particular

the literature review focuses on the effects of dietary Na^+ and K^+ intake on BP and on consequential target organ damage. Chapter 3 describes the methodology employed in the study and in chapter 4 the results obtained are presented. Chapter 5 discusses the findings in context to the scientific literature and chapter 6 concludes and highlights the limitations and clinical implications for this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Salt-sensitivity

Hypertension is characterized by a SBP exceeding 140 mm Hg and a DBP greater than 90 mm Hg (Vikrant and Tiwari, 2001) and can be classified as either primary or secondary hypertension. Primary hypertension accounts for approximately 95% of all cases of hypertension (Carretero and Oparil, 2000; Oparil *et al.*, 2003) and will thus be the focus of this literature review. A number of causative factors are associated with the development of primary hypertension including insulin resistance, obesity, kidney disease, genetics, aging and other lifestyle related factors such as lack of physical activity (Lindgärde *et al.*, 1987; Perry *et al.*, 1994; Oparil *et al.*, 2003). However the exact mechanisms underlying the development of primary hypertension remain unclear (Cutler, 1996; Carretero and Oparil, 2000). In addition to these factors, diet also plays an important role in the development of primary hypertension. Among dietary factors playing an important role in the development of primary hypertension, dietary Na^+ intake is considered the single most important modifiable contributor towards the development of primary hypertension (de Wardener and McGregor; 2002). Nevertheless the extent to which BP responds to changes in Na^+ intake varies widely among individuals (Campese *et al.*, 1994; Siani *et al.*, 2000; Weinberger, 1996). While a high Na^+ intake raises BP in some individuals, no variation with Na^+ (or even a decrease in BP) has been observed in some other individuals (de la Sierra *et al.*, 2002). These observations suggest that some individuals are salt-sensitive whilst others are salt-resistant. However, no standard criteria is currently applied uniformly to distinguish between salt-sensitive and salt-resistant individuals (Meneton *et al.*, 2005). Various criteria have been used to define salt-sensitivity. Weinberger et al (1986) defined salt-sensitivity as a 10% increase in mean arterial pressure (MAP)

when a high Na^+ diet was administered compared with a low Na^+ load (Weinberger *et al.*, 1986). Using this criterion Weinberger *et al* (1986) reported that approximately 51% hypertensive individuals and 26% normotensive individuals were considered salt-sensitive (Weinberger *et al.*, 1986). Since then a number of researchers have attempted to define salt-sensitivity (Burnier *et al.*, 2000; de la Sierra *et al.*, 2002; Gerdts *et al.*, 1999; Gill *et al.*, 1991; Larrousse *et al.*, 2006; Overlack *et al.*, 1993; Sullivan *et al.*, 1991; Weinberger, 2006).

Although there are several definitions suggested to identify salt-sensitive individuals, studies have consistently shown that hypertensive individuals are more frequently salt-sensitive than normotensive individuals (He *et al.*, 1998; Weinberger, 2006). In addition certain population groups are also believed to be more at risk to develop hypertension due to Na^+ intake (Campese, 1997; Wright *et al.*, 2003).

In this regard there is considerable evidence to support salt-sensitivity as an important pathophysiological mechanism responsible for hypertension in persons of African ancestry. This evidence is derived from a number of studies, many of which were intervention studies involving comparisons of the effect of dietary Na^+ restriction or supplementation on BP (Aviv *et al.*, 2004; Beevers, 2002; Falkner and Kushner, 1991; He *et al.*, 1998; He *et al.*, 2009; Vollmer *et al.*, 2001; Weinberger *et al.*, 1986; Wright *et al.*, 2003). However, the mechanisms involved in the higher incidence of SS-HTN in groups of African ancestry are poorly understood. One explanation may be the fundamental differences in the kidney's ability to maintain Na^+ homeostasis in groups of African ancestry compared to other ethnic groups (Bochud *et al.*, 2009; Luft *et al.*, 1982). Nevertheless in considering the pathogenesis of SS-HTN, some studies have suggested that it is

not only a high Na^+ intake that is involved in the development of SS-HTN, but also a low K^+ intake (Aviv *et al.*, 2004; Hedayati *et al.*, 2012; Weinberger *et al.*, 1986). In this regard, studies have shown that prehistoric diets were low in Na^+ and high in K^+ intake (Eaton *et al.*, 1988; Hunt and Cappuccio, 2014; Tobian, 1988). According to Darwin, the human body has been biologically designed to function efficiently with a low Na^+ and a high K^+ diet by conserving Na^+ and excreting K^+ (Tobian, 1997). In contrast to prehistoric diets, the modern westernized diet is extremely high in Na^+ and low in K^+ (Bertram *et al.*, 2012; Jain *et al.*, 2014; Penton *et al.*, 2015). Such a diet with a chronic excess of Na^+ and deficit in K^+ has reportedly been associated with SS-HTN and kidney failure (Adrogué and Madias, 2014). The section below will focus on what is known about the pathophysiology of how Na^+ and K^+ contribute to SS-HTN.

2.2 Sodium

Sodium (Na^+) is the principal cation in the extracellular fluid (ECF) and plays an essential role in the regulation of ECF volume and BP (Geerling *et al.*, 2008; Robertson *et al.*, 2003). Na^+ is found in a variety of foods such as meat and shellfish and is very abundant in most processed foods (WHO, 2006). Data from around the world have reported that modern societies consume far more Na^+ than they physiologically require (Brown *et al.*, 2009; Capuccio and Capewell, 2015; Frassetto *et al.*, 2001). Historically, Na^+ was added to food for food preservation (MacGregor and de Wardener, 1998; Multhauf, 1978), but with modern canning, refrigeration and other methods of food preservation, the need for Na^+ as a preservative has declined (Capuccio and Capewell, 2015). Nevertheless many people have become accustomed to the taste of Na^+ which is why the

consumption of Na^+ in modern societies exceed the current recommendations of less than 1.5g /day (Frassetto *et al.*, 2001; Kearney, 2010; Lloyd-Jones *et al.*, 2010; WHO, 2013). International guidelines on cardiovascular disease prevention recommend reducing Na^+ intake to 1g/day by the year 2025, a level that has not been reached by any country (Appel *et al.*, 2011, WHO, 2013). In black South Africans, the average Na^+ intake is 3.1 g/day (Charlton *et al.*, 2005), which is higher than the 2.4 g/day recommended by the South African Hypertension Guidelines (Seedat *et al.*, 2006; WHO, 2013). Given the high Na^+ intake in modern society, understanding the detrimental effects of Na^+ on BP is clearly of clinical importance.

2.2.1 High sodium intake related increases in blood pressure

It is generally accepted that a high Na^+ consumption is associated with increased BP. The hypertensive effects of a high Na^+ intake was first demonstrated in 1904 by Ambard and Beaujard. Their study showed that the patients' BP was significantly elevated with an increased dietary Na^+ intake and, inversely, lowered when Na^+ intake was reduced (Ambard and Beaujard, 1904). Since then numerous epidemiological studies, meta-analyses and intervention trials have investigated the effects of dietary Na^+ on BP (Appel *et al.*, 2006; Danaei *et al.*, 2009; Cook *et al.*, 2007; Cook *et al.*, 2009; Umesawa *et al.*, 2008). Epidemiological studies have shown that populations consuming low levels of Na^+ have a lower incidence of hypertension (Carvalho *et al.*, 1989; Denton, 1997; James and Baker, 1995). However when these populations migrate to western societies where higher Na^+ levels are consumed, a significant rise in BP occurs (Siani *et al.*, 2000; Weinberger, 1996). Similarly, the large-scale International

Study of Salt and BP (INTERSALT) reported a positive relationship between dietary Na^+ intake and BP (Elliot *et al.*, 1996). Other large population-based studies have confirmed the association between Na^+ and elevated BP (Beard *et al.*, 1997; Law *et al.*, 1991; Yamori *et al.*, 1990). A marked increase in BP due to high Na^+ intake has also been observed in cross-sectional studies (Draaijer *et al.*, 1995; Elliot *et al.*, 1996; Gerdts *et al.*, 1994; Meneton *et al.*, 2005; Sharma *et al.*, 1989; Rodrigues *et al.*, 2015). However, intervention studies examining the effect of high dietary Na^+ intake on BP have reported conflicting results, where some (Sacks *et al.*, 2001, Cappucio *et al.*, 1997) but not others (Ruppert *et al.*, 1993; Todd *et al.*, 2012) have reported changes in BP due to Na^+ intake.

In addition, animal studies including Dahl salt-sensitive (DSS) (Dahl *et al.*, 1962), spontaneously hypertensive (SHR) (Koga *et al.*, 2008), Sprague Dawley (SD) (Giardina *et al.*, 2001; Sofala et al., 2002) and Wistar (Huang and Johns, 2000; Walkowska *et al.*, 2015) rats and chimpanzees (Denton *et al.*, 1995, Elliot *et al.*, 2007) have mainly demonstrated that high Na^+ diets increase BP. Similar to humans, animals show varying degrees of salt-sensitivity or salt-resistance (Stocker *et al.*, 2010). It is well known that the DSS rats and SHRs show large increases in BP with high Na^+ intake (Siegel *et al.*, 2004; Wu *et al.*, 2000) whereas in SD rats the effect of high Na^+ intake on BP has been inconsistent (Farjah *et al.*, 2004, Giardina *et al.*, 2001, Sofola *et al.*, 2002, Titze *et al.*, 2006). In this regard some studies have reported a moderate increase in BP with high Na^+ intake suggesting that SD rats are salt-sensitive (Adegunloye and Sofola, 1996; Giardina et al., 2001; Gu *et al.*, 2008). In contrast, others have found no significant changes in BP with a high Na^+ intake (Debinski *et al.*, 1990; Farjah *et al.*, 2004; Osborn and Hornfeldt, 1998; Titze *et al.*, 2006), indicating that the salt-sensitivity of SD rats

remains controversial. Part of the controversy may be due to the different study protocols and short duration of the studies. Nevertheless both humans and animal studies convincingly show that increasing Na^+ intake above the physiological requirements can cause an increase in BP. A number of studies have investigated the possible mechanisms responsible for the increase in BP in response to a high Na^+ intake, which will be highlighted in the following section.

2.2.2 Mechanisms of sodium-induced salt-sensitive hypertension

Despite extensive animal and clinical investigations, the mechanisms by which the increase in Na^+ intake leads to the development of SS-HTN are complex and not completely understood. Although a number of mechanisms involved in the development of SS-HTN have been proposed, the most frequently suggested mechanism seems to be the inability of the kidneys to excrete large amounts of Na^+ leading to sodium retention (Kobori *et al.*, 2007; Meneton *et al.*, 2005; Navar *et al.*, 1997). Indeed the kidney is the main site for Na^+ handling hence changes in Na^+ handling by the kidney may be a causal factor in the pathogenesis of SS-HTN. Under normal physiological conditions, high Na^+ intake stimulates thirst-induced increases in fluid intake (Stolarz-Skrzypek *et al.*, 2013). This leads to an increase in blood volume which persists only for a short time until water and Na^+ excretion restores the circulating volume and osmolality (Stolarz-Skrzypek *et al.*, 2013). However a decreased capacity of the kidneys to excrete Na^+ leads to Na^+ and water retention and subsequently increased ECF volume resulting in increased BP (Khalil, 2006; Pao, 2014).

In addition to the fluid and Na^+ regulating roles, another mechanism of SS-HTN driven by the kidney is via the regulation of BP by means of a variety of hormones

including renin, angiotensin II and aldosterone, known as the renin angiotensin aldosterone system (RAAS) (Crowley *et al.*, 2005; Weinberger, 2006). The RAAS is a feedback mechanism that maintains BP and Na^+ balance (Harrison-Bernard, 2009). Renin is an enzyme synthesized and released from the juxtaglomerular cells of the kidney in response to a reduced Na^+ delivery at the macula densa of the kidney, a decrease in renal perfusion pressure and a decrease in blood volume (Harrison-Bernard, 2009). Renin cleaves angiotensinogen to form angiotensin I which is converted to angiotensin II (Harrison-Bernard, 2009; Navar *et al.*, 1996). Angiotensin II causes constriction of peripheral vessels, which in turn increase total peripheral resistance (TPR) (Harrison-Bernard, 2009; Navar *et al.*, 1996) and ultimately increases BP (Navar *et al.*, 1996; Visser *et al.*, 2008). Angiotensin II also stimulates aldosterone production from the adrenal glands. Aldosterone increases renal reabsorption of Na^+, with a subsequent increase in water reabsorption by osmosis and therefore urinary loss of Na^+ and water is reduced (Harrison-Bernard, 2009; Navar *et al.*, 1996).This results in an increased blood volume which causes an increase in BP. In addition angiotensin II causes an increased release of antidiuretic hormone (ADH) from the posterior pituitary gland which increases fluid retention in the kidneys, also resulting in a greater blood volume and hence BP (Harrison-Bernard, 2009; Navar *et al.*, 1996).

A large body of evidence has reported that salt-sensitive individuals have low plasma renin activity (PRA) , the standard laboratory measure of systemic RAS in humans (Franco and Oparil, 2006; Mulatero *et al.*, 2007; Rayner *et al.*, 2001; Sagnella, 2001; Sahay, 2012). Indeed blunted renal Na^+ excretion results in an expansion of the ECF volume which leads to a decrease in renin release from the juxtaglomerular apparatus as there is a higher perfusion pressure at the

juxtaglomerular cells and supresses the activity of the systemic renin angiotensin system (RAS) (Visser *et al.*, 2008; Stocker *et al.*, 2010; Stolarz-Skrzypek *et al.*, 2013; Weinberger, 2006). The reduced PRA in salt-sensitive individuals does not exclude local angiotensin II activity in the vascular endothelium, the kidneys, and the brain (Johnston, 1992; Kobori *et al.*, 2007; Orth and Ritz, 2001). Hence it is therefore possible to have activation of local RAS within the kidney with suppressed renin in the systemic circulation (Johnston, 1992; Kobori *et al.*, 2007; Orth and Ritz, 2001). Studies have reported that high Na^+ intake results in increased activation of local intrarenal RAS which may result in increased TPR and BP (Kobori *et al.*, 2010; Michel *et al.*, 2014, Vogt *et al.*, 2004). Similarly in rat studies a decreased PRA and an upregulation of intrarenal RAS in DSS rats, SHR and SD rats receiving a high Na^+ intake has also been reported (Campbell *et al.*, 1996; Chandramohan *et al.*, 2008; Kobori *et al.*, 2003; Kobori *et al.*, 2010; Lara *et al.*, 2012).

A third mechanism that has been suggested in the development of SS-HTN involves the vascular endothelium. The endothelium plays a major role in the maintenance of vascular tone and BP through the release of various relaxing and contracting factors (Bauer and Sotníková, 2010). The endothelium releases endothelial-derived relaxing factors such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) (Bauer and Sotníková, 2010). NO is synthesized from L-arginine by an enzyme called nitric oxide synthase (Förstermann and Münzel, 2006). NO diffuses from endothelial cells into the vascular smooth muscle (VSM) and stimulates guanylate cyclase (Bauer and Sotníková, 2010; Lucas *et al.*, 2000). The conversion of cyclic guanosine triphosphate into cyclic guanosine monophosphate (cGMP) leads to the activation

of cGMP-dependent protein kinase which decreases intracellular Ca^{2+} and ultimately leads to VSM relaxation (Bauer and Sotníková, 2010). Several vasodilators, including acetylcholine (ACh), are known to produce endothelium-dependent vasodilation through the release or synthesis of NO in the endothelium of arteries (Ali and Schumacket, 2002; Kagota *et al.*, 2002; Konishi and Su, 1983; Qiu *et al.*, 2001). Previous studies have shown that a high Na^+ intake negatively affects the endothelial function. Tzemos *et al* (2008) showed that Na^+ loading (200 mmol/day) resulted in an impaired endothelial function in sixteen healthy normotensive individuals. This study showed that Na^+ loading was associated with less endothelium-dependent vasodilation in response to ACh (Tzemos *et al.*, 2008). In addition, Miyoshi *et al* (1997) reported impaired ACh-induced endothelium-dependent vasodilation in 6 salt-sensitive individuals. These findings were also confirmed by other studies that showed impaired Ach-induced vasodilation with high Na^+ intake (Bragulat *et al.*, 2001; DuPont *et al.*, 2013). Similarly, animal studies have demonstrated that a high Na^+ intake leads to an impaired relaxation of blood vessels to vasodilator stimuli (Fujiwara *et al.*, 2000; Lenda et al., 2000; Nashida *et al.*, 1998; Sofola *et al.*, 1997). High Na^+ intake produced an impaired endothelium-dependent relaxation induced by ACh in SHR (Kagota *et al.*, 2001; Konishi and Su, 1983). Similar observations have been reported in DSS rats (Kobori *et al.*, 2003; Lüscher *et al.*, 1987). However, discrepancies exist in the effect of high dietary Na^+ intake on vasodilation in normotensive SD and Wistar rats. Some studies have shown impaired endothelium-dependent relaxation with high Na^+ intake (Banday *et al.*, 2008; Giardina *et al.*, 2001; Payne *et al.*, 2004) while others have reported no changes (Simões *et al.*, 2013). Nevertheless, despite the discrepancies, the majority of the

evidence supports the possibility that impaired endothelium-dependent vasodilation may occur in animals fed a high Na^+ diet because of an impaired production of vasodilators by the endothelium (Sylvester *et al.*, 2002; Zhu *et al.*, 2004). Moreover impaired endothelium-dependent vasodilation may be due to increased release of vasoconstrictors in response to vasoactive stimuli that normally causes vasodilation (Zhu *et al.*, 2004).

In addition to the changes in the release of and response to local vasodilators the increase in BP may also be explained in part by an enhanced reactivity to the activation of α_1-adrenergic receptors in the vessel wall (Adegunloye and Sofola, 1997; dos Santos *et al.*, 2005; Obiefuna *et al.*, 1991; Sofola *et al.*, 2002). Animal studies have reported that a high dietary Na^+ intake increases contraction induced by α_1-adrenoceptor agonist (Adegunloye and Sofola, 1997; dos Santos *et al.*, 2006; Obiefuna *et al.*, 1991; Sofola *et al.*, 2002; Tanoue *et al.*, 2002) which ultimately leads to an increased TPR. Studies have reported that a high Na^+ intake causes enhanced sensitivity to noradrenaline (Obriefuna *et al.*, 1991) or phenylephrine (Smith *et al.*, 2003) at the α-adrenergic receptor level and hence results in the increased stimulation of α-adrenergic receptors (Smith *et al.*, 2003). The stimulation of α-adrenergic receptors by agonists such as phenylephrine and noradrenaline causes activation of phospholipase C and increases production of inositol triphosphate (IP3) and diacylglycerol (Deth and van Breemen, 1974; Smith *et al.*, 2003). IP3 and diacylglycerol act as second messengers and increases VSM intracellular calcium (Ca^{2+}) concentration. Ca^{2+} is a key molecule in the contraction process where Ca^{2+} binds to calmodulin forming a complex which activates myosin light chain kinase leading to VSM contraction (Deth and van

Breemen, 1974). Hence high Na^+ intake can cause increased VSM contraction through the activation of α-adrenergic receptors.

In addition to the direct vasodilator and vasoconstrictor influence, a high dietary Na^+ intake also inhibits the Na^+/K^+-ATPase pump which results in increased intracellular Na^+ concentration in the VSM cells (Adrogue and Madias, 2014; Iwamoto and Kita, 2006). The increased intracellular Na^+ concentration in turn stimulates the Na^+/Ca^{2+} exchanger (which transports Ca^{2+} into the cells) and intracellular Ca^{2+} concentration is increased (Adrogue and Madias, 2014). With an increased intracellular Ca^{2+}, tonicity of the VSM is enhanced which causes vasoconstriction and ultimately increased TPR and BP (Adrogué and Madias, 2014; Blaystein and Hamlyn, 1991, Quednau *et al.*, 2004; Iwamoto, 2006).

In summary, Na^+ results in elevated BP via a number of mechanisms. Certainly these mechanisms explain the development of hypertension in long term high Na^+ exposure, but also acute changes in BP due to a Na^+ load, as seen in normotensive individuals. However whether high Na^+ intake increases BP in specifically normotensive rats is controversial and needs further elucidation. Furthermore, the mechanism involved in the possible development of high BP in previously normotensive animals during high Na^+ exposure is unclear and needs further investigation.

Despite the detrimental effects of Na^+ on BP, a high Na^+ intake has also been implicated in the development of target organ damage comprising the heart, kidneys and vasculature, independent of BP. These Na^+-induced target organ changes will be discussed below.

2.2.3 Sodium-induced target organ damage

2.2.3.1 The effect of high sodium intake on the heart

Left ventricular hypertrophy detected on echocardiography is a strong and independent predictor of cardiovascular morbidity and mortality (Langenfeld and Schmieder, 1995; Schmieder and Messerli, 2000). It is well known that increased BP can lead to a variety of structural and functional changes in the heart, however elevated Na^+ can also cause structural and functional changes independent of BP. In both normotensive and hypertensive subjects, diastolic filling and left ventricular mass have been found to be positively correlated with urinary Na^+ excretion, independent of BP (Daniels *et al.*, 1990; du Cailar *et al.*, 1992; du Cailar *et al.*, 2002; Tuomilehto et al., 2001; Schmieder and Messerli, 1988). In addition to human studies, studies in DSS rats (de Simone *et al.*, 1993), SHRs (Ahn *et al.*, 2004; Frohlich *et al.*, 1993; Varagic *et al.*, 2006) and Wistar rats (Fields *et al.*, 1991; Kihara *et al.*, 1985; Lal *et al.*, 2003) have shown increased left ventricular mass due to high Na^+ intake. Hence high Na^+ intake can cause BP-independent structural changes to the heart.

2.2.3.2 The effect of high sodium intake on the kidneys

Chronic kidney disease is a worldwide public health challenge and is associated with increased cardiovascular events and premature death (Couser *et al.*, 2011; Sarnak *et al.*, 2003; Stenvinkel, 2010). High Na^+ intake may affect the rate of the progression of kidney disease through both BP-dependent and BP-independent effects (de Wardener and MacGregor, 2002; Heerspink *et al.*, 2012; Marijke *et al.*, 2003). Human studies have demonstrated a positive relationship between high Na^+ intake and increased proteinuria (du Cailar *et al.*, 2002; Campese *et al.*, 1991;

Verhave *et al.*, 2004; Weir *et al.*, 2012; Yu *et al.*, 1998), which is a major risk factor for the development of kidney disease (Hsu et al., 2009; Iseki et al., 2003). In animal studies, a high Na^+ diet has also been associated with renal injury. Studies of high Na^+ intake between 7 days and 16 weeks have reported renal hypertrophy and fibrosis in DSS rats (McCormick *et al.*, 1989), SD rats (Gu *et al.*, 2008), SHRs and Wistar rats (Kihara *et al.*, 1985, Yu *et al.*, 1998). Matavelli *et al* (2007) showed in SHRs that high Na^+ intake resulted in increased proteinuria from the second week on the high Na^+ diet. Similarly other animal studies have reported increased urinary protein excretion and decreased renal plasma flow in SHRs, DSS, SD, and Wistar rats (Blizard *et al.*, 1991; Gu *et al.*, 2008; Maitland *et al.*, 2006; Varagic *et al.*, 2006; Yu *et al.*, 1998). Taken altogether these findings support the notion of a strong causal relationship between high Na^+ intake and renal injury.

2.2.3.3 The effect of high sodium intake on the blood vessels

As discussed in section 2.2.2 above, Na^+ has a detrimental effect on the vessels, which leads to the development of high blood pressure. Despite these effects a high Na^+ intake also affects vascular function in the long term which may lead to arterial stiffness and endothelial dysfunction (Safar *et al.*, 2000; Simon and Illyes, 2001; de Wardener and MacGregor, 2002; Zhu *et al.*, 2004). Pulse wave velocity, a classical measure of arterial stiffness is a strong independent predictor of cardiovascular risk (Blacher *et al.*, 1999; Glasser *et al.*, 1997; Safar *et al.*, 2002). Although high BP is associated with the development of arterial stiffness, several clinical studies have shown a BP-independent correlation between Na^+ intake and arterial stiffness (Safar *et al.*, 2000; Mercier *et al.*, 2007). In a study conducted by

Avolio and colleagues (1985), pulse wave velocity was measured in 2 groups living in either a rural or urban community. The pulse wave velocity after adjustment for BP was significantly lower in the rural community than in the urban community consuming high Na^+. Similarly in a group of 34 hypertensive subjects, high Na^+ intake for four weeks significantly increased pulse wave velocity (Todd *et al.*, 2010). In addition other cross sectional and intervention studies have shown an increase in pulse wave velocity in subjects consuming high dietary Na^+ independent of BP (Avolio *et al.*, 1986; He *et al.*, 2009; Seals *et al.*, 2001; Todd *et al.*, 2010). Taken together these findings suggest that high Na^+ intake has a BP-independent effect on arterial stiffness and hence cardiovascular disease risk.

Moreover high Na^+ has been reported to cause endothelial dysfunction. Endothelial dysfunction plays an important role in the progression of atherosclerosis and predicts cardiovascular mortality (Luscher *et al.*, 1993; Shimbo *et al.*, 2007; Suwaidi *et al.*, 2000; Yeboah *et al.*, 2007). High Na^+ intake has been shown to impair endothelial function assessed by flow-mediated dilation (FMD) (Bragulat *et al.*, 2001; Dickinson *et al.*, 2009; Tzemos *et al.*, 2008) a non-invasive assessment of endothelial function (Raitakari and Celermajer, 2000; Shimbo *et al.*, 2007; Yeboah *et al.*, 2007). Dickinson *et al* (2011) reported reduced FMD within 30 minutes of a high Na^+ meal. More recently, 7 days of high Na^+ intake reduced FMD in both normotensive men and women (Lennon-Edwards *et al.*, 2014). In addition the deleterious changes in the vasculature from high Na^+ intake have been documented in SHRs, DSS, Wistar and SD rats independent of BP (Frohlich *et al.*, 1993; Lenda *et al.*, 2000; Lenda *et al.*, 2002; Matavelli *et al.*, 2007; Yu *et al.*, 1998; Zhu *et al.*, 2007). Therefore taken together these studies suggest that high Na^+ intake has BP-independent effects on target organ damage.

However the majority of these studies were performed in hypertensive individuals and rat strains. Hence the effect of Na^+ intake on target organs in normotensive rat strains needs further investigation. Nevertheless current and past literature richly reflects the importance of decreasing Na^+ intake to reduce BP and associated cardiovascular outcomes. One potential strategy that may be effective in decreasing Na^+ levels is to promote Na^+ excretion. This leads to the importance of consuming adequate dietary K^+ levels as it is known to have natriuretic properties which may reduce BP (Brandis *et al.*, 1972; Pamnani *et al.*, 2000; Perucca and Bankir, 2007). In contrast to the abundant literature on the effects of dietary Na^+ on BP, dietary K^+ has received less attention often being viewed as a minor factor in the development of SS-HTN (Adrogué and Madias, 2014; Kanbay *et al.*, 2013). However there is mounting evidence indicating a role of dietary K^+ intake in BP regulation. The mechanisms of how dietary K^+ intake leads to a decrease in BP will be discussed below.

2.3 Potassium

Potassium (K^+) is the most abundant intracellular ion and is important in maintaining total body fluid and electrolyte balance in the body. K^+ is abundant in beans, potatoes, mushrooms, bananas, avocados, guavas and many other fruit and vegetables (Cohn *et al.*, 2002). Despite the wide variety of foods rich in K^+, various populations around the world consume less than the recommended amount of K^+ (Stamler *et al.*, 2003; van Mierlo *et al.*, 2010). The current recommended K^+ intake for adults is at least 90 mmol/day (WHO, 2012). However, studies done in SA ethnic groups showed that K^+ consumption is below 50-70 mmol/day especially in black Africans (Charlton *et al.*, 2005; Matlou *et al.*, 1986;

van Mierlo *et al.*, 2010). Nevertheless, studies suggested that moving towards the recommended K^+ intake has health benefits especially in people of African descent at risk for the development of SS-HTN (Chobanian *et al.*, 2003; Gupta and Guptha, 2010; Weaver, 2013; van Mierlo *et al.*, 2010; Siani *et al.*, 1991; Whelton *et al.*, 1997; WHO, 2012). However, what is the evidence that K^+ intake will have health benefits, specifically related to BP?

2.3.1 High potassium intake related decreases in blood pressure

Epidemiological, clinical and animal studies have reported that dietary K^+ supplementation may decrease BP (Braschi *et al.*, 2008; Gu *et al.*, 2001; He and MacGregor, 2001; Kawano *et al.*, 1998; Khaw and Barret-Connor, 1987; Kido *et al.*, 2008). Several epidemiologic studies from widely divergent geographic locations have consistently showed an inverse relation between K^+ intake and the prevalence of hypertension. A meta-analysis of 19 randomized control trials (RCTs) found that K^+ supplementation with dosages ranging between 48 and 120 mmol/day significantly reduced SBP by 8.2 mm Hg and DBP by 4.5 mm Hg in hypertensive individuals (Cappuccio and MacGregor, 1991). Another meta-analysis of 33 RCTs showed that K^+ supplementation was associated with a reduction in SBP by 1.8 mm Hg and 2.5 mmHg in normotensive and hypertensive persons respectively (Whelton *et al.*, 1997). These studies suggest that K^+ supplementation beneficially affects BP regulation not only in hypertensive individuals but also in normotensive individuals. In contrast a recent meta-analysis of 5 RCTs, however, found no significant effect of K^+ supplementation on BP (Dickinson *et al.*, 2006). The authors acknowledged the small number of participants and the heterogeneity between the trials (Dickinson *et al.*, 2006).

Although previous studies suggest that K^+ supplementation may reduce BP, the results have been inconsistent across different studies in part because of different study protocol designs.

Despite these inconsistencies among the general population, K^+ supplementation significantly lowers BP among salt-sensitive individuals especially in groups of African descent (Fujita and Ando, 1984; Grimm *et al.*, 1990; Liu *et al.*, 2013, Siani *et al.*, 1987; Svetkey *et al.*, 1987; Wilson *et al.*, 1999). Studies have consistently showed that groups of African descent have a lower dietary K^+ intake compared to groups of European descent (Aviv *et al.*, 2004; Hajjar *et al.*, 2001; Sorof *et al.*, 1997). This could explain why there is a higher frequency of salt-sensitivity in groups of African descent compared to groups of European descent (Aviv *et al.*, 2004; Hajjar *et al.*, 2001; Sowers *et al.*, 1988). In this regard, the extent of BP reduction from K^+ supplementation may be greater in groups of African descent than in groups of European descent (Aviv *et al.*, 2004, Hajjar *et al.*, 2001; Whelton *et al.*, 1997). Indeed Whelton and colleagues (1997) showed that K^+ supplementation reduced BP to a greater extent in groups of African descent as compared to groups of European decent. Similarly a greater reduction of BP due to K^+ supplementation in groups of African descent compared to groups of European descent has been observed in other studies (Aviv *et al.*, 2004; Turban *et al.*, 2008; Weaver, 2013). These findings suggest that BP in groups of African descent is particularly sensitive to K^+. Furthermore these findings are important considering that groups of African descent have a higher prevalence of hypertension and are more salt-sensitive than groups of European descent.

Animal studies have also provided convincing evidence supporting the beneficial role of K^+ supplementation on BP (Haddy *et al.*, 1991; Manger *et al.*, 2003;

Reusser *et al.*, 1994; Tobian *et al.*, 1997). In several rat models of hypertension, including the DSS rats (Zheng *et al.*, 2012; Zicha *et al.*, 2010), SHRs (Jodas *et al.*, 2014; Tobian *et al.*, 1984) and DOCA-salt rats (Wang et al., 2005) , K^+ supplementation decreased BP. However, K^+ supplementation seems to have little effect on BP in normotensive rats but have protective effects on end organ damage (Meldrum, 1990; Rigsby *et al.*, 2008). Rigsby and colleagues (2008) reported that dietary K^+ intake had no effect on BP in normotensive Wistar rats, however it improved cerebral vascular structure and reduced the amount of damage caused by cerebral ischemia in normotensive rats (Rigsby *et al.*, 2008). Below is an overview of some of the possible mechanisms of the antihypertensive effects of K^+ supplementation.

2.3.2 Mechanisms of the antihypertensive effect of potassium supplementation

A number of possible underlying mechanisms for the antihypertensive effect of K^+ supplementation have been reported, including enhanced natriuresis (Brandis *et al.*, 1972; Pamnani *et al.*, 2000; Perucca and Bankir, 2007), reduced renal renin release (Bauer *et al.*, 1979; Penton et al., 2015) and improved vasodilation (Amberg et al., 2003, Haddy *et al.*, 2006). Firstly, several studies suggest that K^+ supplementation causes natriuresis and is thought to be mediated by an inhibition of Na^+ reabsorption in the proximal tubules of the kidneys (Brandis *et al.*, 1972; Pamnani *et al.*, 2000, Perucca and Bankir, 2007). Indeed K^+ supplementation in dogs (Keith and Binger., 1935) and rats (Vander, 1970; Wright *et al.*, 1971) produced prompt natriuresis.

Secondly, given both acutely and chronically K^+ acts directly on the kidneys to inhibit renin release (Bauer *et al.*, 1979; Penton *et al.*, 2015; Vander, 1970).

Although the mechanisms are only partially understood, the suppression of renin release by K^+ supplementation may result from a depression of proximal tubule Na^+ reabsorption and an increased Na^+ delivery to the macula densa of the kidney (Sealey *et al.*, 1970; Vander, 1970).

In addition, K^+ supplementation plays an essential role in the regulation of vascular tone (Stolarz-Skrzypek *et al.*, 2013). Several studies have shown that K^+ supplementation improves vascular relaxation by increasing the generation and release of NO (Zhou *et al.*, 1999; Zhou *et al.*, 2000). Zhou and colleagues (2000) reported that K^+ supplementation improves endothelium-dependent release of NO and vasodilation in carotid arteries of DSS rats. (Zhou *et al.*, 2000). A number of studies showed similar results in the aortas of DSS rats (Raij *et al.*, 1988; Sudhir *et al.*, 1993). Furthermore, others have suggested that K^+ causes endothelial dependent vasodilation by hyperpolarizing the endothelium through the activation of the Na^+/K^+-ATPase pump (Amberg *et al.*, 2003; Edwards and Weston, 2004; Haddy *et al.*, 2006; Houston *et al.*, 2011). The Na^+/K^+-ATPase pump has important effects on the contractile state of VSM, which in turn influence blood flow and BP (Haddy, 1983). Activation of the Na^+/K^+-ATPase pump results in the hyperpolarization of the vascular smooth muscle cells, decreases cytosolic Ca^{2+}concentration, promotes vasodilatation and hence decreases TPR and BP (Amberg *et al.*, 2003; Busse *et al.*, 2002; Haddy *et al.*, 2006; Houston, 2011; McGuire *et al.*, 2001).

2.3.3 Protective effects of K^+ supplementation on cardiovascular diseases

In addition to its effect on BP, there is evidence suggesting that K^+ supplementation reduces the risk of stroke (D'Elia *et al.*, 2011; Larsson *et al.*,

2011; O'Donnell *et al.*, 2011) and prevents kidney damage through BP-independent mechanisms (Mattheson *et al.*, 2012). The cardio-protective effects of K^+ supplementation have been hypothesized as the basis for the low CVD rates in populations consuming vegetarian diets (Reddy and Kata, 2004; Ascherio *et al.*, 1991). Khaw and Barrett-Connor (1987) found that a 10-mmol/day of K^+ supplementation was associated with a 40% reduction in stroke-associated mortality independent of other known cardiovascular risk factors, including hypertension. The Framingham Study also reported a BP-independent 22% reduction in the risk of stroke associated with an increased K^+ intake (Gillman *et al.*, 1995). Furthermore, studies in hypertensive rats have shown that high K^+ intake prevents the development of renal vascular, glomerular and tubular damage (Ellis *et al.*, 1992; He and Macgregor, 2008; Tobian *et al.*, 1984; Wang *et al.*, 2005). In addition K^+ supplementation protects against excess Na^+-induced vascular cardiac diastolic dysfunction in DSS rats (Matsui *et al.*, 2006) and reverses cardiac hypertrophy in DOCA-salt induced rats (Wang *et al.*, 2005). Taken together, the above findings suggest that K^+ supplementation may provide protection not only against BP but also CVD.

In addition to its positive effects on lowering BP, K^+ supplementation can mitigate the negative effects of elevated Na^+ consumption on BP (Bulpitt, 2000; Morris *et al.*, 1999). In an intervention study conducted by Sacks and colleagues (2001), 421 pre-hypertensive and hypertensive individuals were randomly assigned to eat either a typical United States (control) diet or the Dietary Approaches to Stop Hypertension (DASH) diet with high, intermediate or low Na^+ intake. The DASH diet is rich in fruit, vegetables, plant based proteins, whole grains and low-fat dairy foods rich in K^+ (Appel *et al.*, 1997; Sacks *et al.*, 2001). Sacks et al., (2001)

reported that the DASH diet had a more pronounced effect on the high Na^+ intake compared to the low Na^+ intake groups (Sacks *et al.*, 2001). Furthermore they concluded that a reduction in Na^+ intake below the recommended 5 g/day in addition to consuming the DASH diet has greater effects on BP in combination than either intervention alone (Sacks *et al.*, 2001). Similarly numerous other studies have shown that a low Na^+ diet combined with the DASH diet has beneficial effects on BP (Bray et al., 2004; Du et al., 2014; Svetkey *et al.*, 2004; Taylor *et al.*, 2010; Vollmer *et al.*, 2001). These studies indicate that dietary K^+ intake in addition to Na^+ intake, may be important when assessing the overall effect on BP and cardiovascular disease risk. In this regard the Na^+ to K^+ urinary excretion ratio (Na^+/K^+) has become an increasingly important marker of BP and CVD risk as studies have shown that a high Na^+/K^+ correlates significantly with an increased SBP and an increased risk of CVD (Cook *et al.*, 2009; Scott *et al.*, 2011). The section below will highlight the evidence for the importance of considering the Na^+/K^+ in association with BP and CVD risk.

2.4 Sodium-to-potassium ratio

The urinary Na^+/K^+ is determined from the collection of urine over a 24 hour period. Total body Na^+ and K^+ are tightly controlled by the kidneys therefore the levels of these electrolytes in urine are considered to be adequate reflections of dietary intake of these ions (Adrogue and Madias, 2007; Cook *et al.*, 2009; Geleijnse *et al.*, 2007; Guyton 1981). A number of studies have utilized the Na^+/K^+ to quantify Na^+ and K^+ intake and its relationship with BP and CVD risk. Khaw and Barrett-Connor (1988) were some of the first researchers to show stronger correlations of the Na^+/K^+ with BP than either electrolyte alone (Khaw and Barret-Connor, 1988). Similarly, data from the INTERSALT study showed that a high

Na^+/K^+ significantly correlated with SBP and DBP (INTERSALT, 1988). Other large scale population based studies have shown similar results where a higher Na^+/K^+ was more strongly associated with increased BP than either Na^+ or K^+ alone (Cook *et al.*, 2007; Smith *et al.*, 1988).

The risk of CVD and the Na^+/K^+ has also been assessed in a meta-analysis using the National Health and Nutrition Examination Survey (NHANES) III data (Yang *et al.*, 2011). This study found that in a United States (U.S) population of all age groups, a higher Na^+/K^+ was associated with a significant increase in risk of CVD (Yang *et al.*, 2011).

A number of cross-sectional studies provide additional support for an elevated Na^+/K^+ as a superior metric to either Na^+ or K^+ alone in the evaluation of BP outcomes (Du *et al.*, 2014; Morris *et al.*, 2006; Taylor *et al.*, 2010). Table 2.1 summarizes the association of the Na^+/K^+ with BP in cross-sectional studies. Majority of the studies reported in Table 2.1 show that hypertensive individuals had higher Na^+/K^+ compared to normotensive individuals (Hedayati *et al.*, 2012; Hu and Tian, 2001; Huggins *et al.*, 2011; Polonia *et al.*, 2006; Zhang *et al.*, 2013). However, some of these studies were limited by the lack of control for confounding variables such as cardiovascular risk factors (Polonia *et al.*, 2006; Hu and Tian, 2001), lack of urine collection (Du *et al.*, 2014; Hu and Tian, 2001; Kim *et al.*, 2014; Ruixing *et al.*, 2008; Zhang *et al.*, 2013) and of 24-hour urinary Na^+ and K^+ excretion (Du *et al.*, 2014; Hu and Tian, 2001; Kim *et al.*, 2014; Ruixing *et al.*, 2008; Zhang *et al.*, 2013), over and underestimation of Na^+ and K^+ intake (Du *et al.*, 2014; Hu and Tian, 2001; Ruixing *et al.*, 2008) and heterogeneous study designs (Polonia *et al.*, 2006).

Table 2.1 Summary of cross-sectional studies on the association of the sodium-to-potassium ratio on blood pressure.

Authors	**Number of participants, % hypertensive**	**Main findings**
Du *et al.*, 2014	16869 10.4% HT	The study showed a significant interaction between the Na^+/K^+ and BP.
Hedayati *et al.*, 2012	3303 36% HT	SBP ↑ by 1.58 mm Hg per 3-unit ↑in Na^+/K^+. The Na^+/K^+ was higher in HT subjects.
Hu and Tian, 2001	762 HT 1306 NT	HT subjects had higher Na^+/K^+. The Na^+/K^+ was positively associated with ↑ BP in both HT and NT subjects.
Huggins *et al.*, 2011	587 43% HT	HT subjects had higher Na^+/K^+. The Na^+/K^+ was strongly associated with SBP in all adjusted and unadjusted models.
Kim *et al.*, 2014	6283 NT	Na^+/K^+ positively related to BP in men.
Polonia *et al.*, 2006	426	Na^+/K^+ was significantly higher in HT subjects
Ruixing *et al.*, 2008	1669 34% HT	Na^+/K^+ positively associated with BP in both male and females
Zhang *et al.*, 2013	10563 2178 HT	Mean Na^+/K^+ was higher in HT subjects. Na^+/K^+ was strongly associated with SBP (1.05 mm Hg for every 0.5 unit ↑ in the Na^+/K^+).

(HT) hypertensive; (NT) normotensive; (Na^+/K^+) sodium-to-potassium ratio; (BP) blood pressure; (SBP) systolic blood pressure

In addition due to the cross-sectional nature of these studies the cause and effect cannot be inferred. Furthermore as mentioned previously the high Na^+ and low K^+ content that characterizes diets of modern societies is believed to contribute to SS-HTN, especially in groups of African ancestry that are considered to be more salt-sensitive. Therefore it is important to consider intervention studies assessing the effect of these dietary cations on BP. In this regard several intervention studies have investigated the effects of varying dietary consumption of Na^+ and K^+ on BP. Table 2.2 summarises the intervention studies on the effects of a high Na^+ and a low K^+ diet on BP in humans where both Na^+ and K^+ intake were controlled. Some of these intervention studies showed that a low K^+ diet exacerbates Na^+-induced increases in BP in both normotensive and hypertensive adults. However most of these studies nonetheless had very small sample sizes (Coruzzi *et al.*, 2001; Krishna and Kapoor 1991; Krishna *et al.*, 1989; Lawton *et al.*, 1990), had a short duration (Coruzzi *et al.*, 2001; Gallen *et al.*, 1998; Krishna and Kapoor 1991; Krishna *et al.*, 1989; Lawton et al., 1990) or did not have prospective follow ups (Gallen *et al.*, 1998; Krishna and Kapoor 1991; Krishna *et al.*, 1989; Morris *et al.*, 1999). In addition the possible mechanisms responsible for these associations are not clear. Moreover due to practical and ethical considerations, long-term Na^+ loading or K^+ restrictions and direct assessments of end-organ damage from tissue samples cannot be carried out in human intervention studies. Hence animal intervention studies are required to appropriately assess the impact of variations in Na^+ and K^+ intake on BP.

Table 2.2 Summary of intervention studies on the effect of a high Na^+ and a low K^+ diet on BP in normotensive and hypertensive subjects.

Authors	Subjects	Study duration	Diet	Findings
Coruzzi *et al.*, 2001	11 HT	10 days	200 mmol/day Na^+ + 18 mmol K^+	High Na^+ and low K^+ intake ↑ SBP by ± 5mm Hg.
Gallen *et al.*, 1998	21 NT	9 days	180 mmol/day Na^+ + 20 mmol/day K^+	High Na^+ and low K^+ ↑ MAP
Krishna *et al.*, 1989	10 NT	9 days	200 mmol/day Na^+ + 10 mmol/day K^+	With high Na^+ and low K^+, MAP ↑ by 5mm Hg and permits further ↑ in BP after saline infusion.
Krishna and Kapoor 1991	12 HT	10 days	120 mmol/day Na^+ + 16 mmol/day K^+	With high Na^+ and low K^+ intake, SBP ↑ by 7mm Hg and DBP ↑ by 6 mm Hg. Low K^+ diet permits further ↑ in BP after saline infusion.
Lawton *et al.*, 1990	11 BHT 10 NT	6 days	400 meq Na^+ + 30 meq K^+	Ambulatory SBP ↑ in both BHT and NT subjects. MAP was ↑ in the BHT subjects however no significant differences were observed in the NT subjects.
Morris *et al.*, 1999	38 NT	10 weeks	15 mmol/day Na^+ + 30 mmol/day K^+ Last 4 weeks 250 mmol/day Na^+	Na^+ loading induced ↑ in BP when K^+ was low (30mmol/day).

(HT) hypertensive; (NT) normotensive; (BHT) border-line hypertensive; (BP) blood pressure; (SBP) systolic blood pressure;

(MAP) Mean arterial pressure

In contrast to the abundant literature on the high Na^+/K^+ ratio and BP in human studies, evidence in animal studies is limited. Table 2.3 summarizes the effect of a high Na^+ and a low K^+ diet on BP in animal studies. The studies reported in Table 2.3 show that in the hypertensive rat strains, a high Na^+ and low K^+ diet increases BP (Dietz *et al.*, 1984; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000). In SHRs and DSS rats a low K^+ diet exacerbates hypertension when administered in combination with a high Na^+ diet (Dietz *et al.*, 1984; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000). Furthermore the increased BP reported in these rat strains is also responsible for renal vascular and functional alterations (Wu *et al.*, 1996; Wu *et al.*, 2000), which could be mediated at least in part by increased sympathetic activity (Dietz *et al.*, 1984; Wu *et al.*, 2000).

However in the normotensive rat strains the effect of a high Na^+ and a low K^+ diet on BP has been inconsistent (Dahl *et al.*, 1972; Dietz *et al.*, 1984; Ray et al., 2001). While a high Na^+ and a low K^+ diet had no effect on BP in WKY rats (Dietz *et al.*, 1984), the diet had significant effects on BP in weanling SD rats (Dahl et al., 1972; Ray *et al.*, 2001). Therefore the evidence in hypertensive rat strains seems to agree, but in normotensive rat strains further elucidation is needed. In addition, although these animal studies have demonstrated that a low K^+ exacerbates high Na^+-induced increases in BP, these studies have some limitations. Hypertension is uncommon in young humans and is more common in older humans, yet most of these studies were conducted in weanling rats (Dahl *et al.*, 1972; Ray *et al.*, 2001; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000). Hence more studies are needed in adult rats to mimic the clinical situation usually encountered in adult humans. In addition most of these studies were done in DSS rats which are well-known salt-

sensitive rats (Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000) and the effects of a westernized diet in adult normotensive rats are not certain.

To my knowledge, only 3 studies have examined the effects of a high Na^+/K^+ on BP in normotensive rat strains. Indeed it is important to examine the effect of dietary Na^+ and K^+ on BP in normotensive rats as normotensive salt-sensitivity predicts future SS-HTN (Barba *et al.*, 1996; Morris *et al.*, 1999; Sullivan, 1991). Moreover, most of the studies that do examine the effects of a high Na^+ and low K^+ intake on BP in normotensive rat strains did not evaluate the possible mechanisms responsible for the changes in BP. Consequently more animal studies are needed to assess the effect of low dietary K^+ intake on BP and end-organ damage. Although some studies have reported increased BP and SS-HTN after a period of Na^+ loading (Dahl *et al.*, 1972; Ray *et al.*, 2001; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000), others have not shown this result (Dietz *et al.*, 1984). Indeed in an attempt to develop a salt-sensitive model of hypertension in rats, in previous studies in our laboratory we have assessed the impact of dietary Na^+ load on BP in the supposedly salt-sensitive SHRs. However, despite these numerous approaches, we have not been successful in producing an increase BP in response to a Na^+ load (unpublished data). A possible reason for the lack of increased BP with a Na^+ load is the high K^+ content of the commercially available rat chow which could blunt the effects of Na^+ loading on BP in rats.

Table 2.3 Summary of animal studies on the effect of a high Na^+ and a low K^+ intake on BP.

Authors	Strain	Study duration	Age	Diet	Findings
Dahl *et al.*, 1972	SD	12 months	Weanling (specific age not given)	4.5% Na^+ + 0.57% K^+ 4.5% Na^+ + 1.91% K^+ 4.5% Na^+ + 5.74% K^+	BP greater in rats receiving less K^+. salt-sensitive rats had higher Na^+ to K^+ ratio
Dietz *et al.*, 1984	SHR WKY	4 weeks	7-11 weeks	1000mmol/kg Na^+ + 100 mmol/kg K^+ 650mmol/kg Na^+ + 250 mmol/kg K^+	High Na^+-low K^+ diet ↑ BP in SHR rats but no effect in WKY rats
Ray *et al.*, 2001	SD	4 weeks	Weanling (specific age not given)	3 weeks Control (0.3% Na^+ +0.05% K^+) K^+ deficit (0.3% Na^+ +< 0.05% K^+) After 3 weeks 6% Na^+ + 0.5% K^+ for 1 week	↑ BP in K^+ deficit diet. ↑ BP on 6% Na^+ + 0.5% K^+
Wu *et al.*, 1995	DSS DSR	4 weeks	Weanling (specific age not given)	8% Na^+ + 0.2 % K^+	Diet ↑BP in both DSS and DSR rats BP higher in DSS rats than DSR rats.
Wu *et al.*, 1996	DSS DSR	4 weeks	Weanling (specific age not given)	8% Na^+ + 0.2 % K^+	Hypertension induced in DSS and DSR rats.
Wu *et al.*, 2000	DSS DSR	4 weeks	Weanling (4-week old)	8% Na^+ + 0.2% K^+	Hypertension induced in DSS and DSR rats.

(↑) increase; (BP) blood pressure; (SHR) spontaneously hypertensive rats; (WKY) Wistar Kyoto; (SD) Sprague-Dawley;

(DSS) Dahl salt-sensitive; (DSR) Dahl salt-resistant

Hence more studies are needed in order to establish an appropriate model of salt-sensitivity in rats and to evaluate possible mechanisms involved in SS-HTN. Furthermore the impact of a low dietary K^+ intake in addition to a high Na^+ load in establishing SS-HTN in normotensive rats needs to be investigated. This knowledge will improve our understanding of the effects of an urbanized lifestyle with a high Na^+ and a low K^+ intake especially in black populations who are believed to be more salt-sensitive.

2.5 Problem statement

SS-HTN is a major risk factor for morbidity and mortality in South Africans, specifically in groups of African descent. Indeed the role of increased dietary Na^+ intake has been studied extensively and suggests that high Na^+ intake does not only have adverse effects on BP but also on the vasculature. Additionally an inverse correlation between dietary K^+ intake and the prevalence of SS-HTN has been documented. Several studies in humans and various rat strains have reported that increases in K^+ intake attenuates increases in BP and has protective effects on the vasculature. However little is known about the effect of a low dietary K^+ intake on BP, specifically in normotensives. Furthermore while these two electrolytes have been independently studied in relation to BP, mounting evidence suggests that the interaction between Na^+ and K^+ may be more important than their independent effects on BP. A combination of high Na^+ intake and low K^+ intake may have additional effects on BP than either high Na^+ or low K^+ intake alone. However to date, most human research that has looked at the combined effects of these electrolytes on BP have been cross-sectional studies where conclusions regarding the cause and effect

cannot be drawn. Secondly in animal models, majority of the studies used DSS rats or SHRs which are well-known model for SS-HTN. Only a limited number of studies that are confounded by methodological issues are available in normotensive rat strains. Moreover the mechanisms by which low K^+ intake might increase BP are not well understood. Hence as normotensive salt-sensitivity is a precursor for the development of SS-HTN, more studies are needed in normotensive rat strains to assess the effects of these electrolytes on BP and the possible underlying mechanisms. Therefore the purpose of the present study is to establish an appropriate model of salt-sensitivity in rats by evaluating whether K^+ intake affects the BP response to a high Na^+ intake in male, normotensive SD rats. The aims of the present study can therefore be summarised as follows:

2.6 Aims

1. To determine the effect of high Na^+ dietary intake on BP in normotensive SD rats
2. To determine whether a decrease in K^+ intake would exacerbate the high Na^+-induced increases in BP in normotensive adult SD rats.
3. To determine whether Na^+ induced BP changes are associated with changes in cardiac structure and function and in the vasculature.

CHAPTER 3

MATERIALS AND METHODS

3.1 Animals

Thirty-six, four-month-old male SD rats weighing 520-540 g were obtained from the Central Animal Service (CAS) unit of the University of the Witwatersrand. Animals were housed individually in cages in a temperature-controlled room with a 12 hour light-dark cycle. All protocols were approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand (AESC number: 2014/07/C).

3.2 Experimental design

An outline of the experimental design is presented in Figure 3.1 below. The rats had two weeks of a familiarization period. During this time, all the rats had *ad libitum* access to drinking water as well as standard rat chow with normal concentrations of K^+ (1.6%) and Na^+ (0.3%). Food and water intake was monitored daily by weighing the respective containers using a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg South Africa). After the two week familiarization period, rats were randomly divided into three groups, each with a sample size of twelve rats. The groups were as follows: standard diet (CON), normal potassium-high sodium diet (NK^+- HNa^+) and low potassium-high sodium diet (LK^+- HNa^+).

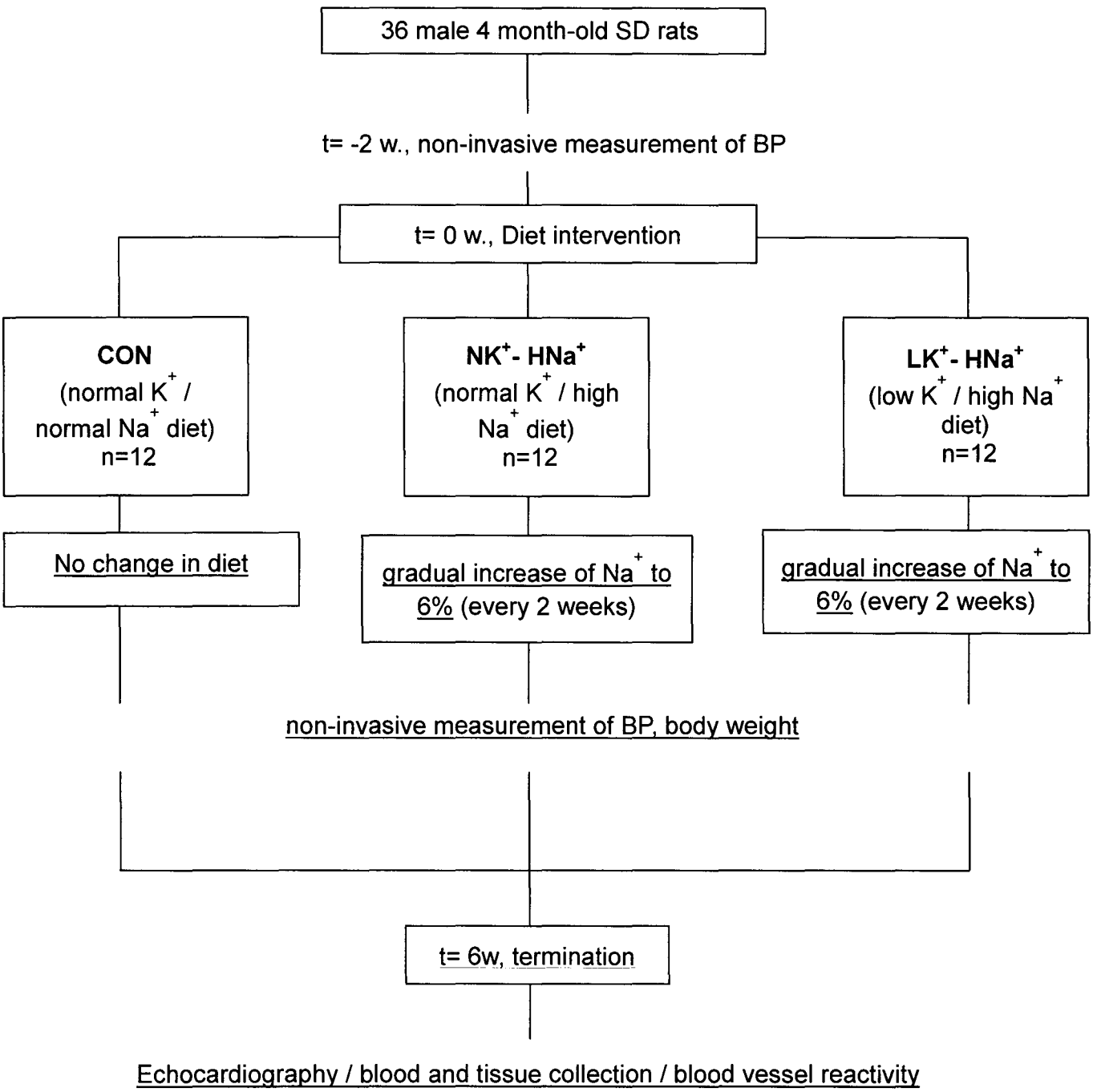


Figure 3.1 A schematic outline of the experimental design and group division with dietary changes.

3.3 Diet intervention

The rats in the CON group received a standard diet with normal K^+ (1.6%) and sodium (0.3%) concentrations. The diets of the NK^+- HNa^+ and LK^+- HNa^+ groups were incremented with 2% Na^+ every 2 weeks for 6 weeks as outlined in the table below (Table 3.1). The rats in the NK^+- HNa^+ group received a diet with normal K^+ (1.6%) and high Na^+ (2 to 6%) concentrations. The rats in the LK^+- HNa^+ group received a diet with low K^+ (0.01%) and high Na^+ (2 to 6%) concentrations. The 2%, 4% and 6% Na^+ concentrations equate to 0.34mol/kg, 0.68mol/kg and 1.03mol/kg of Na^+ respectively. Na^+ content of 6-8% in rat food is considered an adequate Na^+ load (Gu et al., 2008; Kido et al., 2008; Sofala et al., 2003). Hence the 6% Na^+ diet was used to ensure that rats receive an adequate Na^+ load to cause cardiovascular changes.

Table 3.1 An outline of the dietary intervention for the three groups.

		CON	**NK^+- HNa^+**	**LK^+- HNa^+**
Familiarization period	Week -2 to week 0	Normal K^+ (1.6%) / normal Na^+ (0.3%) diet	Normal K^+ (1.6%) / normal Na^+ (0.3%) diet	Normal K^+ (1.6%) / normal Na^+ (0.3%) diet
Diet intervention	Week 0 to week 2	No change in diet	Normal K^+ (1.6%) / 2% Na^+	Low K^+ (0.01%) / 2% Na^+
	Week 2 to week 4		Normal K^+ (1.6%) / 4% Na^+	Low K^+ (0.01%) / 4% Na^+
	Week 4 to week 6		Normal K^+ (1.6%) / 6% Na^+	Low K^+ (0.01%) / 6% Na^+

3.4 Diet composition and preparation

During the familiarization period all the rats received commercial rat chow (Epol, Centurion, South Africa) which contained 1.6% K^+ and 0.3% Na^+. During the dietary intervention the animals received a K^+ deficient diet (MP Biomedicals, Fountain Parkway, United States) which contained 0.01% K^+ and 0.3% Na^+. The K^+ deficient diet was in powder form and 5% water was added to mix. NaCl and KCl were added to the K^+ deficient diet according to the groups' requirements. The rat food was prepared into small pellets and the pellets were allowed to dry for 2 days.

3.5 Measurements and procedures

3.5.1 Non-invasive blood pressure (BP) measurements

Systolic and diastolic BP was measured once a week using a tail-cuff technique which employs Volume Pressure Recording (VPR) technology (non-invasive CODA BP system, Kent Scientific) (Feng and DiPetrillo, 2009). The apparatus calibrated automatically. The VPR sensor utilizes a specially designed differential pressure transducer to non-invasively measure the blood volume in the tail. VPR utilizes a volumetric method to measure the blood flow and blood volume in the tail. Rats were habituated for two weeks before the first measurement in order to enable them to adapt to the procedure. To obtain BP, a conscious rat was placed in a restrainer. Measurements were taken at midday to avoid diurnal variation. BP was determined by the mean of 10 readings.

3.5.2 Echocardiography

After the six week dietary intervention, two-dimensional targeted M-mode echocardiography was performed using a 7.0 MHz transducer and a ACUSON CYPRESS portable ultrasound machine (Siemens Medical Division, USA), as previously described (Woodiwiss *et al.*, 2001, Norton *et al.*, 2002). Rats were anaesthetised 15 minutes before the procedure by intramuscular injections of ketamine ($100mg.kg^{-1}$) and xylazine ($5mg.kg^{-1}$). Once the rats were under anaesthesia, final body mass was recorded. The chest of the rat was shaved and the rat was placed in a prone position in a container with an open window over which the chest area was positioned. The high resolution ultrasonic probe was placed on the chest wall of the rat to obtain echocardiographic images.

considered to be of high quality if the endocardial surface of both the anterior wall and the posterior wall were clearly visible throughout systole and diastole (Figure 3.2). Left ventricular internal dimensions and posterior wall thickness were measured at the end of systole and at the end of diastole according to the American Society for Echocardiography's leading edge method (Sahn *et al.*, 1978).

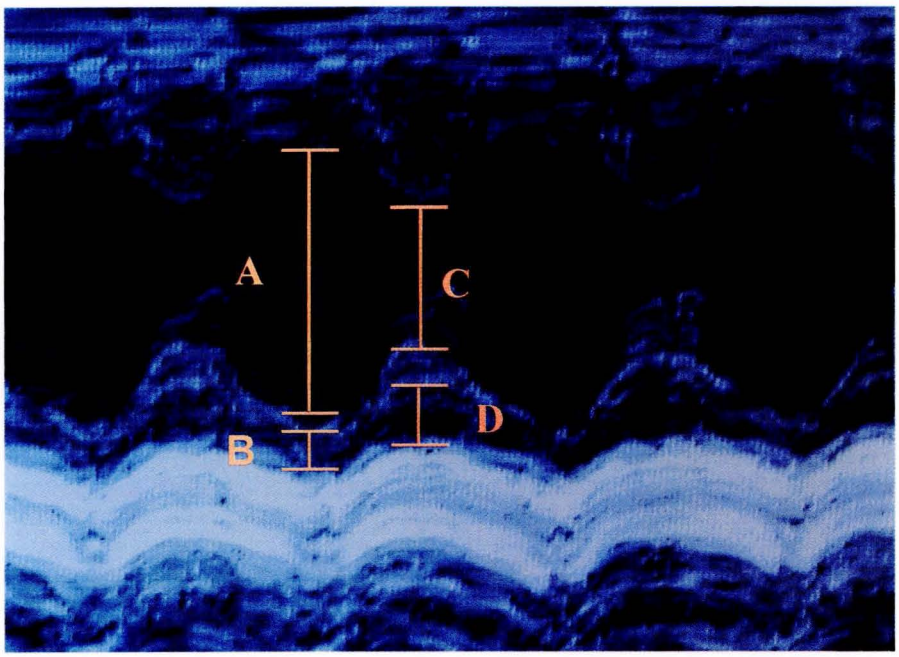


Figure 3.2 Typical M-mode echocardiographic image of the left ventricle in a male Sprague Dawley rat. A = left ventricular end diastolic internal diameter (LVEDD), B = left ventricular end systolic diameter (LVESD), C = left ventricular end diastolic internal diameter posterior wall thickness (LVEDD PWT), D = left ventricular end systolic posterior internal diameter wall thickness (LVESD PWT).

Left ventricular endocardial fractional shortening (FS_{end}) and midwall fractional shortening (FS_{mid}) were the indices used to determine chamber and myocardial

Left ventricular endocardial fractional shortening (FS_{end}) and midwall fractional shortening (FS_{mid}) were the indices used to determine chamber and myocardial function, respectively (Norton *et al.*, 2002). The equations below were used to calculate the FS_{end} and FS_{mid}.

$$FS_{end} = \frac{\text{LV EDD-LV ESD}}{\text{LV EDD}} \times 100$$

Where

LV EDD = Left ventricular end diastolic internal diameter

LV ESD = Left ventricular end systolic internal diameter

$$FS_{mid} = \frac{\text{(LV EDD+LVED PWT)-(LV ESD+LVES PWT)}}{\text{(LV EDD +LVED PWT)}} \times 100$$

Where

LVED PWT = Left ventricular end diastolic posterior wall thickness

LVES PWT = Left ventricular end systolic posterior wall thickness

3.5.3 Blood and urine sampling

Blood was obtained at termination by cardiac puncture. 4 ml of whole blood was centrifuged for 10 minutes at 1400 rpm and 2 ml of serum was collected and stored at -20°C until analysis. Blood samples were used to determine creatinine clearance.

Urine sampling was done at the end of the 6 week dietary intervention. Rats were placed individually in a metabolic cage overnight for 12 hours (fasted but with access to water). Urine was collected the following morning using a measuring cylinder, centrifuged for 10 minutes at 1400rpm and stored at -20°C until analysis. Urine

samples were used to determine creatinine clearance and 24-hour Na^+ and K^+ output. Creatinine clearance was used to estimate the glomerular filtration rate. The equation below was used to calculate creatinine clearance (El Salam *et al.*, 2014).

$$\text{Creatinine clearance} = \frac{\text{Urine creatinine (mmol)}}{\text{Serum creatinine (mmol)}} \times \frac{\text{Urine volume (ml)}}{\text{Time (hours) X 60}}$$

3.5.4 Tissue sample measurements

Immediately after echocardiography, the rats were administered more anaesthetic and the hearts and right kidneys were harvested from the animals and weighed. The left and right ventricles were separated and weighed.

Tibia from the left leg was de-fleshed, using a scalpel, and the length measured with a string and ruler. The tibial length was measured to account for variations in the growth of rats. According to Yin and colleagues (1982), the measurement of tibia length reflects a better reference of body size as it can represent relative lean body size throughout the life of the rat and it is said to be physiologically linked to the metabolic demands exerted on the heart by the body.

3.5.5 Vascular reactivity

After the echocardiography, the anaesthetised rats underwent a thoracotomy and the heart was removed from the thoracic cavity. Thoracic aorta, kidneys and mesentery were also removed and placed in a cold modified Krebs-Ringer bicarbonate (control) solution (mmol/l: 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.1glucose). Arteries were then isolated. Rings of renal and mesenteric arteries (2 mm long) were cleaned of fat and connective tissue and

suspended in a wire myograph (model 610M, Danish Myo Technology, Aahrus, Denmark). The Wire Myograph system is designed to measure the reactivity of small isolated blood vessels of 100 µm to 2 mm in diameter. Arteries were mounted as a ring and threaded over two parallel stainless steel wires. The wires were secured to two supports or "jaws" with one support attached to a precision micrometer (allowing manual control of vessel circumference and stretch). The other support was attached to a force transducer for measurements of force/tension development. Mounted arterial segments were then immersed in organ chambers containing Krebs buffer (37°C) aerated with 95% O_2 and 5% CO_2. The rings were stretched progressively to their optimal resting tension and were allowed to equilibrate for 60 minutes. Active force developments of ≥ 2 mN in mesenteric arteries and ≥ 2.5 mN in renal arteries were considered optimum for experiments to proceed. KCl (80mM) was used to induce contraction and to check the viability of the preparations.

Vasoactive drugs were then added into the chamber and the concentration-response (contractions or relaxations) curves of the preparation for the different vasoactive drugs were determined under isometric conditions (Christon *et al.*, 2005). Contraction responses to Phenylephrine (Sigma, St. Louis MO) (Phe, 1 nM - 0.1mM) and Potassium chloride (KCl, 1 mM to 120mM) were recorded. Blood vessels were dilated with Acetylcholine (Sigma, St. Louis MO) (ACh, 0.1 nM to 100 µM) and Sodium nitroprusside (Sigma, St. Louis MO) (SNP, 1nM to 100µM) under phenylephrine-induced contraction (Phe concentration=0.3-1µM). ACh is an endothelium-dependant vasodilator and SNP is an endothelium independent vasodilator.

3.6 Data analysis

Data analysis was performed using SAS software v. 9.3 (SAS institute Inc, Carry, NC). Data was expressed as means ± SEM. A one-way analysis of variance (ANOVA) with a Tukey post *hoc test* was used to determine differences in vascular reactivity, urinary Na^+/K^+ excretion, kidney and cardiac weight weights, left ventricular diameters and systolic and diastolic function between the groups. A repeated measure two-way ANOVA followed by a Tukey post *hoc test* was used to determine differences in body weights, systolic and diastolic blood pressures, food and water intake over the six week dietary intervention. Differences were considered statistically significant at $P \leq 0.05$.

CHAPTER 4

RESULTS

4.1 The effect of a high sodium and low potassium diet on water intake, food intake and body weight

The effects of variations in dietary sodium and potassium on weekly water intake, food intake and body weights during the 6 week dietary intervention are shown in Figure 4.1. Following the 6 week dietary intervention, weekly water intake (Figure 4.1 A) was significantly increased in the NK^+- HNa^+ and LK^+- HNa^+ groups compared to the CON group ($P<0.0001$). The water intake was significantly increased in the NK^+- HNa^+ and LK^+- HNa^+ groups from week 1 to 6 compared to week 0 ($P<0.0001$), but there was no change in water intake for the CON group over time. No significant differences in weekly water intake were observed between the NK^+- HNa^+ and LK^+- HNa^+ groups ($P>0.05$). Food intake (Figure 4.1B) was significantly decreased in the NK^+- HNa^+ and LK^+- HNa^+ groups compared to the CON group during the first week of the dietary intervention ($P=0.03$ and $P=0.05$ respectively). For the rest of the study duration there were no differences between the groups or over time compared to week 0 for any of the groups (all $P>0.05$). Following the 6 week dietary intervention the average Na^+ intake (mmol/day) in the NK^+-HNa^+ and LK^+-HNa^+ was 33.85 ± 1.20 and 34.12 ± 1.59 respectively. Despite differences in water and food intake, there were no significant differences in body weight (Figure 4.1 C) between the study groups ($P >0.05$).No significant differeneces in tibia lengths (mm) were observed between the study groups (CON= 47.8; NK^+-HNa^+= 48.4; LK^+-HNa^+ = 48.1; $P>0.05$).

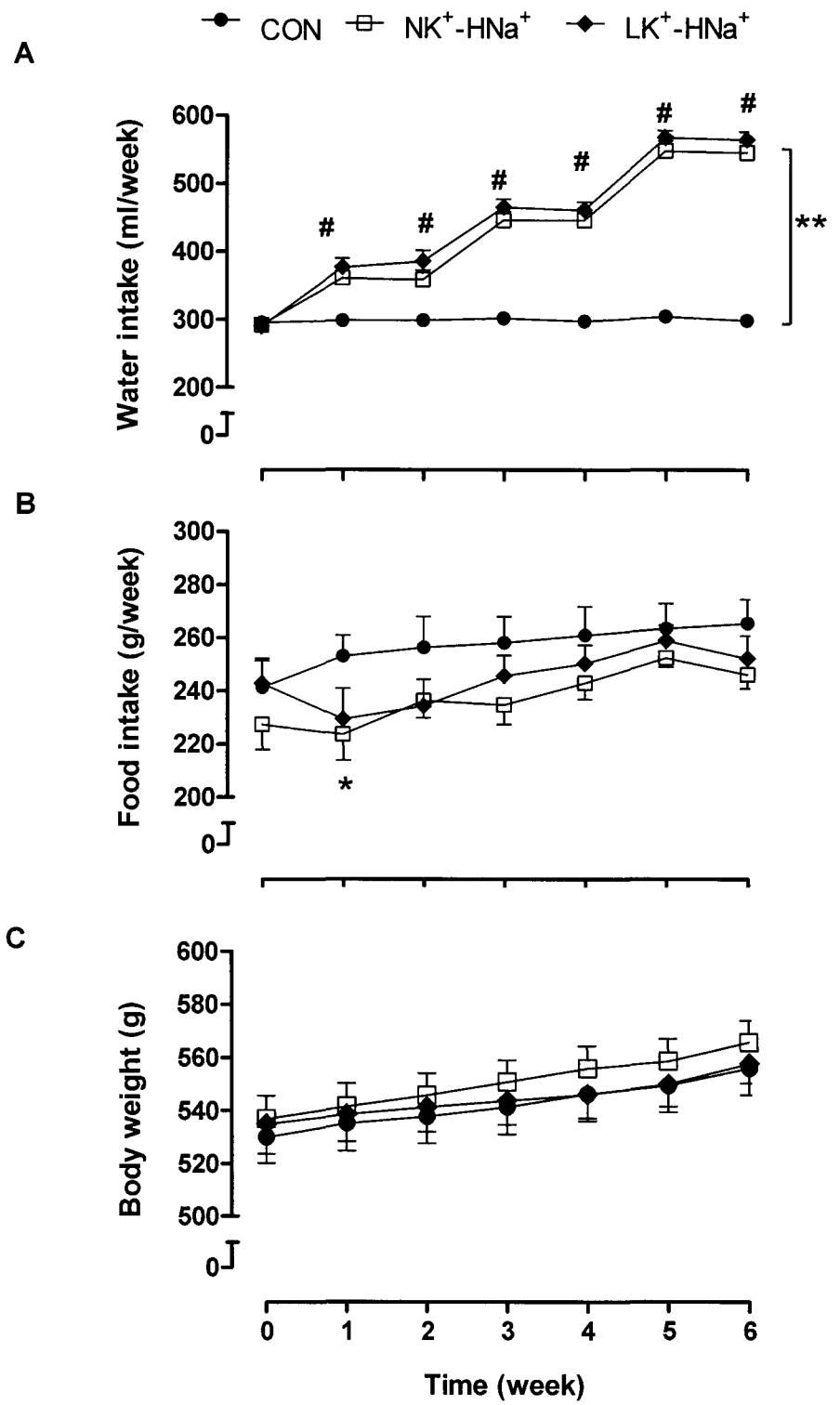


Figure 4.1 Changes in weekly water intake (A), food intake (B) and body weights (C) in male Sprague-Dawley rats. Data expressed as means ± SEM, n=12 per group. **P< 0.001 NK^+-HNa^+ and LK^+-HNa^+ groups versus CON group. # P < 0.01 changes over time in the NK^+-HNa^+ and the LK^+-HNa^+ group versus week 0. *P < 0.05 NK^+-HNa^+ and LK^+-HNa^+ groups versus CON group. CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

4.2 The effect of a high sodium and low potassium diet on urinary parameters

The effects of the dietary sodium and potassium intervention on the urine volume, urinary sodium excretion, urinary potassium excretion and urinary sodium to potassium ratio (Na^+/K^+) at termination are shown in Table 4.1. After 6 weeks of the dietary intervention, no significant differences were observed in urine volume between the different groups ($P > 0.05$). The U_{Na}^+ was significantly greater in the NK^+-HNa^+ and LK^+-HNa^+ groups compared to the CON groups (both $P < 0.0001$). The U_K^+ was significantly lower in the LK^+-HNa^+ group compared to the CON and NK^+-HNa^+ groups (both $P<0.0001$). The urinary Na^+/K^+ ratio was significantly higher in the LK^+-HNa^+ compared to CON and NK^+-HNa^+ groups (both $P < 0.0001$).

Table 4.1 Effect of dietary sodium and potassium on urinary parameters in male Sprague Dawley rats.

	CON	NK^+- HNa^+	LK^+- HNa^+
Urine volume(mL)	6.2 ± 0.8	6.3 ± 0.7	7.7 ± 0.7
Urinary Na^+ excretion (mmol/12h)	392 ± 9	1335 ± 17*	1747 ± 18$^{\#}$
Urinary K^+ excretion (mmol/12h)	623 ± 18	465 ± 10	112 ± 1$^{\#\dagger}$
Na^+/K^+	1.4 ± 0.7	3.8 ± 0.8	16.1± 1.6$^{\#\dagger}$

Data expressed as means ± SEM, n=12 per group. *P < 0.01 NK^+-HNa^+ group versus CON group, $^{\#}$ P < 0.01 LK^+-HNa^+ group versus CON group, † P < 0.01 LK^+-HNa^+ group versus NK^+-HNa^+ group. Na^+/K^+, urinary sodium to potassium ratio; CON, normal potassium-normal sodium diet; NK^+-HNa^+ , normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

4.3 The effect of a high sodium and low potassium diet on blood pressure

Figure 4.2 shows the weekly measures of systolic (SBP) and diastolic (DBP) blood pressures during the dietary intervention, from baseline (week 0) until termination (week 6). The SBP and DBP for the CON group did not change during the 6 week dietary intervention compared to baseline ($P>0.05$). The LK^+-HNa^+ group displayed a significant increase in SBP on weeks 3 (135 mm Hg), 5 (136 mm Hg) and 6 (134 mm Hg) compared with week 0 (120 mm Hg) ($P=0.01$, $P= 0.01$and $P=0.03$ respectively). The increase in SBP was not different between NK^+-HNa^+ and LK^+-HNa^+ ($P>0.05$). Following the 6 weeks of dietary intervention, the SBP was significantly higher in the NK^+-HNa^+ and the LK^+-HNa^+ groups compared to the CON group ($P=0.05$ and $P=0.04$ respectively) (Figure 4.2A).

The LK^+-HNa^+ group displayed a significant increase in DBP on weeks 3 (97 mm Hg), 5 (96 mm Hg) and 6 (94 mm Hg) compared with week 0 (81 mm Hg) ($P=0.001$, $P= 0.003$ and $P= 0.009$ respectively). The increase in DBP was not different between NK^+-HNa^+ and LK^+-HNa^+ ($P>0.05$). Following 6 weeks of dietary intervention, the DBP was significantly higher in the NK^+-HNa^+ and LK^+-HNa^+ groups compared to the CON group ($P=0.05$ and $P=0.02$, respectively) (Figure 4.2B).

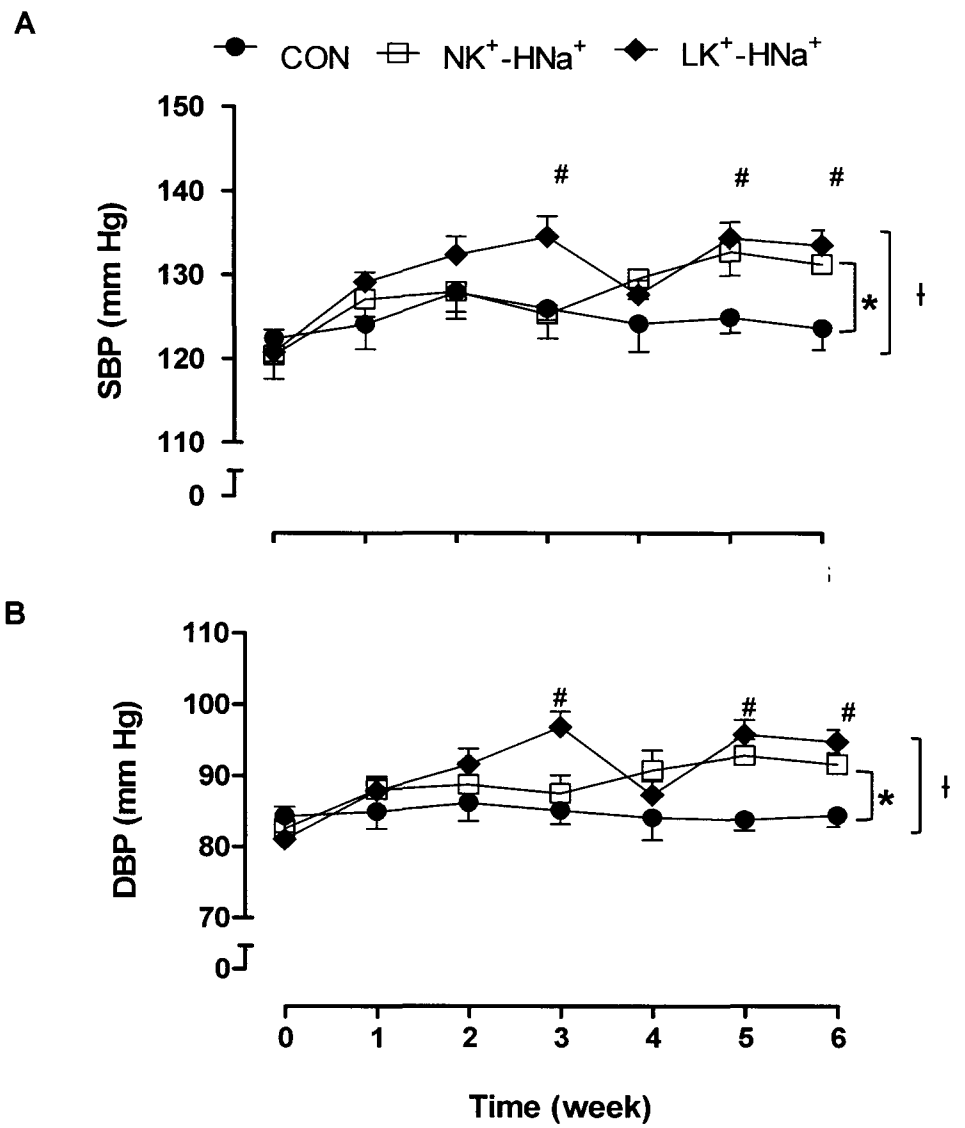


Figure 4.2 Impact of dietary sodium and potassium on weekly systolic (A) and diastolic (B) blood pressures in male Sprague Dawley rats. Data expressed as means ± SEM, n=12 per group. *P < 0.05 NK^+-HNa^+ group versus CON group. †P < 0.05 LK^+-HNa^+ group versus CON group. # P < 0.01 changes over time in the LK^+-HNa^+ group versus week 0. SBP, systolic blood pressure; DBP, diastolic blood pressure; CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

4.4 The effect of a high sodium and low potassium diet on renal function

The effects of the dietary sodium and potassium intake on creatinine clearance and right kidney (RKW) weight at termination are shown in Table 4.1. After the 6 week dietary intervention, no significant differences in the creatinine clearance were onserved between the different groups ($P > 0.05$). The RKW was significantly heavier in the LK^+-HNa^+ group compared to the CON ($P= 0.006$) and the NK^+-HNa^+ ($P= 0.008$) groups. No significant differences were noted in the RKW in the NK^+-HNa^+ group compared to the CON group ($P> 0.05$).

Table 4.2 Effect of dietary sodium and potassium on renal function in male Sprague Dawley rats.

	CON	NK^+- HNa^+	LK^+- HNa^+
Creatinine clearance (ml/min)	0.46 ± 0.17	0.33± 0.08	0.29± 0.03
RKW (g)	1.7 ± 0.1	1.7 ± 0.04	$1.9 \pm 0.1^{\#\dagger}$

Data expressed as means ± SEM, n=12 per group. $^{\#}P < 0.01$ LK^+-HNa^+ group versus CON group, $^{\dagger}P < 0.01$ LK^+-HNa^+ group versus NK^+-HNa^+ group. RKW, right kidney weight; CON, normal potassium-normal sodium diet; NK^+-HNa^+ , normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

4.5 The effect of a high sodium and low potassium diet on heart weight, dimension and function

The heart weight, heart dimensions and left ventricular function of the three groups at termination is shown in Table 4.3 Following 6 weeks of dietary intervention, there were no significant differences in any of the variables between the three groups ($P > 0.05$).

Table 4.3 Effect of dietary sodium and potassium on heart weight, dimensions and function in male Sprague-Dawley rats.

	CON	NK^+-HNa^+	LK^+-HNa^+
HW (g)	1.33 ± 0.03	1.50 ± 0.08	1.47 ± 0.04
RVW (g)	0.27 ± 0.02	0.30 ± 0.01	0.31 ± 0.02
LVW (g)	0.96 ± 0.02	1.10 ± 0.07	1.06 ± 0.03
HW/BW X 100	0.24 ± 0.01	0.27 ± 0.01	0.26 ± 0.01
RVW/BW x 100	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
LVW/BW x 100	0.17 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
LVEDD (mm)	7.23 ± 0.17	7.26 ± 0.33	7.24 ± 0.17
LVESD (mm)	3.57 ± 0.15	3.83 ± 0.32	3.43 ± 0.19
PWED (mm)	1.94 ± 0.05	2.13 ± 0.05	1.90 ± 0.09
PWES (mm)	2.69 ± 0.06	2.88 ± 0.07	2.76 ± 0.08
FS_{end} (%)	50.75 ± 1.37	47.93 ± 2.13	52.91 ± 1.87
FS_{mid} (%)	31.72 ± 0.93	28.61 ± 1.01	32.30 ± 1.63

HW, heart weight; RVW, right ventricular weight; LVW, left ventricular weight; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameters; PWED, posterior wall thickness in diastole; PWES, posterior wall thickness in systole; FS_{end}, endocardial fractional shortening, and FS_{mid}, midwall fractional shortening; CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

4.6 The effect of a high sodium and low potassium diet on vascular responses

4.6.1 Dose-responses to phenylephrine and potassium chloride

Concentration-response curves to phenylephrine (Phe) (Figure 4.3A and B) and potassium chloride (KCl) (Figure 4.3C and D) were constructed in mesenteric (Figure 4.3, left panel) and renal arteries (Figure 4.3, right panel), respectively. The Phe-induced contractions were significantly shifted to the left in NK^+-HNa^+ group compared to CON group (P=0.02) in mesenteric arteries. No significant differences were observed in the Phe-induced contractions between NK^+-HNa^+ and LK^+-HNa^+ groups (P>0.05) in both mesenteric and renal arteries.

The EC_{50} and E_{max} values derived from these curves are presented in table 4.4 The EC_{50} of Phe in mesenteric arteries in the NK^+-HNa^+ group was significantly lower compared to the CON group (P=0.02). The EC_{50} in the LK^+-HNa^+ group in mesenteric arteries showed a trend toward significance compared to the EC_{50} in the Con group (P=0.06). No significant differences in the concentration-response curves, EC_{50} and E_{max} of KCl were noted between the groups in both mesenteric and renal arteries (all, P >0.05).

4.6.2 Relaxation responses to acetylcholine and sodium nitroprusside

Concentration-response curves to sodium nitroprusside (SNP) (Figure 4.4 A and B) and acetylcholine (ACh) (Figure 4.4 C and D) were constructed in mesenteric (Figure 4.4, left panel) and renal arteries (Figure 4.4, right panel), respectively. The EC_{50} and E_{max} values derived from these curves are presented in table 4.5. No significant

differences in the concentration-response curves, EC_{50} and E_{max} of SNP and ACh were noted between the groups in both mesenteric and renal arteries ($P>0.05$).

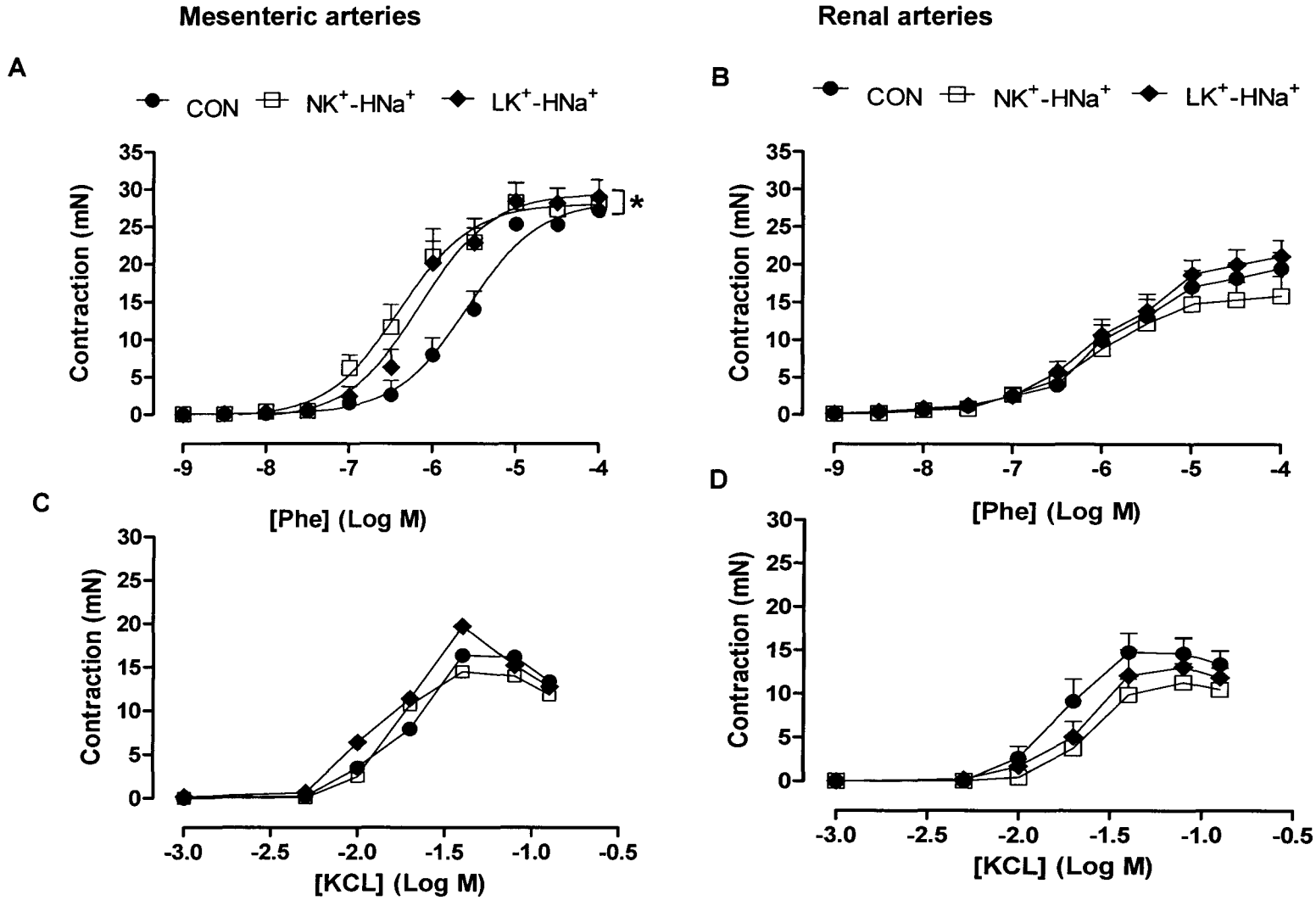


Figure 4.3 Effect of dietary sodium and potassium on contractile responses to phenylephrine (A and B) and potassium chloride KCl (C and D) in mesenteric (left panel) and renal arteries (right panel). Data expressed as means ± SEM, n=9 per group. *P< 0.05 NK^+-HNa^+ versus CON group. Phe, Phenylephrine; KCl, Potassium chloride; CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

Table 4.4 Contraction responses to phenylephrine and potassium chloride in mesenteric and renal arteries after the dietary intervention in male Sprague-Dawley rats.

		Mesenteric arteries			Renal arteries		
		CON	**NK^+-HNa^+**	**LK^+-HNa^+**	**CON**	**NK^+-HNa^+**	**LK^+-HNa^+**
Phe	EC_{50}(μM)	3.2±0.79	0.9 ±0.59*	1.1± 0.26	1.88±0.63	0.94±0.34	1.88±0.10
	E_{max}(mN)	28.99±1.53	29.61±3.22	29.82± 2.28	19.38±2.11	16.12±2.81	21.36±2.39
KCl	EC_{50} (mM)	23±1.00	20±1.00	20±1.00	20±1.00	40±1.00	30±1.00
	E_{max}(mN)	20.98±3.90	17.09±1.98	18.87 ± 1.59	18.75±1.99	16.28±3.77	17.20±4.35

Data expressed as means ± SEM, n=9 per group. *P< 0.05 NK^+-HNa+ versus CON group. Phe, Phenylephrine; KCl, Potassium chloride; EC_{50}, concentration that induces half the maximum contraction; E_{max}, maximum contraction response; E_{C50} expressed in μmole for Phe and mmole for KCl and E_{max} expressed in mN. CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

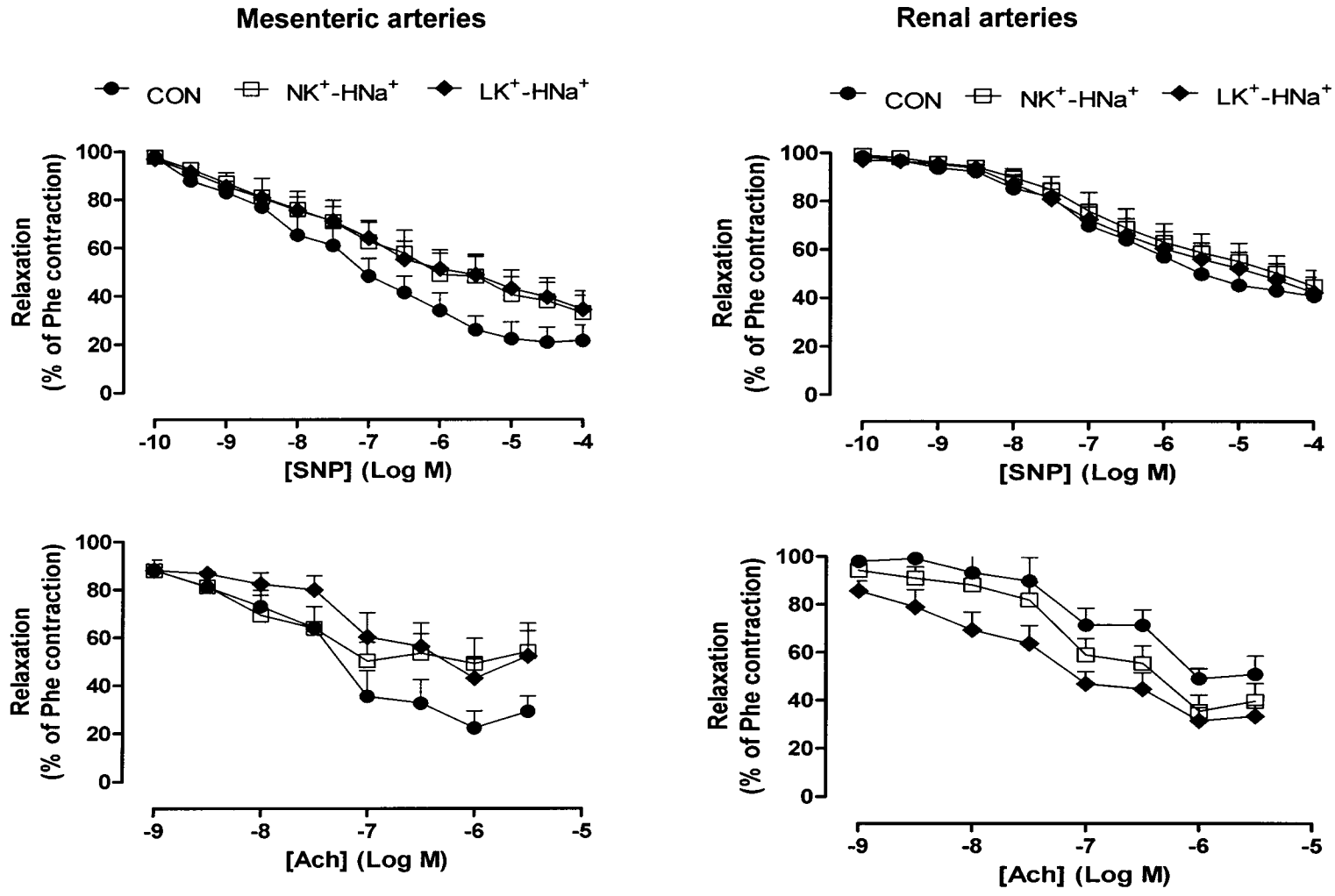


Figure 4.4 Effect of dietary sodium and potassium on relaxation responses to sodium nitroprusside (A and B) and acetylcholine (C and D) in mesenteric (left panel) and renal arteries (right panel). Data expressed as means ± SEM, n=9 per group. SNP, Sodium nitroprusside; ACh, Acetylcholine; Con, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

Table 4.5 Relaxation responses to sodium and acetylcholine in mesenteric and renal arteries after the dietary intervention in male Sprague-Dawley rats.

		Mesenteric arteries			Renal arteries		
		CON	**NK^+-HNa^+**	**LK^+-HNa^+**	**CON**	**NK^+-HNa^+**	**LK^+-HNa^+**
SNP	EC_{50} (μM)	0.08±0.03	0.53±0.39	0.63±0.55	0.68±0.38	1.3±0.068	0.25±0.007
	E_{max}(mN)	25.40±5.85	39.67±6.65	42.46±7.22	43±4.35	49.77±6.22	49.12±6.45
ACh	EC_{50} (μM)	0.15±0.071	0.07±0.022	0.090±0.033	0.03±0.01	0.02±0.00	0.02±0.01
	E_{max}(mN)	19.01±5.57	45.57±11.27	46.36±11.51	40.48±8.52	30.91±7.34	29.05±5.93

Data expressed as means ± SEM, n=9 per group. SNP, Sodium nitroprusside; A.Ch, Acetylcholine; E_{C50}, concentration that induces half the maximum contraction; E_{max}, maximum contraction response; EC_{50} expressed in μmole and E_{max} expressed as % of Phe-induced contraction. CON, normal potassium-normal sodium diet; NK^+-HNa^+, normal potassium-high sodium diet; LK^+-HNa^+, low potassium-high sodium diet.

CHAPTER 5

DISCUSSION

5.1 Introduction

The present study examined the effects of a high Na^+ and a low K^+ intake on BP in four-month-old SD rats. The main findings of the current study are that following a 6 week dietary period, high Na^+ intake induces an increase in BP. In addition high Na^+ intake induces greater phenylephrine-induced vascular contractility which may be responsible for the increase in BP. However a low K^+ diet does not exercerbate the effects of the high Na^+ intake. It may be important to note that a high Na^+ intake concurrently with a low K^+ intake causes an increase in kidney weight.

5.2 Changes in water intake, food intake, body weight and tibia length during the dietary intervention period

In the present study, an increase in water intake was observed once the diet was supplemented with Na^+. As Na^+ concentrations were increased every two weeks, rats were drinking more water than when fed a normal Na^+ diet. This suggests that consumption of high Na^+ intake provided a rapid stimulus of thirst. Indeed, it is well documented in various rat strains that the daily water intake is elevated proportionally as dietary Na^+ intake is increased (Gamble *et al.*, 1929; Sticker *et al.*, 2003). In both human and animal studies, when the osmolality is increased as a result of increased Na^+ intake, thirst is stimulated and water intake increases (Gilman, 1937; McKinley and Johnson, 2004; Ramsay and Thrasher, 1990; de Wardener *et al.*, 2004).

In the present study, once more when Na^+ was introduced in the diet, rats ate less in the first week compared to the control group. The lower food consumption in the high Na^+ intake groups may be explained by a lower palatability or the novelty of the diet (Sticker *et al.*, 2003). Following the first week of the diet intervention, rats increased

their food intake and food intake was similar in the three groups at the end of the 6 week dietary intervention. Despite the differences in food intake between the groups, body weights and tibia lengths were similar throughout the diet intervention for the 3 different experimental groups. This suggests that either the lower food intake with the increase in Na^+ in the first week was not sufficient to cause a decrease in the rat body weights or the loss in body weight due to a lower food intake may have been compensated for by the increased water intake. Therefore the results of the present study imply that a high Na^+ intake increased water intake without affecting body growth.

5.3 Changes in urinary parameters after the dietary intervention period

Animal studies have shown that a high Na^+ intake increases urinary water concentration due to increased water intake (Denton *et al.*, 1985; He *et al.*, 2001). Indeed a high Na^+ intake stimulates thirst, increases fluid intake, in order to reduce plasma osmolality and hence increases urinary volume (Shore *et al.*, 1988). Although the literature provides evidence that high Na^+ increases urinary water excretion, the present study did not show any differences in the urinary volume between the three groups. Similar to the present study, previous studies have showed unaltered urine volume in DSS rats receiving a high Na^+ intake (Kurokawa *et al.*, 2015; Simchon *et al.*, 1989; Spinelli *et al.*, 1987). One possible reason for the unaltered urine volume could be due to an increase in ADH secretion. Since extracellular Na^+ concentration is a determinant of osmolality, variations in Na^+ intake would be expected to influence ADH secretion. The high Na^+ intake could have stimulated ADH secretion which caused water reabsorption in the distal tubule and collecting duct and consequentially

reduced water loss in urine (Cowley *et al.*, 1983; Kjeldsen *et al.*, 1985; Spinelli *et al.*, 1987). Another possible reason for the unaltered urine volume despite increased water intake is that the rats were placed in metabolic cages and fasted overnight in order to collect urine. It is possible that the rats did not drink as much water during these 12 hours as usual since they were deprived of food. This may have altered urine excretion volume during the collection period. Alternatively the metabolic cages expose rats to stress which could have interfered with the total urine volume collected as has been seen in previous studies (Gil *et al.*, 1999; Stechman *et al.*, 2010; Vadjei *et al.*, 1990).

Urinary Na^+ excretion can provide a good estimate of Na^+ intake since 95% of ingested Na^+ is excreted in urine (Schachter *et al.*, 1980). Indeed in the present study urinary Na^+ excretion increased in the high Na^+ intake groups, reflecting the increased Na^+ in the diet. In addition, urinary K^+ excretion may also provide a good estimate of K^+ intake (Tasevska *et al.*, 2006). In the present study, a low K^+ diet in conjunction with a high Na^+ diet for 6 weeks resulted in a marked decrease in urinary K^+ excretion and increased the Na^+/K^+ compared to the other groups, confirming the greater Na^+ intake in conjunction with the lower K^+ intake. The present study is in agreement with previous studies showing an increased Na^+/K^+ due to a low K^+ intake (Du *et al.*, 2014; Coruzzi *et al.*, 2001; Krishna *et al.*, 1989; Morris *et al.*, 1999; Zhang *et al.*, 2013).

5.4 Changes in blood pressure during the dietary intervention period

In the present study, the consumption of a high Na^+ diet for 6 weeks resulted in a significant increase in SBP and DBP compared to the rats receiving the control diet. An increase in SBP and DBP was observed on the third week when rats were

receiving a 4% Na^+ diet and on the fifth and sixth week when rats were receiving a 6% Na^+ diet.

Indeed many clinical studies suggest that a high Na^+ can increase BP in both hypertensive and normotensive individuals (Draaijer *et al.*, 1995; Elliot *et al.*, 1996; Gerdts *et al.*, 1994; Hedayati *et al.*, 2012; Hu and Tian, 2001; Huggins *et al.*, 2011; Meneton *et al.*, 2005; Polonia *et al.*, 2006; Rodrigues *et al.*, 2015; Sharma *et al.*, 1989; Weinberger, 1996; Zhang *et al.*, 2013). In addition, high Na^+ intake is known to result in increases in BP in various rat strains especially in genetically selected DSS rats (Adegunloye and Sofola, 1996; Dahl *et al.*, 1962; Giardina *et al.*, 2001; Gu *et al.*, 2008; Huang and Johns, 2000; Sofala *et al.*, 2002; Walkowska *et al.*, 2015; Wu *et al.*, 1995; Wu *et al.*, 2000). However the effect of high Na^+ intake on BP has been studied to a lesser extent in normotensive rats such as SD rats. In the present study, no significant increase in SBP and DBP was observed on the first and second week when rats were receiving a 2% Na^+ diet. These results are consistent with previous findings in male SD rats showing no changes in BP after two weeks, despite being on an 8% Na^+ diet (Debinski *et al.*, 1990; Farjah *et al.*, 2004; Jernigan *et al.*, 2007; Gu *et al.*, 2008; Mattson and Higgins, 1996; Titze *et al.*, 2006). Therefore the results of the present study may imply that a short-term high Na^+ intake is insufficient to cause changes in BP and that a longer duration intervention may be needed.

In this regard we have demonstrated that on a 4% Na^+ diet from week 3 to 4 and a 6% Na^+ diet from week 5 to 6, SBP and DBP significantly increased compared to the control group. These findings suggest that 4% and 6% Na^+ intake can induce increases in BP in normotensive rats. Although there was an increase in BP in the high Na^+ groups SBP increases did not exceed 140 mm Hg, and hence the rats did

not develop hypertension. Indeed Gu *et al* (2008) reported that an 8% Na^+ intake for 8 weeks causes hypertension in SD rats. Hence a greater Na^+ intake with a longer diet intervention may have caused hypertension in the normotensive rats.

Moreover, as highlighted in chapter 2 the relationship between a high Na^+ and low K^+ intake with BP has been found to be stronger than Na^+ alone. In the present study, consumption of a low K^+ diet did not exacerbate the high Na^+-induced increases in BP. These results are in contrast to previous human and animal studies. Krishna and colleagues (1989) investigated the effects of dietary K^+ on BP using either low (10 mmol/day) or normal (90 mmol/day) amounts of K^+ in conjunction with a high Na^+ intake (120-200 mmol/day) in normotensive men. They reported no change in BP during normal K^+ intake, but increased BP (4.1 mm Hg) with low K^+ intake (Krishna *et al.*, 1989). The same participants were given a saline infusion which further increased BP in those on the low K^+ diet but had no effect on those consuming the normal K^+ diet (Krishna *et al.*, 1989). Similarly, Coruzzi and colleagues (2001) showed that there was a reduction in urinary K^+ excretion which was associated with increased SBP in patients receiving high Na^+ intake (200 mmol/day) and a low K^+ (18 mmol/day) diet. Morris *et al* (1999) reported that when dietary K^+ intake was reduced, dietary Na^+ loading induced a significant increase in BP.

Moreover the evidence of the effect a high Na^+ and low K^+ intake on BP has been reported in animal studies. In weanling hypertensive rat strains, studies have reported that a low K^+ intake exacerbates Na^+-induced increase in BP (Dietz *et al.*, 1984; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000). Interestingly, the high Na^+ and low K^+ diet induced SS-HTN only after 4 weeks on a high Na^+ (8%) diet (Dietz *et al.*, 1984; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000). Similar to the effects observed in

the weanling hypertensive rat strains (Dietz *et al.*, 1984; Wu *et al.*, 1995; Wu *et al.*, 1996; Wu *et al.*, 2000), a low K^+ diet with a high Na^+ intake induced SS-HTN in weanling normotensive rat strains only after 4 weeks on the dietary intervention (Ray *et al.*, 2001). Ray et al (2001) reported that a low K^+ diet with a high Na^+ intake resulted in a more marked increase in BP compared to rats only receiving a high Na^+ diet. Weanling SD rats receiving a normal Na^+ diet (0.3%) combined with a low K^+ (< 0.05%) diet for 3 weeks had higher SBPs (114± 7.4 mm Hg) than the rats a normal Na^+ combined with a normal K^+ diet (91 ± 5.3 mm Hg) (Ray *et al.*, 2001). In order to determine whether the increase in BP was associated with salt-sensitivity, rats were switched to a high Na^+ (6% Na^+) diet for 1 week (Ray *et al.*, 2001). The high Na^+ diet resulted in marked increases in SBP (155 ± 14 mm Hg) in the rats receiving a low K^+ diet compared to the receiving a normal K^+ diet (123 ± 8.9 mm Hg) (Ray *et al.*, 2001). The study concluded that dietary K^+ deficiency increases BP and induces salt-sensitivity in weanling normotensive rats (Ray *et al.*, 2001).However, in the present study, when a high Na^+ diet was combined with a low K^+ diet no significant changes in BP were observed compared to the rats receiving a normal K^+ diet in conjunction with a high Na^+ intake. It is possible that a low dietary K^+ intake could have a greater effect in younger than older rats as it has been reported that young rats are more sensitive to changes in dietary K^+ and Na^+ than adult rats (Ray *et al.*, 2001; Wu *et al.*, 1995).

Moreover Dahl et al (1972) showed that when weanling rats receive a high Na^+ and low K^+ diet long-term (12 months) significant BP changes occurred. In the prior study Dahl *et al.*, 1972 showed that the lower the K^+ intake in conjunction with a constant high Na^+ intake, the higher the BP after the 12 months dietary intervention (Dahl *et*

al., 1972). Therefore the 6 week dietary intervention period in the present study may have been too short for a low K^+ diet to worsen the high Na^+-induced increases in BP.

5.5 Changes in renal function after the dietary intervention period

In the present study neither high dietary Na^+ alone or in combination with low dietary K^+ had any effect on normal renal function as indicated by creatinine clearance. This is in contrast with other studies that have shown profound effects of a high Na^+ and a low K^+ diet on renal function. These changes include a reduction in creatinine clearance accompanied by a decline in the number of functioning nephrons (Muntwyler and Griffin, 1953; Ray *et al.*, 2001; Suga *et al.*, 2002;Yachantha *et al.*, 2009; Wu *et al.*, 2000). Suga et al (2002) reported reduced creatinine clearance in 3 month old SD rats receiving a low K^+ (0.01%) diet for 10 weeks. Hence the discrepancies may be due to the shorter duration of the present study.

In the present study, the consumption of a high Na^+ diet did not alter the weights of the right kidneys. However the right kidney weight was significantly heavier in the rats receiving a high Na^+ and a low K^+ diet. The present finding is consistent with previous studies where others have shown that a high Na^+ intake concurrently with a low K^+ intake causes enlargement of the kidneys and striking changes in their tubular structure leading to kidney failure (Brokaw, 1953; Elger *et al.*, 1992; Ray *et al.*, 2001; Suga *et al.*, 2001; Wu *et al.*, 2000). However, the precise mechanism by which low K^+ intake increases kidney weight was not elucidated. One possibility is that there was an increase in extracellular volume in the kidneys and hence increasing the kidney weight. However, more studies are needed to determine pathogenic mechanisms of the increased kidney weight due to high Na^+ and low K^+ intake.

5.6 Changes in cardiac function after the dietary intervention period

In order to determine the effect of increased BP in response to high Na^+ intake, heart dimensions and function were measured. In the present study the consumption of a high Na^+ diet resulted in no significant changes in the hearts dimensions and function compared to the control diet. These results are in contrast with previous studies. Previous studies have reported that a high Na^+ (8%) diet for 8 weeks results in left ventricular hypertrophy in normotensive weanling Wistar rats (Yu *et al.*, 1998; Lal *et al.*, 2003). Moreover, Cordaillat *et al* (2010) investigated the effects of a high Na^+ (8%) diet on cardiac structure and function in weanling SD rats for 5 months. They reported that the high Na^+ intake for 5 months resulted in left ventricular hypertrophy without changes of systolic and diastolic function (Cordaillat *et al.*, 2010). Therefore the results of the present study may imply that a 6 week high Na^+ dietary intervention may have been too short to cause structural changes to the heart.

Moreover human studies have suggested that a high Na^+ to K^+ ratio is more strongly associated with left ventricular hypertrophy than Na^+ intake alone (Cook *et al.*, 2009; Daniels *et al.*, 1990; du Cailar *et al.*, 1992; du Cailar *et al.*, 2002; Rodriguez *et al.*, 2011; Tuomilehto *et al.*, 2001; Schmieder and Messerli, 1988). Yet in the present study, no high Na^+ or low K^+-induced increases of the left ventricular wall were demonstrated after the 6 week dietary intervention period. It is possible that the duration of the present study was not extensive enough to cause changes in the hearts structure and function. Despite increases in BP after the increased Na^+ intake, the rats were not hypertensive; hence it is unlikely that the rats would have developed hypertension induced left ventricular hypertrophy or changes in cardiac function.

Nevertheless there was also no direct effect of the high Na^+ on the cardiac structure or function. Hence more studies with a longer duration dietary intervention where the rats do develop hypertension are needed to determine whether high Na^+ and low K^+ intake directly or indirectly may cause cardiac changes.

5.7 Vascular reactivity

5.7.1 Vasoconstrictor responses in mesenteric and renal arteries after the dietary intervention period

In the present study, the consumption of a Na^+ diet increased the vascular responsiveness to phenylephrine in mesenteric arteries. This is in agreement with previous studies that have reported enhanced phenylephrine contractility in isolated aortic rings (Adegunloye and Sofola, 1997; Obiefuna *et al.*, 1991) and perfused mesenteric beds (dos Santos *et al.*, 2005; Sofola *et al.*, 2002). The increased vascular reactivity to phenylephrine may be explained in part by an increase in the sensitivity to phenylephrine at the α-receptor level (Smith *et al.*, 2003).

In addition, no changes in phenylephrine-induced contractions were observed in the renal arteries suggesting that enhanced phenylephrine-induced contractions by high Na^+ intake is not generalized to all vascular beds. Since mesenteric arteries are resistance arteries, they contribute substantially more to changes in TPR than renal arteries. Hence the short-term effect of high Na^+ intake may be primarily on resistance arteries. Indeed, Yu and colleagues demonstrated no changes in renal vascular function after 4 weeks of high Na^+ intake (Yu *et al.*, 1998). However increased renal media wall to lumen ratio and fibrosis were reported in this study which could have led to the increase in BP. In this regard structural changes in the

blood vessel walls could represent an adaptive phenomenon in which the blood vessel wall thickens in response to an increase in BP (Simon *et al.*, 2001; Tanoue *et al.*, 2002). Therefore further studies are needed to assess the effect of high Na^+ intake on vascular structural changes in both renal and mesenteric arteries.

In the present study, a low K^+ did not affect phenylephrine-induced contractions as the contractions were similar between the rats receiving a normal and a low K^+ diet. To my knowledge, this is the first study to report on the effects of a low K^+ on vascular responsiveness. Previous studies have only assessed the effects of high K^+ intake on vascular responsiveness (Amberg *et al.*, 2003; Dietz *et al.*, 1984; Edwards and Weston, 2004; Haddy *et al.*, 2006; Houston *et al.*, 2011; Raij *et al.*, 1988; Sudhir *et al.*, 1993). Dietz et al (1984) reported that the sensitivity of VSM to noradrenaline is enhanced in weanling hypertensive SHRs on a high Na^+ diet. However these changes in noradrenaline are reversed when the concentration of K^+ in a high Na^+ diet is increased. Therefore these results show that K^+ supplementation reduces VSM vasoconstriction in the hypertensive rat strain (Dietz *et al.*, 1984). However in normotensive WKY rats no K^+ supplementation effect was observed (Dietz *et al.*, 1984). Therefore maybe a low K^+ diet could be more sensitive in hypertensive rat strains as compared to normotensive rat strains.

Furthermore the present study determined whether high Na^+ intake and a low K^+ was associated with changes in the vascular reactivity to KCl. KCl causes membrane depolarization and stimulates Ca^{2+} entry through voltage gated Ca^{2+} channels (Khalil and van Breemen, 1995). In the present study, no differences in reactivity to KCl were observed between any of the groups, either in mesenteric or renal arteries. Conversely, Smith *et al* (2003) reported that high Na^+ (8%) causes increased KCl

contractions in aortic rings of 3 month old SD rats after 9 days on the diet. However in the present study high Na^+ did not cause increased membrane depolarization. This could be due to the lower Na^+ concentration (6%) in the diet. Therefore, in the present study, it is more likely that high Na^+ intake leads to increased BP by increasing contraction-induced by α_1-adrenoceptor agonists rather than increasing membrane depolarization in mesenteric arteries.

5.7.2 Vasodilator responses in mesenteric and renal arteries after the dietary intervention period

In the present study, the ACh-induced vasodilation in both renal and mesenteric arteries was similar in the different experimental groups. Impaired endothelium-dependent relaxation has been reported in mesenteric arteries (Sofola *et al.*, 2002) and the aorta (Adegunloye and Sofola, 1997) of rat models of SS-HTN in response to ACh. Studies have reported that a high Na^+ intake in normotensive rats causes impaired endothelium-dependent arteriolar responses to ACh and most probably because of the suppression of local NO activity (DuPont *et al.*, 2013; Kagota *et al.*, 2001; Lenda *et al.*, 2000). Indeed, high Na^+ diet for 6 weeks caused a 40% reduction of renal vasodilator effects of ACh (Fiore *et al.*, 2011). Moreover studies have suggested that high Na^+ and high K^+ levels have complementary effects to reduce availability of NO leading to VSM contraction (Raij *et al.*, 1988; Zhou *et al.*, 2000). However, the effect of a low K^+ diet in conjunction with a high Na^+ is unknown. The present study indicate that the endothelial capacity to release endothelial derived relaxing factor (EDRF) is not modified in mesenteric and renal arteries after 6 weeks

of a high Na^+ intake. Moreover the combination of high Na^+ and low K^+ intake did not impair endothelium-dependent vasodilation in both mesenteric and renal arteries.

Previous studies have only assessed the effect of K^+ supplementation in conjunction with a high Na^+ diet (Raij *et al.*, 1988; Zhou *et al.*, 2000). Zhou *et al* (2000) and Raij *et al* (1988) reported that K^+ supplementation improves endothelium-depend NO-mediated relaxation in response to ACh in DSS rats. It is noteworthy that these were weanling hypertensive DSS rat, therefore maybe the effects of K^+ is more sensitive in weanling hypertensive rat strains rather than normotensive rat strains.

Furthermore the present study determined whether high Na^+ intake was associated with changes in the vascular reactivity to SNP. SNP spontaneously generates NO via an endothelium-independent manner (Kagota *et al.*, 2001). In this study, the responsiveness of mesenteric and renal arteries to SNP was not different between the groups. This study is in agreement with previous reports showing the inability of high Na^+ intake in changing vascular responsiveness to SNP (Boegehold, 1993; Frisbee and Lombard, 1998; Lenda *et al.*, 2000). These observations suggests that the ability of VSM to respond to the NO donor and hence the guanylate cyclase enzyme which mediates the action of SNP is not altered by high Na^+ intake. Moreover a low K^+ diet did not alter the responsiveness of mesenteric and renal arteries to SNP. Therefore additional experiments are necessary to identify the endothelial factors that might be altered after high Na^+ and low K^+ intake.

CHAPTER 6

CONCLUSIONS

6.1 Conclusions

The present study investigated the effects of a 6-week high dietary Na^+ and low dietary K^+ intake on BP in four-month-old male SD rats. The present study shows that 6 weeks of high Na^+ intake increases BP and increases vascular responsiveness to phenylephrine in mesenteric arteries. These findings suggest that the greater phenylephrine-induced mesenteric vascular contractility could be responsible for the increase in BP. Nevertheless the rats did not develop hypertension as animals remained normotensive after the 6-weeks dietary intervention. In addition, a 6 week high Na^+ intake does not affect heart dimensions and function. This suggests that a 6 week high Na^+ intake does not cause cardiac changes associated with the increase in BP, possibly because the rats did not become hypertensive. The data presented is novel in showing that a low K^+ intake has little effect on Na^+-induced increases in BP however it can cause an increase in kidney weight. Although the present study has not shown the mechanisms involved in the increase in kidney weight, it serves as a platform for future studies.

6.2 Limitations and future studies

The possible limitations to this study are as follows; first, the present study focused on the short-term effect of a high Na^+ and low K^+ intake on BP. Although the intervention did result in significant BP changes in the normotensive rats, the rats did not develop hypertension and hence the duration may have been too short. Future studies should investigate the long-term effect of the diet as it may result in more adverse outcomes.

Second, due to the high cost of the K^+ dificient diet (R50 000 for 20Kg) only three dietary groups could be investigated. Hence future studies should also investigated the effects of a low K^+ with normal Na^+ diet on BP. Third, SBP and DBP were measured using the non-invasive tail cuff technique. The main limitation of this method is that measurements can only be performed in restrained rats which could have induced stress to the animals and hence affected BP. However the stress was minimized by habituating the rats to the restrainers and the BP machine for two weeks before data collection commenced. Future studies should use the telemetry technique which is more reliable and is used to determine the accuracy of non-invasive blood pressure measurements by implanting radio transmitters at the carotid artery of the rats at the beginning of the experiment. Thourth, no histology was performed to see whether the low K^+ resulted in any histological changes in the kidney due to the increase in kidney size. Hence future studies need to assess the histological changes to the kidney. Fourth, mesenteric and renal arteries were mounted on two steel wires for isometric force measurements (wire-myograph) which is not the most physiological technique as part of the endothelial surface is under pressure exerted by the wires which could possibly damage the vessels. Future studies should use the pressure perfused vessel technique which develops myogenic tone and is more physiological and sensitive than the wire-myograph. Moreover, only the functional changes in mesenteric and renal arteries were assessed. Future studies should look at the structural changes in these blood vessel walls as structural changes could cause arterial wall thickness in response to an increased BP. Finally, as urinary Na^+ and K^+ concentrations were measured, future studies should measure plasma Na^+ and K^+ concentrations to compare the findings.

6.3 Possible clinical implications and recommendations

By confirming that high Na^+ intake increases BP and causes pathological changes in the vasculature, it remains of paramount importance to reinforce a low Na^+ diet as a strategy for the prevention and treatment of SS-HTN. In addition the importance of maintaining adequate K^+ intake needs further investigations. Although low K^+ intake only had little effect on Na^+ induced increases in BP, low K^+ could cause detrimental effects on the kidney. Hence further studies are needed to assess the effects of dietary K^+ on target organ changes. In addition a better understanding of the mechanisms involved in the regulation of BP by Na^+ and K^+ is needed. This will provide important insights into understanding salt sensitivity and hence guide dietary strategies to prevent SS-HTN.

APPENDIX A

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/07/C

APPLICANT: A Millen

SCHOOL: Physiology

LOCATION: Faculty of Health Sciences

PROJECT TITLE: *Interaction between low dietary potassium and high dietary sodium intake on blood pressure in adult rats. A pilot study*

Number and Species

60 Rats

Approval was given for to the use of animals for the project described above at an AESC meeting held on 25 February 2014. This approval remains valid until 24 February 2016.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None.

Signed: _____ (Chairperson, AESC) Date: 24th April 2014

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 22/04/2014

cc: Supervisor: N/A
Director: CAS

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