Dietary sodium intake and its relationship to adiposity in young black and white adults: The African-PREDICT study

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** Any opinion, finding, and conclusions or recommendations expressed in this material are those of the authors and therefore, the NRF does not accept any liability in this regard.*

PREFACE

This study, "*Dietary sodium intake and its relationship to adiposity in young black and white adults: The African-PREDICT study*" forms part of the dissertation for the degree Master of Health Science in Cardiovascular Physiology at the North-West University of Ms SH Crouch. The dissertation is compiled in the article format as described and recommended by the North-West University. Following this format, the chapter outline is as follows:

Chapter 1: Background, Motivation and Literature Overview

Chapter 2: Methodology

Chapter 3: Research Manuscript for Publication

Chapter 4: Final Remarks and Recommendations for Future Studies

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AUTHOR CONTRIBUTIONS

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Responsible for conducting literature search; writing of the initial research proposal and ethics application. Performing cardiovascular measurements within the African-PREDICT study, and analysing urine samples in the laboratory. Writing the literature study; performing statistical analysis; as well as the design, planning and writing of the manuscript and dissertation.

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The following is a statement of the co-authors confirming their individual roles in the study and giving their permission that the manuscript may form part of this dissertation.

Prof. AE Schutte	**Dr. LF Gafane-Matemane**	**Dr. LJ Ware**
Prof. HS Kruger	**Dr. T Van Zyl**	**Dr. B Swanepoel**

SUMMARY

Motivation

It is well-known that both a high-salt diet and obesity are risk factors for the development of hypertension and cardiovascular disease. Several studies have suggested a link between dietary sodium and obesity. However, recent literature suggests this relationship may be independent of energy intake. In addition, a series of novel studies suggest that sodium may be stored in the skin, however, the effect this may have on the relationship between dietary sodium and obesity remains unknown. The African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT) study provides us with the ideal population to investigate the sodium intake-adiposity link, as participants are young and apparently healthy with detailed nutritional, anthropometric and cardiovascular measures.

Aim

The aim of this study was to determine whether there was a relationship between sodium intake and obesity-related measures in a young healthy, black and white South African population, when adjusting for potential confounders.

Methods

This study used cross-sectional data from the first 761 participants with complete data sets at baseline. Data with regard to age, sex, ethnicity and dietary intake were collected using various questionnaires. Socio-economic status was calculated using a point system adapted from the Kuppuswamy's Socioeconomic Status Scale 2010. Participants provided a 24hr urine sample for analysis. Anthropometric measurements and bioelectrical impedance were collected as well as physical activity (accelerometery) and 24hr ambulatory blood pressure. Additionally, venous blood samples were taken from the brachial vein branches and analysed for a wide range of biochemical markers.

Results

Based on 24hr urine sodium analysis, the total group consumed on average 7.65 g of salt per day with 79.9% consuming above the daily recommended salt intake of 5 g per day. In the total population, 46% were classified as overweight or obese (26% overweight, body mass index (BMI) 25-29.9 kg/m^2; 20% obese, BMI >30 kg/m^2). In Pearson correlations, all anthropometric measures correlated with 24hr sodium, with body surface area (BSA) showing the strongest correlations. Multivariate-adjusted regression analysis of associations between either BSA or BMI and 24hr urinary sodium showed both BSA and BMI associated positively with 24hr urinary sodium in the total group, black and white men (unadjusted model; all $p \le 0.032$) but not in women. In Model 1, adjusted for total energy expenditure (TEE) only, BSA associated positively with 24hr urinary sodium in both the total group and white women (all $p \le 0.037$). Following adjustment for energy intake only (Model 2), BSA remained associated with 24hr urinary sodium in the total group and additionally in black and white men (all $p \le 0.012$), BMI also associated positively with 24hr urinary sodium in the total group and white men (both $p \le 0.026$). Model 3, adjusted for both TEE and energy intake, showed an association between BSA and 24hr urinary sodium in the total group only ($p=0.005$). In Model 4, we replicated the analysis of a previous study that found a positive relationship between BMI and sodium intake when adjusting for age, socio-economic score, TEE, dietary energy intake, self-reported tobacco and alcohol use. We also found a significant positive association but only between BSA and 24hr urinary sodium in the total group and white women (both $p \le 0.043$). Lastly, we ran a fully adjusted model including systolic blood pressure and a range of additional covariates (age, socio-economic status, cotinine, gamma glutamyl transferase, aldosterone, C-reactive protein, low and high-density lipoprotein cholesterol and glucose; Model 5). In this model, BSA, but not BMI, remained significantly associated with 24hr urinary sodium in the total group ($p=0.039$). Within the total group, when using the fully adjusted model, we additionally tested for associations between all other measures of obesity and 24hr urinary sodium using the same models and no consistent independent associations were found.

Conclusion

In conclusion, we found a continuous and robust positive relationship between BSA and 24hr urinary sodium in the total population independent of energy intake, expenditure and numerous potential confounds. As BSA is also used as an estimate of skin surface area, the relevance of this finding in terms of obesity remains unclear, especially as salt intake was not independently associated with any of the more traditional obesity markers.

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Table 1. Multiple regression analysis with varying body surface area formulae as dependent variables and 24hr urinary sodium as independent variable.

LIST OF ABBREVIATIONS

AEE-	Activity Energy Expenditure
African-PREDICT-	African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension
ABPM-	24hr Ambulatory Blood Pressure
BMI-	Body Mass Index
BSA-	Body Surface Area
CRP-	C-reactive Protein
CKD-EPI-	Chronic Kidney Disease Epidemiology
CVD-	Cardiovascular Disease
DBP-	Diastolic Blood Pressure
EDTA-	Ethylene-Diamine-Tetraacetic Acid
ELISA-	Enzyme-Linked Immunosorbent Assay
eGFR-	Estimated Glomerular Filtration Rate
GGT-	Gamma Glutamyltransferase
GPAQ-	Global Physical Activity Questionnaire
HDL-C-	High-density Lipoprotein Cholesterol
HIV-	Human Immunodeficiency Virus
HREC-	Health Research Ethics Committee
LDL-C-	Low-density Lipoprotein Cholesterol
MAPK/ERK-	Mitogen-Activated Protein Kinase/ Extracellular-Signal-Regulated Kinase
MRI-	Magnetic Resonance Imaging
MRC-	Medical Research Council
MVPA-	Moderate and Vigorous Physical Activity combined
NRF-	National Research
PAHO-	Pan American Health Organisation
PPAR delta-	Peroxisome Proliferator-Activated Receptors Delta
RAAS-	Renin-Angiotensin-Aldosterone System

REDCap-	Research Electronic Data Capture
RMR-	Resting Metabolic Rate
SBP-	Systolic Blood Pressure
SES-	Socio-Economic Status
SSB-	Sugar Sweetened Beverages
TEE-	Total Energy Expenditure
WHO-	World Health Organisation

Chapter 1

Background, Motivation and Literature Overview

1. Background and Motivation

For the majority of human existence salt was consumed only through what was contained in naturally found foods, amounting to an intake of less than 0.25 g salt daily.[1] This however has drastically changed due to salt now being used as a preservative, increased consumption of high salt, energy dense foods and dietary changes related to urbanisation.[1, 2] The average salt intake across a number of countries is now approximately 9-12 g/d.[3] The World Health Organisation (WHO) recommends a daily salt intake of less than 5 g/d.[4] The formula for the conversion of salt (i.e. sodium chloride) to sodium is as follows: 1g salt (sodium chloride) = 390 mg sodium.[5] The reasoning for recommendation to lower daily salt intake is due to evidence that elevated sodium intake is related to an increased risk for stroke and cardiovascular disease (CVD).[6] In numerous studies, a link between high sodium intake and an increase in blood pressure has been found,[7, 8] as well as an increased risk for the development of CVD and stroke.[9, 10] Studies have also indicated a clear link between a reduction in sodium intake and reduced blood pressure,[11] which translated to a reduced risk for the development of CVD.[12] A reduction in sodium results in a decrease in blood pressure in both normotensive and hypertensive individuals.[13]

Globally obesity has also been linked to increased blood pressure and risk of CVD.[14] Being overweight or obese may account for approximately 15-30% of coronary heart disease deaths.[15] Estimates suggest that approximately one billion adults worldwide are overweight, of which approximately 300 million are obese.[16] In 2002, significant differences were found between South African men and women in obesity prevalence, with 29.2% of South African men and 56.6% of women being obese.[17] However, 2016 statistics found nearly 40% of South African men and 70% of women to be either overweight or obese.[18]

Recently it was suggested that high sodium intake is related to and may be a risk factor for, obesity.[3, 19-25] Ma *et al.* found that higher sodium consumption was associated with a 20% higher risk of central obesity.[19] It was suggested that a diet high in sodium may have a negative effect on body composition by leading to an increase in body fat as well as a reduction in fat free mass.[3] A recent review found that high sodium consumption was not only associated with an increase in body mass index (BMI) but also a 4.75 cm increase in waist circumference.[23]

There are a number of theories as to how this potentially occurs,[3] several of which suggest it is a function of an increased energy intake. It has been shown that dietary sodium may increase fluid intake and therefore the intake of sugar-sweetened beverages (SSB).[25, 26] The increased intake of SSB results in increased obesity.[25] A second potential mechanism may be increased intake of high-energy processed foods containing a high sodium level.[3] However, several studies suggest that the association between a high sodium intake and obesity may be independent of energy intake.[20-22, 24] The direct mechanism by which this occurs is unknown though there are several potential mechanisms involving increased extracellular fluid volume,[27, 28] salt sensitivity,[29] altered fat metabolism,[19, 30] genetic factors,[31,32] and sodium-to-potassium ratio.[33]

Another factor to consider when looking at both obesity and sodium intake is physical activity. A physically active lifestyle is known to impact energy expenditure,[34, 35] affect resting metabolic rate,[36] and also contributes to the loss of sodium that occurs during sweating.[37, 38]

From the above it is clear that both a high sodium intake and obesity are risk factors for hypertension and CVD. The possibility of a relationship between sodium intake and obesity independent of high energy intake, suggests that high sodium intake may even pose an additional cardiovascular risk. The African Prospective study on the Early Detection and Identification of Cardiovascular Disease and Hypertension (African-PREDICT) provides us with the ideal population to investigate the sodium intake-adiposity link, as participants of the study are young and apparently healthy. This allows for investigation of the physiology underpinning this interaction without influence from diagnosed conditions. In addition, the African-PREDICT study includes a bi-ethnic population allowing for investigation of potential ethnic differences. This is important as black individuals are known to be more salt sensitive and more susceptible to hypertension development.[39, 40]

In the ensuing literature overview, the focus will be on the potential link between dietary sodium intake and obesity, both energy dependent and independent. With this overview, the most recent literature on potential confounders to this link was accessed, such as physical activity, ethnicity and gender differences.

2. Salt

A daily salt intake of less than 5 g/d or 1.7 g/d of sodium is recommended (WHO).[4] A study found that in 2010 the global mean consumption of sodium was 3.95 g/d, with 181 out of 187 countries, which makes up approximately 99% of the adult population, exceeding the WHO daily sodium intake recommendation.[41] South Africa is no exception. In South Africa it was shown recently that more than two-thirds of the population consumed over 5 g/d of salt with the average consumption around 7.2 g/d.[42, 43] Overall, in 2015, 69% of adults had salt intakes above the WHO recommendation, with 28% consuming more than twice this level (>10 g/day) and 11% consuming at least three times the recommended level.[43]

With high sodium intake associated with an increase in blood pressure,[7] and risk for CVD and stroke,[9, 10] alongside the elevated rates of hypertension observed in South Africa,[44] it is of the utmost importance that measures be put in place to reduce salt intake. A study has found that 60% of salt consumed by South Africans is from nondiscretionary salt intake through processed foods.[45] Therefore, in June 2016 a new salt legislation was officially implemented to regulate the salt content of processed foods.[46] Research has suggested that reduction of a mere 0.85 g of salt per day per person could result in a decrease of 7400 cardiovascular-related deaths each year.[46]

Apart from the cardiovascular risks associated with a high sodium diet, it seems to have other detrimental health effects, such as reduced bone density.[47] This is due to a diet high in salt resulting in a decrease in calcium reabsorption.[48] A high sodium diet may additionally be associated with increased risk for the development of kidney stones.[49]

However, while a high sodium diet is definitively associated with adverse health effects, one study reported that a diet extremely low in sodium is also associated with increased risk for mortality.[50]

One mechanism by which a high sodium diet could result in increased blood pressure is through changes in the extracellular fluid volume. Murphy *et al.* (1950) found that individuals placed on the low sodium Kempner rice diet (high carbohydrate, high fluid, low protein, low fat, low sodium, high potassium diet mostly consisting of rice and fruits)[51] showed a 12% reduction in extracellular fluid volume. This decrease was accompanied by a decrease in arterial blood pressure.[52, 53] These results were replicated by Watkin *et al.* in individuals on the above mentioned rice diet showing a 15% decrease in extracellular fluid.[53] When participants' sodium intake was increased by 3 g/d, an associated rise in blood pressure towards baseline levels were seen.[53]

Ledingham postulated that the relationship between extracellular fluid volume expansion and hypertension is caused by the associated increase in cardiac output that is seen with increased extracellular fluid resulting in an increase in blood pressure.[53, 54] Due to diuresis, extracellular fluid returned to normal.[53, 54] As cardiac output then returns to normal a subsequent rise in total peripheral resistance occurs.[53, 54] This results in a reduction in volume expansion and normalising systemic flow while maintaining a high blood pressure.[53,54]

3. Obesity

Obesity is defined as "a condition that is characterised by excessive accumulation and storage of fat in the body and that in an adult is typically indicated by a body mass index (BMI) of 30 or greater".[55] BMI is calculated using the following formula: BMI = Weight (Kg)/Height (m)^2. Obesity is a complex condition and is in broader terms the result of a chronic pattern of imbalance between higher energy intake and lower energy expenditure.[56] Obesity is linked to a decreased life expectancy and is a continuous, rising global problem.[57,58]

In South African adults, there has been a 10.8 % increase in the prevalence of obesity in men and a 30% increase in women between 2002 and 2016.[17, 18] In addition to this, the Heart and Stroke Foundation has stated that in South Africa 1 in 4 girls and 1 in 5 boys between the ages of 2 and 14 years are now either overweight or obese.[59] A recent study evaluating global trends in BMI found southern African children presented with the largest increase in prevalence of obesity in any region over the last 42 years.[60] This is of grave concern as being overweight or obese during childhood or adolescence has been linked to increased risk and earlier onset of chronic diseases such as diabetes.[61-65] In 2002 approximately 7% of deaths in South Africa were as a result of excess body weight,[66] due to the significant increases in obesity prevalence in South Africa, it is probable that this figure also escalated over the past 15 years.

Obesity is a major risk factor for diabetes, CVD, several forms of cancer, pulmonary, osteoarticular and metabolic diseases.[56] Obese individuals have a higher heart rate, systolic blood pressure and diastolic blood pressure in comparison to non-obese individuals.[67] Mounting evidence has suggested that visceral obesity may be the most important risk factor in terms of the development of hypertension and cardiovascular disease.[68] One study has found that more than two-thirds of deaths related to an increased BMI were as a result of cardiovascular disease. Additionally, global mortality related to BMI has risen by a staggering 28% in the last 25 years.[69]

The exact mechanisms that cause obesity-related hypertension are not fully understood. It is thought that the rise in blood pressure is caused by volume overload which is linked to the activation of the renin-angiotensin-aldosterone system (RAAS) and activation of the sympathetic nervous system (Figure 1).[70, 71] In addition, both endothelial dysfunction and renal functional abnormalities play a role in the development of hypertension and are associated with obesity.[71] Activation of the sympathetic nervous system is thought to be mediated through leptin which is an adipocytokine produced by adipose cells.[70, 72] Obesity is associated with dysregulation of adipokines (such as adiponectin and leptin) and is characterised by a condition of 'leptin resistance' which has far-reaching consequences on the cardiovascular system.[14, 72-74] The second process thought to be involved in the activation of the sympathetic nervous system is the stimulation of pro-opiomelanocortin (POMC) neurons in the pituitary gland which in turn produce a number of biologically active enzymes.[70, 75] Lastly, obesity may influence blood pressure through the activation of central nervous system melanocortin 4 receptors which play an important role in maintaining homeostasis in humans.[70, 76]

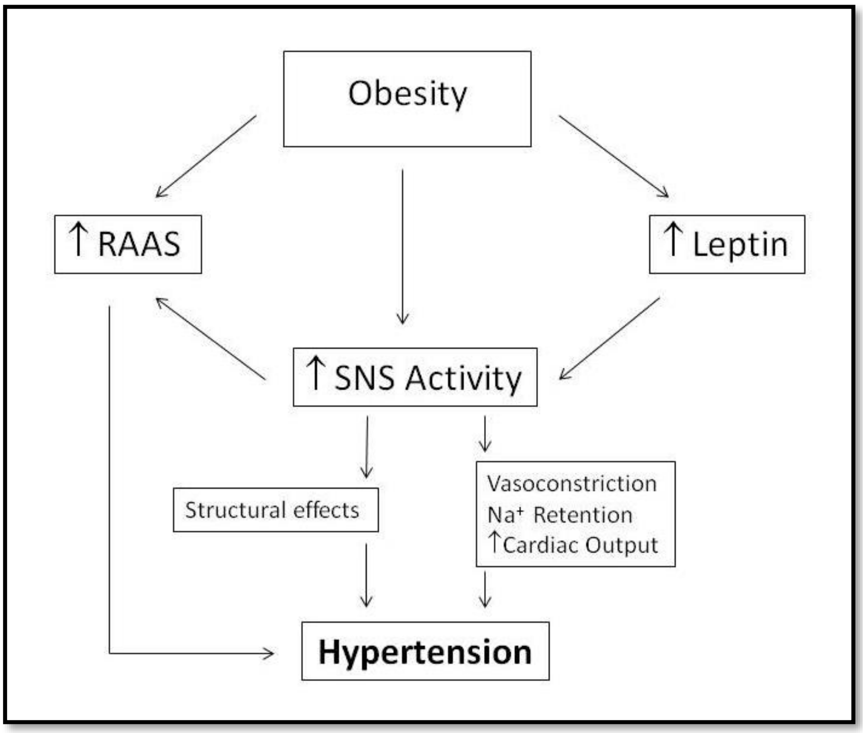

Figure 1. Relationship between obesity and hypertension (Adapted from Masuo *et al*.).[77]
Abbreviations: RAAS, Renin angiotensin aldosterone system; SNS, Sympathetic nervous system; Na, Sodium.

It is known that impaired pressure natriuresis plays a role in chronic hypertension.[78] It was recently shown that obesity may also play a role in this process as obesity has been found to both increase sodium reabsorption as well as impair pressure natriuresis in turn resulting in hypertension.[78]

A study performed on the link between BMI and blood pressure found that for each 10% increase in BMI, systolic blood pressure increased by 3.85 mmHg and diastolic blood pressure increased by 1.79 mmHg.[79] A second study across three populations in Africa and Asia also reported that BMI was significantly and positively correlated with both systolic blood pressure and diastolic blood pressure in all three populations.[80] Concurrently it was shown that even modest weight loss results in a decrease in blood pressure in both normotensive and hypertensive individuals.[81]

4. Link between salt and obesity

Below are presented the proposed mechanisms by which sodium intake may relate to and potentially be a risk factor for, increased body size and obesity. These mechanisms are divided into the categories of those thought to be related to increased energy intake (energy dependent) and those not (energy independent).

4.1. Energy Dependent Mechanisms

4.1.1 Sugar-Sweetened Beverages and Energy Dense Foods

Research suggests that dietary sodium intake can be used to predict fluid intake as salt stimulates thirst.[26] For each additional 1 g/d of salt there is an associated 46 g/d greater intake of fluid.[25] In those who consume SSB, the 1 g/d increase in salt consumption resulted in a 17 g/d increase in consumption of SSB.[25] Individuals consuming more than 250 g/d of SSB are 26% more likely to be overweight or obese,[25] suggesting higher sodium intake increases the risk of obesity through increased SSB consumption.[25] In 2010, South Africans were found to consume on average 254 Coca-Cola products (SSB) per person per year, which is exuberantly high when compared to Kenya, Nigeria or Russia where consumption was only 40, 28 and 68 Coca-Cola products respectively, or compared to the 89 Coca-Cola products consumed per person per year on average worldwide.[66, 82]

The majority of SSB are sweetened using high fructose corn syrup.[83] The absorption, digestion and metabolism of fructose differs largely from that of glucose.[83] Fructose does not result in the stimulation of either insulin or leptin, therefore resulting in increased energy consumption.[83] Additionally, fructose favours de novo lipogenesis, [83] which is an enzymatic pathway for the conversion of carbohydrates into fats.[84] These fatty acids are then esterified to storage triacylglycerols. Therefore, increased de novo lipogenesis as a result of increased fructose intake may result in increased risk of obesity.[85]

Larsen *et al.* (2013) suggested that another potential mechanism linking adiposity with a diet high in sodium, is the increased intake of processed foods as these foods often contain high sodium concentrations.[3] Additionally processed foods are often energy dense, [3] which in turn leads to an increase in total energy intake, resulting in an increase in body fat.[3]

4.1.2 Salt and Hunger

It has long been the general consensus that a diet high in sodium results in increased thirst and urine volume.[86] However, recent studies suggested that high sodium intake results in hunger as opposed to thirst.[87] According to Rakavo *et al*. (2017) increased sodium intake results in body water conservation and decreased fluid intake.[86] Kitada *et al*. (2017) demonstrated that renal concentration mechanisms promote salt excretion while maintaining high water reabsorption.[88] This results in limited natriuretic osmotic diuresis leading to concurrent extracellular volume conservation and concentration of salt excreted into urine.[88] The process relies on urea recycling by the kidneys and production by the liver.[88] The hepatic and extrahepatic urea osmolyte production processes are particularly energy intense.[88] Therefore, to prevent hepatic ketogenesis and glucocorticoid-driven muscle catabolism, increased food intake is required,[88] such that high sodium intake results in hunger as opposed to thirst.[87]

4.2 Energy Independent Mechanisms

4.2.1 Extracellular Fluid Volume

Very early studies indicated that salt deficiency leads to a reduction in extracellular fluid in man.[89] As such, it is unsurprising that increased dietary sodium intake results in an increase in extracellular fluid, fluid retention and potentially leads to weight gain.[27] Visser *et al*. (2009) showed that, when moving from a diet low in sodium (1150 mg Na/day) to a diet that is high in sodium (4598 mg Na/day), the corresponding increase in extracellular fluid volume was higher in individuals with a greater BMI.[28] This suggests that the higher the BMI of an individual the more sodium elicits fluid retention.[28] However, not all sodium loading studies have shown an increase in body weight suggesting that excess sodium, if not excreted, may be stored in other tissues where it is not osmotically active.[90, 91] (See section 5.3)

4.2.2 Altered Metabolism

It was found in rats that higher sodium intake may also lead to increased adiposity by increasing leptin levels and adipocyte hypertrophy, potentially due to an increased lipogenic capacity of white adipose tissue.[30] This implies that sodium in some way alters the metabolism of fats.[19] Obesity in and of itself is associated with a state of increased leptin and decreased adiponectin levels.[92, 93]

While there appears to be little evidence for a direct relationship between sodium intake and adiponectin, the potential associated increase in adiposity would be expected to result in decreased adiponectin levels.[92, 93] However this relationship appears complex. In a mouse model, Baudrand *et al*.(2014) found a 5-fold increase in adiponectin levels while on a low sodium diet when compared to those on a high sodium diet.[94] However, findings on the sodium-adiponectin relationship are controversial as a human study indicated the opposite, finding a decrease in adiponectin in men on a low sodium diet.[95]

Zhao *et al*. (2016) suggested a direct relationship between salt and adiponectin through the expression of peroxisome proliferator-activated receptors delta (PPARδ), linked to increased production of adiponectin.[96] Mice on a high sodium diet were found to have elevated levels of PPARδ, therefore suggesting a direct relationship between sodium and adiponectin.[96] Additionally, plasma adiponectin levels were found to be negatively associated with blood pressure.[97] Kamari *et al.* found an increase in adiponectin in rats on a high sodium diet independent of changes in blood pressure, further suggesting a direct positive relationship between adiponectin and salt.[98]

As stated earlier it has been suggested that sodium may alter the metabolism of fats in the body.[19] Recently it was proposed that a diet high in salt may induce adipogenesis.[56] This increase in adipogenesis occurred via the enhancement of the MAPK/ERK1/2 pathway in both 3T3-L1 adipocytes and co-culture with macrophages.[56]

In summary, there appears to be a number of potential mechansims whereby a diet high in sodium may result in increase adiposity.

4.2.3 Genetics

It is important not to ignore potential genetic factors. While this study does not focus on genetics, there have been some interesting genetic findings that further support the potential of sodium to directly influence obesity and explaining variation in this relationship by ethnicity. Lee *et al.* found that girls with the hetero/mutant allele of the CYP11β2 gene (involved in the synthesis of aldosterone)[31] showed an increased incidence of obesity when there was a significant increase in dietary sodium intake.[99] This suggests that certain individuals have a genetic predisposition to gain weight on a high sodium diet. Additionally, specific polymorphisms in the GRK4 gene (coding for a protein kinase found in the kidney and involved in sodium transport) have been related to ethnic differences in sodium handling and hypertension.[100, 101] These and other gene polymorphisms associated with hypertension may be particularly frequent in black Africans,[102] contributing to decreased sodium excretion.[32] Polymorphisms in the adiponectin gene may also modulate blood pressure in response to both low and high sodium diets.[103]

4.2.4 Sodium-to-Potassium Ratio

There also seems to be a link between the dietary sodium-to-potassium ratio and obesity.[42, 104] A diet high in sodium and low in potassium is linked to an increase in total body fat percentage.[33] This sodium-to-potassium ratio becomes more important with age. Khaw *et al.*

found that the positive correlation between blood pressure and sodium-potassium ratio increased with age.[105] Dietary potassium additionally plays an important role in salt sensitivity.[106] This potassium is obtained from food such as fruits (e.g. bananas and avocados), vegetables (e.g. spinach and asparagus), milk, almonds and yogurt.[107] A study found that salt sensitivity in black men occurs in individuals with insufficient potassium in their diet.[108] When dietary potassium levels were increased to within the normal recommended range, salt sensitivity was suppressed.[108] This is of particular importance in a South Africa as it has been shown that 91-93% of the population does not reach the daily recommended intake of potassium.[42,43]

5. Factors that may potentially Confound the Sodium-Obesity Link

5.1 Physical Activity

Physical activity has been linked to a reduction in adiposity,[34, 35] as well as a reduction in body sodium.[37, 38] An overall reduction in weight in obese individuals may lead to a reduction in blood pressure.[80] Furthermore, a reduction in sodium is also linked to decrease blood pressure.[7]

In addition, physical activity may have an effect on resting metabolic rate (RMR). RMR makes up approximately 70% of daily energy expenditure and is therefore extremely important in maintenance of body weight.[36] RMR is closely associated with body composition and, in particular, fat free mass.[36, 109] Therefore, physical activity leading to a reduction in fat mass and an increase in fat free mass may result in increased RMR.[36, 109] Animal studies have shown that single physical activity events result in increased RMR.[110] An added health benefit associated with weight loss in obese individuals is a decrease in blood pressure.[80] One study found a negative correlation between physical activity and the occurrence of hypertension, with the total reduction in blood pressure averaging 3.4/2.4 mmHg.[111]

Furthermore, physical activity is linked to a loss of sodium through sweat.[37, 38] This in turn suggests that individuals who take part in regular physical activity need to replenish their sodium levels to compensate for sodium loss.[37, 38] Therefore, individuals that consume high levels of sodium may be able to partly compensate for their intake through regular physical activity.[112]

5.3 Ethnic and Gender Differences

It is well known that black individuals are more salt sensitive when compared to white individuals.[39] Kawasaki *et al.* (1978) defined salt sensitivity as a 10% or more increase in blood pressure in response to a salt load.[113] One study found that while on a high sodium diet, salt sensitive individuals gained more weight than non-salt sensitive individuals.[114] In addition, black populations are shown to be more prone to the development of hypertension.[40] A study performed by Sowers *et al.* (1988) investigated the possibility that hypertension in black individuals may in part be due to impaired renal excretion of salt.[39] (See section 4.2.3) It was found that salt sensitivity is not confined to hypertensive black individuals but is also present in normotensive black individuals.[39] While black individuals often present with salt sensitive hypertension this is not the only contributing factor to the development of hypertension in black populations.[40, 115] The specific mechanisms behind salt sensitivity are complex and include both genetic and environmental factors.[106] However, salt sensitive individuals were shown to share a number of similar characteristics such as: increased blood pressure response to changes in sodium; higher baseline blood pressure; a shift in the blood pressure-natriuresis relationship; lower aldosterone levels; and suppressed renin levels during sodium depletion.[116]

As mentioned above, black populations have been shown to have higher sodium retention,[117] with a consequent higher extracellular fluid volume. As a corrective mechanism, black individuals display suppressed plasma renin levels.[40, 118] Furthermore, suppressed plasma renin was shown to be associated with increased adiposity in black individuals.[29] Since adiposity in this study was assessed using BMI, it is not clear whether BMI reflects adipose tissue or increased fluid volume. With this said, one study evaluating the sodium retention in black and white female adolescents found that while black adolescents did present with higher sodium retention than their white counterparts, there was no corresponding increase in weight or extracellular fluid. This suggests the retained sodium is stored in a non-extracellular compartment.[119] The authors speculated that this sodium is stored in bone, however, as discussed below in section 5.3, a second possibility is that it is stored in the skin and skeletal muscle.[119]

In addition to black individuals being more salt sensitive than white individuals, men may be more salt sensitive than women, however there is some literature that disputes this,[120-122] though results may differ depending on the specific protocol used.[121] For example, when evaluating a decrease in blood pressure while on a low sodium diet, 14% of boys were found to be salt sensitive in comparison to 22% of girls.[121] However, when testing the increase of blood pressure on a high sodium diet, 31% of boys were found to be salt sensitive in comparison to only 18% of girls.[121] Globally men on average consume 10% more salt than women,[123] this was also found to be true in a South African population.[42]

Furthermore, studies have indicated that there are both ethnic and gender differences in the prevalence of obesity. According to the 2016 SA Demographic and Health Survey, 67% of black and 69% of white women were either overweight or obese (BMI >25 kg/m^2) in comparison to the 30% of black and 28% of white women with a normal BMI (18.5-24.5 kg/m^2).[124] When looking at men 27% of the black and 74% of the white populations where either overweight or obese with 62% of the black and 24% of the white men being classified as having a normal BMI.[124] When investigating the prevalence of severe obesity, it was found that 20 % of black South African women had a BMI >35 kg/m^2 in comparison to only 2.1% of black men while the prevalence of a BMI >35 kg/m^2 in white women and men was 14.5 and14.1 respectively.[124]

5.3 Skin Sodium Storage and Volume

The maintenance of sodium concentrations in the body are mediated by the kidneys.[125] Additionally the kidneys' mediation of sodium includes the control of blood volume and therefore blood pressure.[126] However, sodium handling is not just a renal matter.[125] Titze and his research group have found that excess sodium intake can be stored in the skin as well as in skeletal muscles.[125, 127, 128] Sodium accumulates in the subcutaneous interstitium through reabsorption from sweat glands.[126] Polyanionic matrix molecules in the interstitium can bind sodium without commensurate water (figure 2).[126] As such, sodium may be stored in the body without the expected fluid retention.[126] During periods of low sodium intake this osmotically inactive sodium is then released back into the body.[126] However, at present the only method of testing sodium storage in the skin is through magnetic resonance imaging (MRI). [128] This new area of research on skin sodium storage leaves many questions unanswered and opens a new field of study in the role of sodium not only in obesity, but also in cardiovascular health.

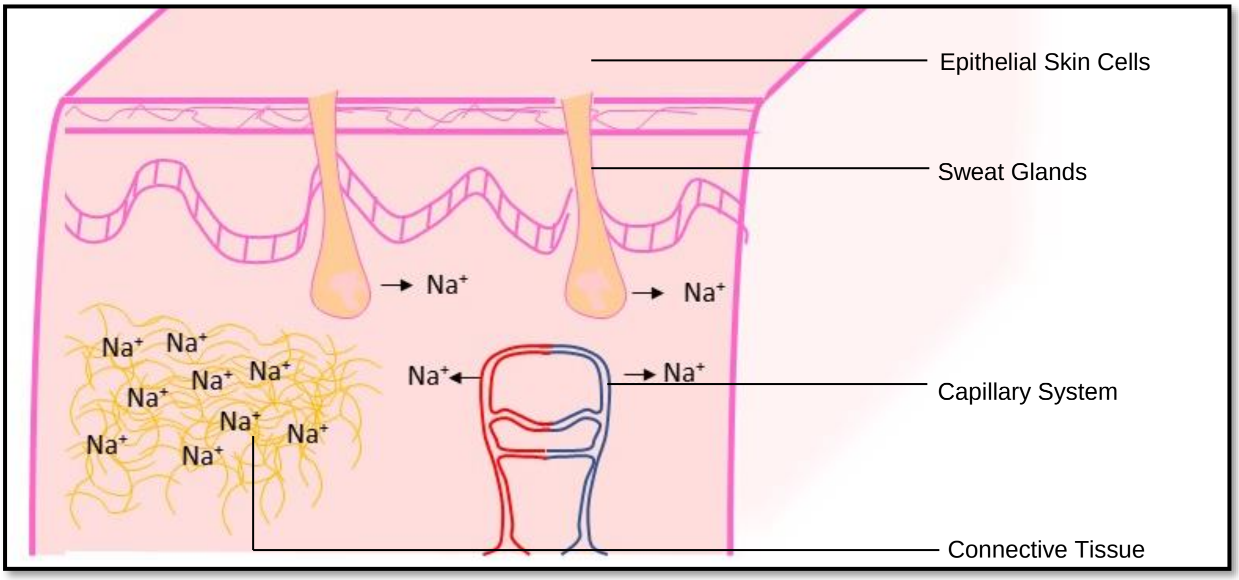

Figure 2. Excess sodium is stored in the skin. (Adapted from Rabelink *et al.*).[126]

This also brings into question whether an increase in skin volume results in an increase in skin sodium storage. As well as whether body surface area is an accurate measure of skin volume. It has been shown that in obese and underweight individuals body surface area is often inaccurate and in obese individuals in particular is often underestimated.[129] However, body surface area is often used to make decisions with regard to medical practice and therefore must provide some adequate level of accuracy.[130]

5.4 Additional Confounders

Smoking and tobacco use have been shown to have a negative association with obesity.[131] In addition to the effect tobacco use has on obesity, it is often associated with a poor quality diet.[132] One study has shown that individuals that use tobacco, alcohol or in particular both were much less likely to consume a low sodium diet.[133] Unlike tobacco use, high alcohol consumption, due to the high calorie nature of alcohol, is positively associated with increased risk for the development of obesity.[134] This is of great concern due to the high rates of both tobacco and alcohol use in South Africa. It has been shown that 3% of black and 15% of white women in South Africa use tobacco products and 36% and 31% of black and white men, respectively.[124] While this study focuses only on the black and white populations in South Africa, tobacco use is even higher in the coloured populations as well as Indian and Asian men.[124] In terms of alcohol consumption, the 2016 SA Demographic and Health Survey found 4.5% and 4.2% of black and white women respectively to have consumed five or more drinks on at least one occasion in the past 30 days while 2.6% and 1.6% showed signs of problem drinking.[124] Additionally, 28.3% of black and 25.7% of white men consumed five or more drinks on at least one occasion in the past five days with 16% and 8.2% showing signs of a drinking problem.[124] Once again, these statistics were much higher in the coloured populations.[124]

Age may also influence the relationship between dietary sodium intake and obesity. Within South Africa there is a direct correlation between BMI and age with BMI shown to steadily increase with age in the general population.[124] However, a study presented a decrease in sodium consumption with increasing age.[43] It has also been shown that socio-economic status may too have an effect on this relationship as a low socio-economic status is often associated with increased prevalence of obesity as well as poor diet however this relationship has been shown to be inconsistent.[135, 136]

While the importance of glucose when examining obesity seems straight forward, as obesity is closely linked to insulin resistance,[137] glucose levels may also be important when looking at salt. One study found that patients with essential hypertension also display insulin resistance associated with a hyperinsulinemic response to oral glucose intake.[138] This insulin resistance is believed to induce hypertension by causing renal sodium and water retention.[138] It has been shown that this insulin resistance is present prior to the development of hypertension and the hypertension can be controlled by a reduction in salt intake.[138]

Aldosterone is another important factor to include when investigating the relationship between dietary sodium and obesity. A study has shown a direct positive correlation between aldosterone levels and obesity.[139] Additionally, hyperaldosteronism has been linked to the development of salt sensitive hypertension.[140]

6. Problem Statement

Both obesity and high dietary sodium intake contribute to the development of high blood pressure and CVD.[7, 8, 14] Studies also suggest a link between high dietary sodium intake and obesity,[2, 19] though it is unclear if this relationship exists in young black and white South Africans and the mechanisms for this. The purpose of this present study is to determine if there is a relationship between sodium intake and obesity-related measures in the young healthy, black and white South African population participating in the African-PREDICT study.

This study could therefore contribute to the knowledge base necessary for better physiological understanding of the relationship between high dietary sodium intake and obesity in a young apparently healthy black and white South African population. This may be of public health importance because, if indeed a relationship does exist, it further amplifies the risks associated with a diet high in sodium.

7. Aim and Objectives

The aim of this study is to determine whether there is a relationship between sodium intake and obesity-related measures in a young healthy, black and white South African population, when adjusting for potential confounders.

The objectives of this study are:

- To determine whether there is a relationship between sodium estimated from 24hr urinary sodium excretion (an accepted surrogate marker for sodium intake) and obesity-related measures (BMI, waist, hip and neck circumference, lean mass %, body fat %, body surface area, waist-to-height ratio, waist-to-hip ratio, serum leptin and adiponectin), independent of potential confounders such as energy intake and expenditure.
- To determine whether there is an interaction of sex or ethnicity on the relationships between sodium intake and the obesity-related measures.

8. Hypotheses

Based on the literature regarding the young population of the African-PREDICT study, the following hypotheses are formulated:

- There will be positive independent correlations between estimated 24hr urinary sodium excretion and BMI, waist circumference, hip circumference, neck circumference, waist-to-height ratio, waist-to-hip ratio, body fat %, body surface area and leptin; as well as negative correlations with adiponectin and lean mass %.
- The above correlations will be more prominent in black than white participants and more prominent in men than women.

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Chapter 2

Methodology

1. Study Design and Participants

This MHSc study is a sub-study of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT). The African-PREDICT study is a longitudinal study that aims to gain new knowledge on early cardiovascular disease related pathophysiology as well as identify early markers or predictors of cardiovascular disease in young apparently healthy South Africans. This will enable the implementation of successful prevention programs in the long term.

The African-PREDICT study recruits young participants from Potchefstroom and surrounding areas in the North West Province, South Africa (Figure 1 and 2). Participants in this study are either black or white, men and women, between the ages of 20-30 years. The study includes apparently healthy individuals with normotensive office blood pressures (<140/90 mmHg) and not diagnosed with human immunodeficiency virus (HIV) or any other previous diagnosis of a chronic disease. Individuals from low, middle and high socioeconomic status groups are specifically included. These participants will be followed every 5 years over a 10-20-year period.

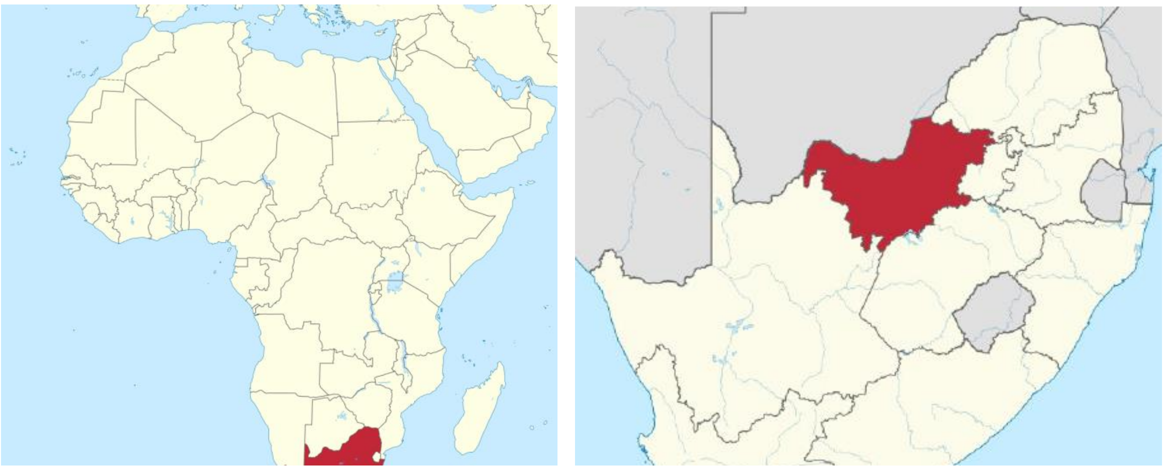

Figure 1: Maps indicating South Africa and the North-West Province.

Figure 2. Map indicating Potchefstroom.

As mentioned in the previous chapter, obesity and high dietary sodium intake contribute to cardiovascular disease.[1-3] Studies also suggest a link between high dietary sodium intake and obesity,[4, 5] though the nature of this relationship is unclear. This study therefore investigated if there was a relationship between sodium intake and obesity-related measures in the African-PREDICT study population.

2. Methodology

2.1 Organisational Procedures

First participants were screened for eligibility based on the inclusion and exclusion criteria listed in Table 1. Second, those participants eligible for participation were then invited to take part in the African-PREDICT study and were provided with detailed information of procedures ahead of time. Participants were requested to fast for 8 hours (overnight) prior to the study day and asked to arrive at the research unit at 08h00 (or transportation provided) after which they were shown around to ensure they were familiar with their surroundings. A maximum of four participants were accommodated on a single day to maintain the quality of the measurements. Following informed consent, participants were requested to provide a spot urine sample, where after they were taken to a private room where a registered research nurse took blood samples (Refer to 2.9). During the study day, participants were rotated around a number of measurement stations with the help of the nurse. In between the stations, participants were requested to complete questionnaires (Refer to 2.2, 2.6 and 2.8). All procedures were performed according to good clinical practice.

Table 1: Justification of inclusion criteria.

Inclusion criteria	**Justification of inclusion/exclusion**
1. Apparently healthy individuals	1. The African-PREDICT study aims to track young healthy individuals over a period of 10-20 years.
2. Between 20-30 years of age	2. As the African-PREDICT study aims to track and evaluate the development and early stages of hypertension, individuals in the age group 20-30 years, proved the ideal population as they are adults that are considered to be in good cardiovascular health.
3. Black or white ethnicity	3. Black individuals in South Africa are at increased risk for the development of hypertension and are therefore included in this study. White individuals are used as a comparison group. Other ethnicities are excluded as the black population has shown to be particularly at risk.[6]
4. Equal distribution of men and women	4. Both men and women are included to allow for the determination of gender differences.
5. Office brachial blood pressure of <140mmHg and 90mmHg	5. As the aim of this study is to observe the development of hypertension, individuals already presenting with hypertension were excluded.

6. HIV uninfected 7. No previous diagnosis or medication for a chronic disease 8. Pregnant or lactating women were not included in the study	6/7. Individuals with a self-reported previous diagnosis of disease were excluded as the aim of this study is to track seemingly healthy individuals. 8. Pregnant or lactating women were excluded due to the known influence of pregnancy hormones on the cardiovascular system.

2.2 General Health and Demographic Questionnaire

Data with regard to age, sex and ethnicity were collected using a General Health and Demographic Questionnaire. The questionnaire included demographic information such as level of education, employment information, household income, smoking, alcohol consumption, medication use and family history. This questionnaire was completed on iPads using a web-based program and took approximately 15 minutes to complete.

2.3 Socio-Economic Status

Socio-economic status was calculated using a point system that was adapted from Kuppuswamy's Socioeconomic Status Scale 2010[7] for a South African environment. In this version adapted for a South African population, participants were scored in three categories: skill level, education and household income. Skill level was classified according to the South African Standard Classification of Occupation (SASCO). These three factors were scored and used to categorise participants into low, middle and high socio-economic groups. Socioeconomic status was scored as both a categorical and continuous variable.

2.4 24hr Urine Collection

Trained research staff explained the process for 24hr urine sample collection and provided participants with all the necessary equipment. Participants were instructed to collect a 24hr urine sample on a day that was convenient for them, which was noted. The first urine of the day was to be discarded and all urine passed thereafter was collected in the provided container, including the first urine of the following morning (day two). The start and finish times were recorded. The protocol for 24hr urine collection follows that of the Pan American Health Organisation/World Health Organisation protocol for population level sodium determination in 24hr urine samples.[8] Incomplete urine collections were defined as a volume less than 300 mL per 24hr and/or a 24hr creatinine excretion of less than 4 mmol or more than 25 mmol in women and less than 6 mmol or more than 30 mmol in men.[9] (Refer to section 2.10 for breakdown of urinary analysis.)

2.5 Anthropometric Measurements

Trained researchers measured weight (kg) to the nearest 0.01 kg (SECA electronic scales, SECA, Birmingham, UK) and height (m) to the nearest 0.1 cm (SECA stadiometer, SECA, Birmingham, UK). Waist, hip and neck circumferences were measured three times using a non-flexible tape measure (Holtain, Crymych, UK) and recorded to the nearest 0.1 cm. The median of the three recordings was used in subsequent analyses. Body mass index (BMI) was calculated using the standard weight (kg)/height (m^2) calculation and waist-to-height ratio was calculated using waist circumference (cm)/height (cm). Body surface area was determined using the Mosteller formula.[10] All measurements were performed on one participant at a time in a temperature controlled private room, following the guidelines of the International Society for the Advancement of Kinanthropometry.[11]

2.6 24hr Dietary Recall Questionnaire

A 24hr dietary recall questionnaire was administered by a trained dietitian or nutritionist on the study day and on two subsequent days (arranged with the participant) to provide an analysis of dietary intake on two week days and one weekend day. This method makes use of a five-step multiple-pass approach which is designed to enhance complete and accurate food recall and reduce respondent burden.[12] Food models, a photo book and other appropriate tools were used to facilitate portion size estimation. The data was then coded, all measurements were converted to grams and the data read into an Excel spreadsheet. Data accuracy was ensured by comparing the data on the spreadsheet with the original questionnaires. This data was then sent to the South African Medical Research Council (MRC) where software developed by the MRC was used to determine macro- and micronutrient intake using the South African Food Composition Tables. The average daily energy intake was then calculated from the recorded three days.

2.7 Ambulatory Blood Pressure

Participants were fitted with a validated 24hr ambulatory blood pressure (ABPM) and echocardiography apparatus (CardioXplore® CE120, Meditech, Budapest, Hungary). The apparatus was programmed to take recordings every 30 minutes during the day (06h00 to 22h00 hours) and every hour during the night (22h00 to 06h00 hours). The ABPM was fitted to each participant at approximately the same time every day (late morning), using an appropriate sized cuff fitted to the participants none dominant arm. Only participants with >70% valid 24hr blood pressure measurements and >20-day time measurements and >7-night time measurements were included in the data analysis.

2.8 Bioelectrical Impedance

Bioelectrical impedance measurements were performed to estimate lean body mass, water and body fat percentage using a Bodystat 1500MDD dual-frequency analyser (Bodystat, Ltd, Ballakaap, British Isles). These measurements were performed by a trained scientist in a temperature controlled private room. Participants were in a supine position while electrodes were placed on the skin of the individual's right hand and foot. A low-level battery generated electrical current was passed through the body and the impedance (at frequencies of 5 kHz and 50 kHz) was measured.

2.9 Physical Activity Measurements

A compact, chest-worn accelerometer device that records heart rate, inter-beat-interval and physical activity in one combined unit was used to objectively measure physical activity. The ActiHeart device (CamNtech Ltd., England, UK) is designed to capture heart rate variability and then calculate total and activity energy expenditure (TEE, AEE) and was worn for a maximum of 7 days. The ActiHeart device was fitted by trained researchers in a temperature controlled private room to ensure privacy. In addition to the ActiHeart device, the Global Physical Activity Questionnaire (GPAQ) was administered. This questionnaire aims to collect information on physical activity participation (both work related and leisure) and was developed by the World Health Organisation (WHO).[13]

2.10 Blood Sampling and Biochemical Analyses

Participants were asked to fast overnight for at least 8 hours prior to attending the research measurement day. Early in the morning venous blood samples were collected from the brachial vein branches, using a sterile winged butterfly infusion set and syringe, by a qualified nurse in a temperature controlled private room. This is an invasive procedure. However, it holds minimal risk for the participant. All participants were aware of the procedure prior to giving informed consent. The samples were prepared according to standardised protocols and stored at -80°C until the time of analysis.

Serum samples were analysed for C-reactive protein (CRP), creatinine, total cholesterol, glucose and gamma glutamyltransferase (GGT) (Cobas Integra 400plus, Roche, Basel, Switzerland). Serum leptin, adiponectin and renin were determined using Quantikine ELISA kits (R&D systems, Minneapolis, MN USA) analysed on a Synergy H4 hybrid microplate reader (BioTek, Winooski, VT, USA). Ethylene-diamine-tetraacetic acid (EDTA) whole blood was used for analysis of glycated haemoglobin (HbA1c) (Cobas Integra 400plus, Roche, Basel, Switzerland). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) apparatus from serum samples. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula.[14] Urinary sodium, potassium and chloride were measured by means of ion-selective electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland) and creatinine concentrations were measured using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland).

Daily urinary sodium and potassium excretion were calculated by multiplying the sodium, potassium and creatinine concentrations (mmol/l) of the 24hr urine by the total 24hr volume of urine (in litres) resulting in the sodium, potassium and creatinine in mmol/day. Daily salt intake was then estimated from 24hr urinary sodium excretion by converting sodium in mmol to mg: sodium (mmol) x 23= sodium (mg)[15] and then applying the conversion: 1g salt (sodium chloride) = 390 mg sodium.[15]

3. Data Management

The African-PREDICT study uses the REDCap (Research Electronic Data Capture, see http://project-redcap.org) system to capture its data. REDCap is a free, secure web-based, electronic database software which can be quickly developed and customised for studies, in a user-friendly manner, for collecting and tracking information and data from research studies. REDCap can additionally be used to schedule patient visits.[16] Dr. Lisa Uys, the data manager for this study was appointed and trained to fulfil this function. When using this system, all laboratory specimens, evaluation forms, reports, data and other records are identified only by the participant number to maintain subject confidentiality. Apart from this system, the data manager ensures that all data is backed up on password protected hard drives. The accuracy of the data used during statistical analysis is ensured by the data being imported directly into Statistica from automatically generated Excel sheets.

A six-digit ID number is used: the first 2 digits correspond with a particular examination where, for example "00" will refer to the baseline phase and "01" to the first follow-up; the next four digits denote the subject’s unique four-digit number. Prior to participation in the study, detailed information is conveyed individually to participants, with opportunities to ask questions. As explained orally and in the participant leaflet attached to the informed consent form, voluntary participation included consent that all personally identifiable information as well as sensitive personally identifiable information would be captured as part of the study with the understanding that this information would be stored and handled very securely and coding would take place promptly. Furthermore, the minimum amount of personally identifiable information was captured within the study and only information that directly contributed towards the aims of the study was captured.

4. Ethical Considerations

The African-PREDICT study and this MHSc study were evaluated and approved by the Health Research Ethics Committee of the North-West University. (Appendix B) Additionally, both this study and the African-PREDICT study adhere to the principles set out in the Declaration of Helsinki. This study has no direct benefit for the participants, but the knowledge obtained during the study will add to the body of literature surrounding sodium intake and its relationship to adiposity. The use of existing data from this study and the relevant above-mentioned methodology would not expose the participants to any additional mental, physical or emotional risks.

Prior to participants being included in the study, all procedures were explained orally and participants obtained an information leaflet about the study. Participants were also given the opportunity to ask any questions and if they wished to participate, written informed consent was obtained.

This study made use of field workers who were able to convey and explain all the study procedures to participants in their home language to ensure that they fully understood before informed consent was obtained. Field workers encouraged and helped participants to ask questions to allow for a two-way communication process.

5. Student Contributions

As a MHSc student, I was involved in both the initial screening phase and the advanced research measurements of the African-PREDICT study. In terms of the initial screening, I was responsible for urine dipstick analysis, glucose and cholesterol testing as well as taking brachial blood pressure measurements and determining blood grouping. In the research phase of the African-PREDICT study, I was responsible for performing the electrocardiography measurements, downloading the data and checking the data for completion and accuracy before it could be added to the master dataset. Additionally, I contributed to laboratory work for this study on specific data collection days. Once blood and urine collection were completed per day, I was involved in ensuring that blood samples were centrifuged and aliquoted into individually marked cryovial tubes and correctly stored in minus 80°C biofreezers. I was further involved in the urine analysis of the 24hr and spot urine samples, for the biochemical analysis of sodium, potassium and chloride making use of ion-selective electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland). I was also involved in measuring urinary creatinine using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland).

6. Statistical Analysis

Statistical analyses explained in detail in Chapter 3.

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Chapter 3

Dietary sodium intake and its relationship to adiposity in young black and white adults:

The African-PREDICT study

Dietary sodium intake and its relationship to adiposity in young black and white adults: The African-PREDICT study

Running title: Salt and Obesity in Healthy young adults.

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(See Appendix A for Author Instructions)

Abstract

Background: Obesity remains a growing public health concern worldwide. Recently studies have found a positive link between dietary salt and obesity. However, the nature of this relationship remains controversial. We therefore investigated the relationship between obesity-related measures (body mass index (BMI); weight; waist, hip and neck circumference; % body fat; % lean mass; waist-to-hip and waist-to-height ratio, leptin, adiponectin and body surface area (BSA) which may also be a measure of skin area) and sodium intake, while controlling for energy intake, expenditure and other potential confounders. This was performed in apparently healthy, young black and white adults, screened for potential pre-existing health conditions. **Methods:** We included 761 adults (20-30 years) with complete 24hr urinary sodium data, as well as detailed anthropometry and bioelectrical impedance measurements. We additionally determined energy intake (dietary questionnaires), expenditure (accelerometery) and collected blood for analysis of leptin, adiponectin, aldosterone, lipids, glucose, C-reactive protein, gamma glutamyltransferase and cotinine. **Results:** In unadjusted linear regression analysis all obesity-related measures, including BMI and BSA, related positively with 24hr urinary sodium ($p \leq 0.008$). However, in multivariate adjusted regression analysis, upon adjustment for energy intake, accelerometery, age, sex, ethnicity and other covariates, only BSA remained independently associated with 24hr urinary sodium ($R^2=0.72$, $\beta=0.05$, $p=0.039$), but not any other measures of body composition. In all models adjusted for total energy expenditure, BMI was no longer positively associated with 24hr urinary sodium. **Conclusion:** We found a consistent and robust positive relationship between BSA and 24hr urinary sodium, independent of energy intake, energy expenditure and numerous other potential confounders – but not with any of the traditional obesity measures. Further studies are needed to investigate the role of BSA and potentially, skin area in salt handling.

Key Words: Dietary Sodium, Obesity, Energy Intake, Energy Expenditure, Salt, Body Surface Area, Anthropometry, Skin.

Introduction

Obesity is a growing public health concern worldwide. Several factors are known to contribute to obesity, but recently, a number of studies have found a positive link between dietary salt and obesity.[1-8] However, the nature of this relationship remains a matter of debate. Compared to individuals on lower sodium diets, those with a high sodium diet have a 20% higher risk of central obesity,[1] while a separate study found an average increase of 4.75 cm in waist circumference associated with a high sodium diet.[6] Some studies suggest the relationship between salt and obesity is simply the result of additional energy consumption that accompanies a high salt diet i.e. increased consumption of high energy-dense processed foods and sugar-sweetened beverages.[2, 8] However, other studies suggest salt may contribute to obesity independent of energy intake via increased extracellular fluid (increased weight via water retention),[9, 10] genetic predisposition,[11] salt sensitivity[12] or altered fat metabolism.[6]

Both high sodium intake and obesity are independent risk factors for hypertension and cardiovascular disease.[13, 14] A relationship between sodium intake and obesity suggests an additional cardiovascular risk associated with high dietary sodium. However, the existing literature demonstrating a relationship between dietary sodium intake and obesity contains important limitations, including the failure to take several key potential confounders into consideration. For example, energy expenditure, smoking, glucose and circulating aldosterone[3, 4, 7] are all known to play an important role in sodium handling, obesity or both. In addition, few studies included a healthy adult population screened for pre-existing health conditions that may influence the sodium/obesity relationship.[1-5] We therefore investigated the relationship between obesity-related measures and sodium intake while controlling for energy intake, expenditure and other potential confounders in apparently healthy, young black and white adults, screened for potential pre-existing health conditions. Our detailed dataset allowed us to evaluate the relationship between 24hr urinary sodium and a range of measures reflecting obesity and overall body composition, namely height, weight, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, body mass index (BMI), body surface area (BSA), fat mass, lean mass, body fat %, serum adiponectin and leptin.

Methods

Study Population

This study forms part of the African-PREDICT study (African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension) which is an on-going study that will recruit and monitor young white and black participants over a period of 10-20 years. The inclusion criteria for this study were: black or white, apparently healthy individuals between the age of 20-30 years; HIV uninfected; office brachial blood pressure <140 mmHg systolic and <90 mmHg diastolic; no previous diagnosis or medication for a chronic disease; and, if female, not currently pregnant or lactating. This study used cross-sectional data from the first 761 participants with complete data sets at baseline. This study was approved by the Health Research Ethics Committee (HREC) of the North-West University (NWU-00050-17-A1), adheres to the Declaration of Helsinki and all participants in the study provided written informed consent.

General Health and Demographic Questionnaire

Data with regard to age, sex and ethnicity were collected using a General Health and Demographic Questionnaire. The questionnaire also included level of education, employment information, household income, self-reported tobacco and alcohol use, medication use and family history.

Socio-economic status was calculated using a point system adapted to the South African context from the Kuppuswamy's Socioeconomic Status Scale 2010.[15] The adapted version scored participants in three categories: 1) skill level; 2) education; and 3) household income. Skill level was classified according to the South African Standard Classification of

Occupation. Socio-economic status was classified as both a continuous and categorical variable.

24hr Urine Collection

Participants were instructed to collect a 24hr urine sample on a day that was convenient for them, the date of which was noted. The first urine of the day was to be discarded and all urine passed thereafter was collected in the provided container, including the first urine of the following morning (day two). The start and finish times were recorded. The protocol for 24hr urine collection follows that of the Pan American Health Organisation/World Health Organisation (PAHO/WHO) protocol for population level sodium determination in 24hr urine samples.[16] Incomplete urine collections were defined as a volume less than 300 mL per 24 hours and/or a 24hr creatinine excretion of <4 mmol or >25 mmol in women and <6 mmol or >30 mmol in men.[17]

Body Composition Measurements

Trained researchers measured weight (kg) (SECA electronic scales, SECA, Birmingham, UK) and height (m) (SECA stadiometer, SECA, Birmingham, UK). Waist, hip and neck circumferences were measured three times using an anthropometric non-flexible tape measure (Holtain, Crymych, UK) and the median values used. Body mass index (BMI) was calculated using the standard weight (kg)/height (m^2) formula and waist-to-height ratio and waist-to-hip ratio were calculated by dividing waist circumference (cm) by height (cm) or hip circumference (cm). Body surface area (BSA) was determined using the Mosteller formula[18] i.e. BSA (m^2) = square root of (height (cm) x weight (kg)/3600). All measurements were performed following the guidelines of the International Society for the Advancement of Kinanthropometry.[19]

Bioelectrical impedance measurements were performed to estimate lean body mass, water and body fat percentage using a Bodystat 1500MDD dual-frequency analyser (Bodystat, Ltd, Ballakaap, UK) after 8 hours fasting and on an empty bladder. Participants were in a supine position while electrodes were placed on the skin of the individual's right hand and foot. A low-level, battery-generated electrical current was passed through the body and the impedance (at frequencies of 5 kHz and 50 kHz) measured.

24hr Dietary Recall Questionnaire

A 24hr dietary recall questionnaire was administered by a dietician or nutritionist on the study day and on two subsequent days (arranged with the participant) to provide an analysis of each participant's dietary intake on two week days and one weekend day. This questionnaire makes use of a five-step multiple-pass approach which is designed to enhance complete and accurate food recall and reduce respondent burden.[20] Food models, a photo book, [21] and other appropriate tools were used to facilitate portion size estimation. The data was then coded according to the South African Food Composition Tables and measurements were converted to grams using standard tables.[22] Energy and nutrient intake (macro- and micronutrient intake) was calculated using the South African Food Composition database[23] and the individual average intakes were calculated from the three recorded days.

Ambulatory Blood Pressure

Participants were fitted with a validated 24hr ambulatory blood pressure monitor (ABPM) and electrocardiography apparatus (CardioXplore® CE120, Meditech, Budapest, Hungary). The apparatus was programmed to take recordings every 30 minutes during the day (06h00 to 22h00 hours) and every hour during the night (22h00 to 06h00 hours). The ABPM was fitted to each participant at approximately the same time every day (late morning), using an appropriate sized cuff fitted to the participant's non-dominant arm. Only participants with $>$70% valid 24hr blood pressure measurements and $>$20 day time measurements and $>$7 night time measurements were included in the data analysis following the criteria recommended by the European Society of Hypertension.[24]

Physical Activity Measurements

A compact, chest-worn accelerometer was used to objectively measure physical activity. The ActiHeart device (CamNtech Ltd., England, UK), designed to capture heart rate variability and movement using a triaxial accelerometer, was worn for a maximum of 7 days and average daily total and activity energy expenditure (total energy expenditure (TEE), activity energy expenditure (AEE)) were calculated. The Global Physical Activity Questionnaire (GPAQ), developed by the WHO,[25] was administered to measure self-reported leisure and work related physical activity and sedentary time.

Blood Sampling and Biochemical Analyses

Participants were asked to fast overnight for at least 8 hours prior to visiting the research facilities. Venous blood samples were collected from the brachial vein branches, using a sterile winged butterfly infusion set and syringe, by a qualified nurse. The samples were prepared according to standardised protocols and stored at -80°C until the time of analysis.

Serum samples were analysed for C-reactive protein, creatinine, total cholesterol, high and low-density lipoprotein cholesterol (HDL-C), glucose and gamma glutamyltransferase (GGT) (Cobas Integra 400plus, Roche, Basel, Switzerland). Serum leptin, adiponectin and renin were determined using Quantikine ELISA kits (R&D systems, Minneapolis, MN USA) and aldosterone was detected using the RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic). Cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) apparatus. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula.[26] Urinary sodium, potassium and chloride were measured by means of ion-selective electrode potentiometry on the Cobas Integra® 400 plus (Roche, Basel, Switzerland) and creatinine concentrations were measured using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland). Daily urinary sodium and potassium excretion (mmol/d) were calculated by multiplying the sodium, potassium and creatinine concentrations (mmol/l) of the 24hr urine by the total 24hr volume of urine (in litres). Daily salt intake was estimated from 24hr urinary sodium excretion by converting sodium in mmol to mg: sodium (mmol) x 23= sodium (mg)[27] and then applying the conversion: 1g salt (sodium chloride) = 390 mg sodium.[27]

Statistical Analysis

Statistica v13.2 (Dell Software, Round Rock, Texas; 2016) was used for data analysis. All continuous variables were checked for normality by visual inspection (QQ plots) and the Kolmogorov-Smirnov test. Variables with non-Gaussian distributions were logarithmically transformed. To evaluate if data should be presented and analysed independently by sex, ethnicity or socio-economic status, we investigated the interactions of these variables on the relationship between BMI, BSA, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, body fat %, lean mass, leptin and adiponectin and 24hr urinary sodium excretion. T-tests, Chi-square tests and Mann-Whitney-U tests (for added salt and sugar) were performed to compare the profiles of black and white men and women. The relationships between obesity-related measures as dependent variables (obesity-related measures) and 24hr urinary sodium excretion (as the main independent variable) were explored using Pearson, partial and multiple regression analyses. In Model 1 we adjusted for TEE only, Model 2 energy intake only and Model 3 both TEE and energy intake. In Model 4 we aimed to replicate the model by Ma *et al,*[1] and adjusted for age, socio-economic score, total energy expenditure, dietary energy intake, self-reported tobacco and alcohol use. Model 5 was adjusted for age, socio-economic score, TEE, energy intake, cotinine, GGT, high density lipoprotein cholesterol, systolic ambulatory blood pressure, glucose, C-reactive protein and aldosterone. The covariates for Model 5 were determined based on bivariate correlations with the dependent variables and 24hr urinary sodium excretion.

Results

As an interaction with sex was present between 24hr urinary sodium and BSA (p=0.019), as well as an interaction with ethnicity between 24hr urinary sodium and neck circumference (p=0.021), data are presented for the total group and additionally, by sex and ethnicity. No further interaction effects were identified (Supplementary Table S1). The general characteristics of the participants are shown in Table 1.

Based on 24hr urine sodium analysis, the total group consumed on average 7.65 g of salt per day with 79.9% consuming above the daily recommended salt intake of 5 g per day. The highest mean salt intakes were observed in white men (mean 8.35 g/day), with 84% of this group consuming above the daily recommended salt intake. While there was no significant difference in 24hr sodium excretion between black and white men, black women had significantly higher sodium excretion than white women (p=0.011). Both white men and women were found to have higher 24hr urinary potassium than their black counterparts (both p<0.01). A higher percentage of black men (94.8%) had a 24hr urinary potassium level below the recommended 90mmol/day,[28] than white men (88.3%; p=0.036) and both black men and women had a higher sodium-to-potassium ratio than whites (p<0.001). In contrast to these findings, analysis of dietary intake data showed that white men and women reported higher mean daily consumption of sodium, as well as energy, total fat, saturated fat and added sugar when compared to black men and women (p<0.001).

In the total population, 46% were classified as overweight or obese (26% overweight, BMI 25-29.9 kg/m^2; 20% obese, BMI >30 kg/m^2). In terms of body composition, white men had higher mean values than black men on all measured variables, including height, weight, BMI, BSA, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, fat mass, lean mass, water and body fat % (all p≤0.017). Leptin concentrations were also higher and adiponectin lower than black men (both p≤0.001). White women showed greater height, lean mass, water and adiponectin, with lower BMI, hip circumference, waist-to-height ratio, body fat %, fat mass, waist circumference, leptin (all $p \leq 0.001$) and renin ($p=0.046$) than black women. When objectively measured energy expenditure was calculated per kilogram bodyweight to adjust for differences in body size, black men presented with higher AEE/kg and TEE/kg ($p<0.001$) than white men. No differences were observed between black and white women.

Comparing self-reported health behaviours, black men reported more tobacco use than white men ($p=0.015$) while white women reported more tobacco use than black women ($p=0.014$), though no significant differences were observed in serum cotinine levels. Self-reported alcohol intake also did not differ between the groups, though the non-specific marker GGT was higher in white, than in black women ($p<0.001$).

Table 1. Characteristics of participants

	Total	Men			Women		
	(N=761)	**Black (N=159)**	**White (N=166)**	***p***	**Black (N=227)**	**White (N=209)**	***p***
Age (years)	24.7 ± 3.03	24.3 ± 0.00	25.3 ± 0.23	0.003	24.7 ± 3.32	24.8 ±2.90	0.78
Socio-economic status				<0.001			<0.001
Low, n (%)	292 (38.4)	101 (63.5)	30 (18.1)		120 (52.9)	41 (19.6)	
Middle, n (%)	197 (25.9)	34 (21.4)	41 (24.7)		65 (28.6)	57 (27.3)	
High, n (%)	272 (35.7)	24 (15.1)	95 (57.2)		42 (18.5)	111 (53.1)	
24hr Urine Analysis							
Sodium (mg/day)	2989 (2828;3104)	3127 (2805;3494)	3265 (3012;3540)	0.58	3035 (2828;3265)	2621 (2414;2851)	0.011
Salt (NaCl g/day)	7.65 (7.24;7.94)	8.04 (7.21;8.96)	8.35 (7.71;9.05)	0.58	7.79 (7.22;8.40)	6.73 (6.17;7.33)	0.011
Above 5g salt/day, n (%)	608 (79.9)	127 (79.9)	140 (84.3)	0.29	182 (80.2)	159 (76.1)	0.30
Potassium (K) (mmol/day)	39.9 (38.2;41.7)	32.9 (19.63;36.6)	49.8 (45.7;54.2)	<0.001	36.4 (33.7;39.4)	42.7 (39.4;46.3)	0.006
Below 90 mmol/day K, n (%)	673 (92.3)	147 (94.8)	143 (88.3)	0.036	195 (92.4)	188 (93.5)	0.66
Sodium-to-Potassium Ratio	3.31 (3.18;3.43)	4.17 (3.86;4.51)	2.85 (2.64;3.08)	<0.001	3.80 (3.60;4.02)	2.69 (2.49;2.90)	<0.001
Creatinine (mmol/day)	10.5 (10.1;10.9)	11.2 (10.3;12.2)	13.5 (12.6;14.4)	0.001	9.23 (8.62;9.89)	9.35 (8.74;10.0)	0.80
Reported Dietary Intake							
Energy Intake (kJ/day)	7486 (7275;7703)	7988 (7499;8508)	9206 (8727;9711)	<0.001	6379 (6049;6728)	7301 (6972;7646)	<0.001
Energy Intake (kcal/day)	1789 (1739;1841)	1909 (1792;2034)	2200 (2086;2321)	<0.001	1525 (1446;1608)	1745 (1666;1827)	<0.001
Salt Intake (mg/day)[a]	1824 (1267;2729)	2132 (1407;2921)	2453 (1767;3330)	0.003	1454 (983;2161)	1731 (1256;2641)	<0.001
Total Fat Intake (g/day)	72.2 ± 35.8	68.2 ± 35.9	95.2 ± 36.0	<0.001	57.6 ± 30.9	74.6 ± 31.5	<0.001
Saturated Fat Intake (g/day)	22.7 ± 12.0	20.5 ± 11.2	30.7 ± 12.2	<0.001	17.0 ± 9.60	24.8 ± 11.0	<0.001
Added Sugar Intake (g/day)[a]	9.50 (0.57;22.1)	2.22 (0.00;14.4)	16.6 (4.80;34.5)	<0.001	5.28 (0.00;15.9)	12.5 (5.22;24.5)	<0.001

Body Composition							
Height (cm)	168 ± 9.39	169 ± 6.91	178 ± 6.09	<0.001	160 ± 5.94	167 ± 6.57	<0.001
Weight (kg)	72.0 ± 17.6	63.9 ±13.9	86.7 ± 15.6	<0.001	70.1 ± 16.2	68.6 ± 16.6	0.34
BMI (kg/m^2)	25.5 ± 5.83	22.1 ± 4.36	27.1 ± 4.97	<0.001	27.5 ± 6.30	24.5 ± 5.54	<0.001
BSA (m^2)	1.82 ± 0.24	1.74 ± 0.20	2.07 ± 0.20	<0.001	1.75 ± 0.21	1.79 ± 0.22	0.11
Waist circumference (cm)	80.6 ± 12.9	76.1 ± 9.64	90.1 ± 12.0	<0.001	80.5 ± 12.4	76.6 ± 12.5	0.001
Neck circumference (cm)	34.3 ± 3.72	35.0 ± 2.06	39.2 ± 2.87	<0.001	32.3 ± 2.39	32.1 ± 2.36	0.48
Hip circumference (cm)	103 ± 12.6	93.5 ±10.3	105 ± 9.27	<0.001	109 ± 13.0	103 ± 11.7	<0.001
Waist-to-hip Ratio	0.78 ± 0.08	0.81 ±0.06	0.85 ± 0.06	<0.001	0.74 ± 0.07	0.74 ± 0.06	0.75
Waist-to-height Ratio	0.48 ± 0.08	0.45 ±0.06	0.50 ± 0.07	<0.001	0.51 ± 0.08	0.46 ± 0.07	<0.001
Body fat (%)	26.2 ± 10.6	17.13 ± 6.23	18.89 ± 6.88	0.017	35.1 ± 8.02	29.2 ± 8.27	<0.001
Fat (kg)	19.5 ± 11.4	11.36 ± 6.57	17.28 ± 9.61	<0.001	25.5 ± 11.4	21.0 ± 11.4	<0.001
Lean mass (kg)	52.9 ± 18.2	54.96 ± 31.1	69.6 ± 7.80	<0.001	44.5 ± 6.21	47.5 ± 6.72	<0.001
Water (L)	36.7 ± 7.82	37.9 ± 5.08	47.7 ± 5.55	<0.001	31.5 ± 4.25	32.9 ± 4.40	<0.001
Biochemical Markers							
Leptin (ng/ml)	13.5 (12.1;15.0)	2.73 (2.12;3.52)	6.89 (5.76;8.24)	<0.001	37.2 (33.3;41.5)	22.5 (19.9;25.4)	<0.001
Adiponectin (ng/ml)	3760 (3540;3995)	3504 (3070;4000)	2583 (2268;2943)	0.001	3922 (3524;4365)	5053 (4556;5604)	<0.001
Total Cholesterol (mmol/L)	4.15 (4.07;4.22)	3.66 (3.53;3.80)	4.72 (4.55;4.89)	<0.001	3.83 (3.71;3.94)	4.46 (4.34;4.59)	<0.001
HDL-C (mmol/L)	1.31 ± 0.38	1.27 ± 0.36	1.14 ± 0.28	<0.001	1.26 ± 0.34	1.52 ± 0.42	<0.001
LDL-C (mmol/L)	2.67 (2.60;2.74)	2.28 (2.15;2.41)	3.22 (3.05;3.40)	<0.001	2.45 (2.32;2.59)	2.78 (2.68;2.89)	<0.001
Triglycerides (mmol/L)	0.82 (0.79;0.85)	0.75 (0.70;0.80)	1.09 (1.00;1.19)	<0.001	0.67 (0.64;0.71)	0.87 (0.81;0.93)	<0.001
C-reactive protein (mg/ml)	1.08 (0.97;1.20)	0.60 (0.48;0.75)	0.86 (0.70;1.04)	0.018	1.93 (1.59;2.36)	1.05 (0.86;1.28)	<0.001
Glucose (mmol/L)	4.68 ± 0.80	4.39 ± 0.88	4.11 ± 0.63	<0.001	4.48 ± 0.76	4.73 ± 0.76	0.001
Renin (pg/ml)	692 (668;716)	822 (769;877)	784 (745;824)	0.25	657 (608;709)	594 (558;632)	0.046
Aldosterone (pg/ml)	69.1 (64.5;74.0)	47.9 (41.3;55.6)	80.7 (72.2;90.2)	<0.001	50.6 (45.2;56.7)	104 (90.85;120)	<0.001

eGFR (ml/min/1.73m^2)	121 ± 22.4	135 ± 16.2	103 ± 16.0	<0.001	136 ± 18.0	108 ± 15.6	<0.001
24hr ABPM							
SBP (mmHg)	116 ± 9.29	119 ± 8.38	124 ± 7.47	<0.001	113 ± 8.18	113 ± 8.62	0.84
DBP (mmHg)	68.7 ± 5.57	69.2 ± 6.09	70.3 ± 5.72	0.10	68.1 ± 5.00	68.0 ± 5.45	0.71
Energy Expenditure							
AEE (kcal/day)	427 ± 214	359 ±165	380 ± 181	0.35	495 ± 236	436 ± 220	0.018
TEE (kcal/day)	2282 ± 463	2220 ± 352	2599 ± 435	<0.001	2215 ± 432	2167 ± 484	0.33
AEE (kcal/kg/day)	6.77 ± 3.05	6.67 ± 2.52	4.37 ± 2.02	<0.001	7.20 ±3.06	6.31 ± 2.98	0.006
TEE (kcal/kg/day)	32.0 ± 4.82	35.2 ± 4.22	30.0 ±4.18	<0.001	32.4 ±4.81	31.6 ± 4.82	0.13
GPAQ							
MVPA (minutes/day)	19.3 (16.8;22.3)	25.7 (18.62;35.6)	20.1 (14.9;27.2)	0.27	14.7 (11.2;19.4)	20.6 (16.2;26.3)	0.074
Sedentary (minutes/day)	353 ± 192	337 ± 201	372 ± 199	0.14	345 ± 189	359 ± 184	0.44
Sedentary-to-MVPA ratio	6.04 (5.36;6.82)	4.78 (3.55;6.43)	7.01 (5.48;8.96)	0.050	5.42 (4.30;6.83)	7.18 (5.85;8.81)	0.073
Health Behaviours							
Self-Reported Smoking, n (%)	165 (21.7)	63 (39.9)	45 (27.1)	0.015	21 (9.25)	36 (17.2)	0.014
Serum cotinine (ng/ml)	3.14 (2.70;3.67)	7.07 (4.68;10.7)	4.65 (3.23;6.71)	0.13	1.87 (1.51;2.31)	2.20 (1.71;2.83)	0.33
Tobacco exposure n (%)	174 (23.4)	58 (37.9)	51 (30.9)	0.19	31 (14.0)	34 (16.6)	0.46
Self-Reported Alcohol, n (%)	416 (55.2)	91 (58.3)	105 (63.3)	0.37	108 (48.4)	112 (53.6)	0.28
γ-glutamyltransferase (U/L)	21.3 (20.3;22.3)	27.8 (25.3;30.6)	25.2 (23.0;27.5)	0.13	22.9 (21.1;24.7)	14.4 (13.4;15.6)	<0.001

Abbreviations: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body mass index; BSA, Body Surface Area; AEE, Activity energy expenditure; TEE, Total energy expenditure; HDL-C High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; MVPA, Moderate and Vigorous Physical Activity combined; K, potassium. Tobacco exposure = Serum cotinine >10ng/ml

Data presented as mean ± SD; or geometric mean 95 C.I.

[a] Data presented as medium (25th and 75th percentiles)

To address our aim, we performed single regression analysis between obesity measurements and 24hr urinary sodium as well as sodium-to-potassium ratio (Supplementary Tables S2 and S3). No consistently significant findings were found between obesity measures and sodium-to-potassium ratio. The 24h urinary sodium correlated positively with all anthropometric measures in the total group ($p \leq 0.008$); and with several measures in the black and white men, namely weight, BMI, BSA, waist circumference, waist-to-hip ratio and waist-to-height ratio (all $p<0.05$), but with no anthropometric measures in black or white women. A negative correlation was present between 24hr urinary sodium and adiponectin only in black women ($p=0.001$). In the single regression analyses, the most prominent and consistent results were observed between BSA and 24hr sodium.

Multivariate-adjusted regression analysis (Table 2) of associations between either BSA or BMI and 24hr urinary sodium showed both BSA and BMI associated positively with 24hr urinary sodium in the total group, black and white men (unadjusted model; all $p \leq 0.032$) but not in women. In Model 1, adjusted for TEE only, BSA associated positively with 24hr urinary sodium in both the total group and white women (all $p \leq 0.037$). Following adjustment for energy intake only (Model 2), BSA remained associated with 24hr urinary sodium in the total group and additionally in black and white men (all $p \leq 0.012$), BMI also associated positively with 24hr urinary sodium in the total group and white men (both $p \leq 0.026$). Model 3, adjusted for both TEE and energy intake, showed an association between BSA and 24hr urinary sodium in the total group only ($p=0.005$). In Model 4, we wanted to replicate analysis of Ma *et al,*[1] who found a positive relationship between BMI and sodium intake when adjusting for age, socio-economic score, TEE, dietary energy intake, self-reported tobacco and alcohol use. We also found a significant positive association but only between BSA and 24hr urinary sodium in the total group and white women (both $p \leq 0.043$). Lastly, we ran a fully adjusted model including systolic blood pressure and a range of additional covariates (age, socio-economic status, cotinine, GGT, aldosterone, C-reactive protein, low and high-density lipoprotein cholesterol and glucose; Model 5). In this model, BSA, but not BMI, remained significantly associated with 24hr urinary sodium in the total group (p=0.039). In all models adjusted for TEE, BMI was no longer significantly associated with 24hr urinary sodium.

Within the total group when using the fully adjusted model, we additionally tested for associations between height, weight, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, fat mass, lean mass, body fat %, adiponectin and leptin with 24hr urinary sodium using the same models and no consistent independent associations were found (Figure 1).

Table 2. Multiple regression analysis with either body surface area or body mass index as dependent variable and 24hr urinary sodium as the main independent variable.

		Body Surface Area					**Body Mass Index**				
		Total[a]	**Black Men**	**White Men**	**Black Women**	**White Women**	**Total[a]**	**Black Men**	**White Men**	**Black Women**	**White Women**
		N=761	**N=159**	**N=166**	**N=227**	**N=209**	**N=761**	**N=159**	**N=166**	**N=227**	**N=209**
Unadjusted Model											
24hr Urinary Sodium	R^2	**0.20**	**0.07**	**0.03**	-0.01	0.01	**0.03**	**0.02**	**0.02**	-0.01	0.01
	β ± SE	**0.14 ± 0.03**	**0.27 ± 0.08**	**0.20 ± 0.08**	0.04 ± 0.07	0.09 ± 0.07	**0.12 ± 0.04**	**0.17 ± 0.08**	**0.17 ± 0.08**	0.05 ± 0.07	0.08 ± 0.07
	p	**<0.001**	**<0.001**	**0.013**	0.55	0.22	**<0.001**	**0.032**	**0.032**	0.45	0.26
Model 1- Adjusted for TEE only											
24hr Urinary Sodium	R^2	**0.63**	0.68	0.37	0.49	**0.63**	0.56	0.58	0.40	0.46	0.63
	β ± SE	**0.07 ± 0.03**	0.05 ± 0.06	0.10 ± 0.07	0.04 ± 0.05	**0.10 ± 0.05**	0.04 ± 0.02	-0.04 ±0.06	0.06 ± 0.07	0.05 ±0.06	0.09 ± 0.05
	p	**0.006**	0.32	0.16	0.43	**0.037**	0.14	0.57	0.39	0.35	0.060
Model 2- Adjusted for energy intake only											
24hr Urinary Sodium	R^2	**0.20**	**0.08**	**0.03**	-0.01	-0.01	**0.03**	0.02	**0.03**	-0.01	0.01
	β ± SE	**0.14 ± 0.03**	**0.25 ± 0.08**	**0.21 ± 0.08**	0.04 ± 0.07	0.09 ± 0.07	**0.12 ± 0.04**	0.16 ± 0.08	**0.19 ± 0.08**	0.05 ± 0.07	0.09 ±0.07
	p	**<0.001**	**0.002**	**0.012**	0.58	0.22	**0.001**	0.053	**0.027**	0.47	0.23
Model 3- Adjusted for TEE and energy intake only											
24hr Urinary Sodium	R^2	**0.64**	0.68	0.36	0.49	0.63	0.56	0.58	0.40	0.46	0.62
	β ± SE	**0.07 ± 0.03**	0.06 ±0.06	0.11 ± 0.08	0.05 ± 0.06	0.10 ± 0.05	0.05 ± 0.03	-0.03 ± 0.06	0.08 ± 0.07	0.06 ± 0.06	0.09 ± 0.05
	p	**0.005**	0.32	0.17	0.36	0.052	0.080	0.64	0.30	0.28	0.063
Model 4[b]- Comparison with Ma *et al*[1]											
24hr Urinary Sodium	R^2	**0.64**	0.68	0.37	0.48	**0.63**	0.56	0.60	0.41	0.46	0.62
	β ± SE	**0.08 ± 0.03**	0.07 ± 0.06	0.11 ± 0.08	0.05 ± 0.06	**0.10 ± 0.05**	0.05 ± 0.03	-0.01 ± 0.07	0.08 ± 0.07	0.07 ± 0.06	0.09 ± 0.05
	p	**0.004**	0.24	0.15	0.35	**0.044**	0.065	0.87	0.28	0.24	0.087
Model 5[c]- Fully Adjusted											
24hr Urinary Sodium	R^2	**0.72**	0.67	0.47	0.61	0.69	0.68	0.64	0.67	0.60	0.70
	β ± SE	**0.05 ± 0.02**	0.04 ± 0.07	0.09 ± 0.07	0.05 ± 0.06	0.04 ± 0.05	0.02 ± 0.03	-0.04 ± 0.08	0.07 ± 0.06	0.06 ± 0.06	0.03 ± 0.05
	p	**0.039**	0.52	0.21	0.35	0.38	0.38	0.58	0.21	0.29	0.52

[a]All adjusted models in the total group also adjusted for sex and ethnicity
[b]Adjusted for age, socio-economic score, total energy expenditure, dietary energy intake, self-reported tobacco and alcohol use.[1]
[c] Adjusted for age, socio-economic score, total energy expenditure, dietary energy intake, cotinine, γ-glutamyltransferase , high density lipoprotein cholesterol, systolic ambulatory blood pressure, glucose, C-Reactive protein, aldosterone.

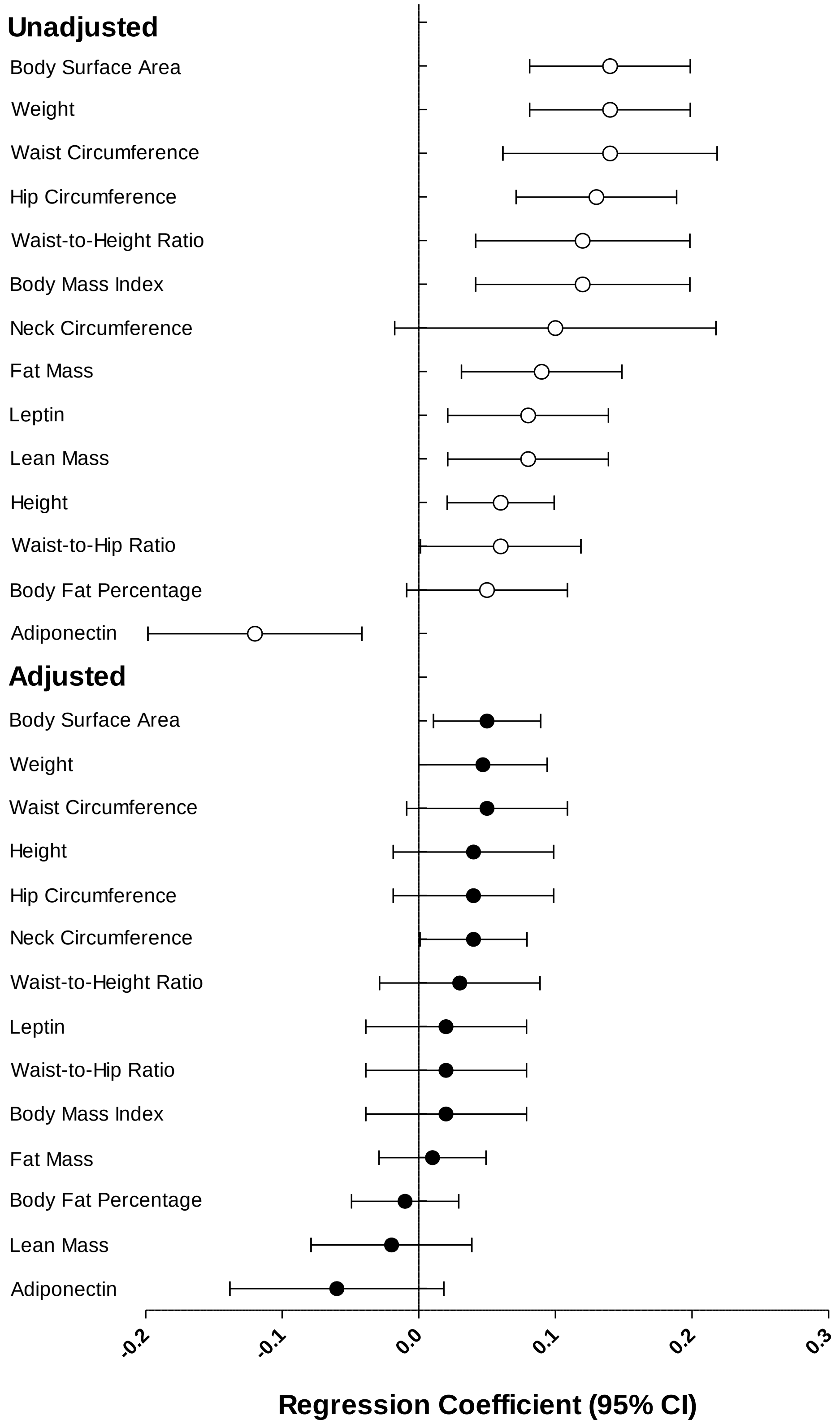

Figure 1: Regression coefficients for the relationship between obesity-related measures and 24hr urinary sodium in unadjusted and adjusted models

Adjusted for: age, socio-economic score, total energy expenditure, dietary energy intake, cotinine, gamma glutamyltransferase, high density lipoprotein cholesterol, systolic ambulatory blood pressure, glucose, C-reactive protein, aldosterone.

Discussion

Our aim was to evaluate if objectively measured sodium intake is associated with obesity in a large, young, healthy adult cohort. We included a comprehensive range of body composition measures and determined the associations thereof with 24hr urinary sodium. A significant positive relationship between 24hr urinary sodium and BMI (as well as other traditional obesity measurements) was apparent in unadjusted models, but was no longer observed after controlling for energy expenditure. However, the relationship between BSA and sodium intake was robust. This link was independent of energy intake, energy expenditure, age, socio-economic status, self-reported tobacco and alcohol use plus several, potentially important biochemical confounders.

Previous findings on the relationship between sodium and obesity, including a study by Ma *et al*[1] (replicated in our Model 4) showed a positive association between BMI and sodium intake, while BSA was not considered.[1, 3-5] In our analysis replicating this study,[1] the link between sodium and BMI reached only borderline significance but disappeared in all models where we adjusted for objectively measured energy expenditure. BSA and sodium intake, however, remained associated even after adjustment for all covariates in the final model.

Despite the known limitations in the accurate estimation of BSA using different formulae in different individuals (obese, tall, short),[29, 30] the question could rather be posed whether BSA is a better reflection of obesity or of total skin area? This may be particularly salient as none of the more traditional measures of obesity (height, weight, BMI, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, fat mass, lean mass, body fat %, adiponectin and leptin) showed an independent association with sodium intake. Further support is found for the hypothesis that BSA may reflect skin area, as it is often used to calculate the degree of injury and make medical decisions on the treatment of patients with burn wounds.[31]

If BSA is indeed a marker for skin area, our findings may highlight the recent reports from the research group of Jens Titze that found sodium to be stored in the skin.[32-34] During sodium loading, skin extracellular matrix molecules are modified allowing for a higher sodium storage capacity.[35] Similarly during long-term low sodium diets the sodium in the skin is released,[36] suggesting that the skin acts as a sodium storage facility. It was also found that tissue sodium storage was closely linked to essential hypertension.[32] As yet, it is unclear if a greater BSA indicates a larger skin surface area available for greater sodium storage and further research is required to understand this relationship.

Consideration of several potential confounders on the relationship between sodium and obesity is important, which is an aspect not fully taken into account in previous studies. For example, tobacco use is known to have a negative association with obesity,[37] and may also be related to poor diet quality and increased sodium intake.[38, 39] The inclusion of serum cotinine as a marker of tobacco exposure in our final model is of particular importance with one quarter of our study population classified as tobacco-exposed (serum cotinine >10ng/ml).[40] Additionally aldosterone may be related to obesity independent of salt intake.[41] Yet, our finding on a positive link between 24hr urinary sodium and BSA was independent of these known confounders.

Our study results should be interpreted within the framework of the strengths and limitations. This study was performed using a bi-ethnic group of young, healthy men and women. Participants were screened prior to participation using strict exclusion criteria for conditions that may affect the results. In terms of strengths, the absence of pre-existing chronic disease afforded us the opportunity to investigate the physiology behind this interaction in adults without influence from pathology. A further strength of our study is the inclusion of multiple potential confounders such as aldosterone – not considered in other studies.[1-5] The large bi-ethnic cohort used in this study allowed us to further investigate the relationship between objectively measured sodium and obesity in both a white and black population, which are reported to differ in frequency of salt sensitivity,[42] as well as in body composition and diet.[43,44] However, within our study we did not expect to find differences in the association between sodium intake and obesity by sex or ethnicity as the initial interaction effects found were minimal. In terms of limitations, the potential for under-reporting when using self-reported dietary intake data is well known.[45] While methods are available to correct for this misreporting, others have suggested this correction is unnecessary when studies include objective energy expenditure measurement.[46] A further limitation is the cross-sectional study design, precluding the assessment of cause and effect. This population will have longitudinal data to address this research question in future. Additionally, the study involves only young adults. However, with almost half of the study cohort overweight or obese and 4 out of 5 eating too much salt, it could be argued that targeting salt and obesity reduction strategies toward young adults' food choices, where interventions may still reduce disease risk, could yield the greatest potential benefit.

In conclusion, in young healthy adults we found a consistent and robust positive relationship between BSA and 24hr urinary sodium, independent of energy intake, energy expenditure and numerous other potential confounders. The relevance of this finding in terms of obesity remains unclear as sodium intake was not independently associated with any of the traditional obesity markers (height, weight, waist circumference, hip circumference, neck circumference, waist-to-hip ratio, waist-to-height ratio, fat mass, lean mass, body fat %, adiponectin and leptin) in the total group. Further studies are needed to investigate the association between body surface area, sodium intake and skin sodium storage.

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Table S1. Interaction Terms

	24hr Urinary sodium per day		
	Ethnicity	**Sex**	**SES**
	p	*p*	*p*
Body Mass Index	0.33	0.49	0.074
Body Surface Area	0.11	**0.019**	0.070
Lean Mass Percentage	0.16	0.083	0.54
Body fat percentage	0.61	0.57	0.19
Waist Circumference	0.26	0.26	0.21
Neck Circumference	**0.021**	0.21	0.074
Hip Circumference	0.63	0.27	0.15
Waist-to-hip ratio	0.14	0.44	0.50
Waist-to-height ratio	0.53	0.65	0.25
Leptin	0.71	0.25	0.65
Adiponectin	0.85	0.084	0.45

Interaction terms p-values calculated with multiple regression analyses.

Table S2: Pearson correlations between 24hr urinary sodium per day, sodium potassium ratio and markers of obesity.

	Anthropometric Measures									Bioelectrical Impedance			
	Weight (kg)	Height (cm)	BMI (kg/m^2)	BSA (m^2)	WC (cm)	HC (cm)	NC (cm)	Waist : Hip Ratio	Waist : Height Ratio	Lean Mass (kg)	Fat (kg)	Body Fat %	Water (L)
Total (N=761)													
24hr Na/ day (mg)	**r =0.144**	**r =0.095**	**r =0.105**	**r =0.149**	**r =0.144**	**r =0.096**	**r =0.152**	**r =0.114**	**r =0.111**	**r =0.109**	r =0.472	r =-0.015	**r =0.161**
	p <0.001	**p =0.008**	**p =0.004**	**p <0.001**	**p <0.001**	**p =0.008**	**p <0.001**	**p =0.002**	**p =0.002**	**p =0.003**	p =0.20	p =0.69	**p <0.001**
Na-to-K Ratio	**r =-0.092**	**r =-0.151**	r =-0.021	**r =-0.114**	r =-0.048	r =-0.064	r =-0.065	r =0.012	r =0.007	r =-0.023	r =-0.020	r =0.008	**r =-0.092**
	p =0.012	**p <0.001**	p =0.57	**p =0.003**	p =0.19	p =0.085	p =0.080	p =0.75	p =0.86	p =0.54	p =0.59	p =0.83	**p =0.014**
Black Men (N=159)													
24hr Na/ day (mg)	**r =0.238**	**r =0.200**	**r =0.170**	**r =0.271**	**r =0.251**	**r =0.196**	**r =0.193**	**r =0.134**	**r =0.187**	r =0.079	r =0.110	r =0.004	**r =0.247**
	p =0.003	**p =0.012**	**p =0.032**	**p =0.001**	**p =0.001**	**p =0.013**	**p =0.015**	**p =0.10**	**p =0.018**	p =0.320	p =0.167	p =0.96	**p =0.002**
Na-to-K Ratio	r =-0.054	r =0.106	r =-0.113	r =0.000	r =-0.005	r =-0.113	r =-0.141	r =0.127	r =-0.041	r =0.124	r =-0.089	r =-0.113	r =0.058
	p =0.50	p =0.19	p =0.16	p =1.00	p =0.95	p =0.16	p =0.080	p =0.13	p =0.61	p =0.12	p =0.27	p =0.16	p =0.47
White Men (N=166)													
24hr Na/ day (mg)	**r =0.190**	r =0.039	**r =0.167**	**r =0.203**	r =0.135	**r =0.193**	**r =0.156**	r =0.050	r =0.118	**r =0.247**	r =0.134	r =0.103	**r =0.259**
	p =0.014	p =0.62	**p =0.032**	**p =0.013**	p =0.084	**p =0.013**	**p =0.045**	p =0.54	p =0.13	**p =0.002**	p =0.089	p =0.19	**p =0.001**
Na-to-K Ratio	r =-0.004	r =-0.071	r =0.012	r =-0.028	r =-0.015	r =-0.002	r =0.040	r =-0.031	r =-0.002	r =0.021	r =0.006	r =0.009	r =0.021
	p =0.96	p =0.37	p =0.88	p =0.74	p =0.85	p =0.98	p =0.62	p =0.71	p =0.99	p =0.80	p =0.94	p =0.91	p =0.80
Black Women (N=227)													
24hr Na/ day (mg)	r =0.046	r =-0.006	r =0.050	r =0.040	r =0.070	r =0.048	r =0.101	r =0.031	r =0.068	r =0.053	r =0.038	r =0.024	r =0.053
	p =0.49	p =0.93	p =0.45	p =0.55	p =0.29	p =0.48	p =0.13	p =0.64	p =0.31	p =0.43	p =0.57	p =0.71	p =0.43
Na-to-K Ratio	r =-0.000	r =-0.160	r =0.053	r =-0.034	r =0.014	r =0.004	r =0.014	r =0.007	r =0.050	r =-0.026	r =0.001	r =-0.017	r =-0.008
	p =0.99	p =0.020	p =0.45	p =0.62	p =0.84	p =0.96	p =0.84	p =0.92	p =0.47	p =0.71	p =0.98	p =0.81	p =0.91
White Women (N=209)													
24hr Na/ day (mg)	r =0.106	r =0.103	r =0.079	r =0.089	r =0.084	r =0.082	r =0.075	r =0.060	r =0.059	r =0.102	r =0.072	r =0.044	r =0.101
	p =0.13	p =0.14	p =0.26	p =0.22	p =0.23	p =0.24	p =0.28	p =0.41	p =0.40	p =0.15	p =0.31	p =0.53	p =0.15
Na-to-K Ratio	r =-0.004	r =-0.085	r =0.021	r =-0.015	r =-0.010	r =0.013	r =-0.083	r =-0.015	r =0.008	r =-0.113	r =0.051	r =0.095	r =-0.125
	p =0.95	p =0.23	p =0.77	p =0.84	p =0.89	p =0.86	p =0.24	p =0.84	p =0.91	p =0.11	p =0.48	p =0.19	p =0.082

Abbreviations: WC, Waist circumference; HC, Hip circumference; NC, Neck circumference;

Table S3: Pearson correlations between 24hr Urinary sodium per day, sodium potassium ratio and biochemical markers of obesity.

	Biochemical Measures	
	Leptin (ng/ml)	Adiponectin (ng/ml)
Total (N=761)		
24hr Na/ day (mg)	r =0.015 p =0.70	r =-0.141 p =0.70
Na-to-K Ratio	r =-0.035 p =0.38	r =0.002 p =0.97
Black Men (N=159)		
24hr Na/ day (mg)	r =0.123 p =0.17	r =-0.034 p =0.70
Na-to-K Ratio	r =-0.071 p =0.44	**r =0.176** **p =0.043**
White Men (N=166)		
24hr Na/ day (mg)	r =0.134 p =0.11	r =-0.052 p =0.053
Na-to-K Ratio	r =0.090 p =0.29	r =-0.045 p =0.059
Black Women (N=227)		
24hr Na/ day (mg)	r =0.019 p =0.79	**r =-0.225** **p =0.001**
Na-to-K Ratio	r =-0.041 p =0.58	r =-0.107 p =0.14
White Women (N=209)		
24hr Na/ day (mg)	r =0.087 p =0.23	r =-0.097 p =0.18
Na-to-K Ratio	r =0.079 p =0.29	r =0.074 p =0.32

Chapter 4

Final Remarks and Recommendations for Future Studies

Introduction

It is well known that there are a number of contributors to the development of obesity such as a sedentary lifestyle,[1] stress,[2] and in some cases also genetic abnormalities.[3] However, the most prominent and well-known factor is an unhealthy diet.[4] Recent observations in the field of obesity research revealed a somewhat unexpected specific dietary contributor. A number of recent studies have suggested that increased dietary sodium intake may play a role in obesity. However, it is unclear if this relationship is dependent or independent of energy intake and expenditure.[5-12] The possibility of a relationship between sodium and obesity is of great concern in terms of public health as both are independent risk factors for the development of hypertension and cardiovascular disease. Hypertension is one of the leading global risk factors for mortality and is responsible for 13% of deaths worldwide.[13, 14] Globally 51% of strokes and 45% of ischemic heart disease deaths can be linked to high systolic blood pressure.[13]

A link between sodium and obesity suggests a further risk associated with a diet high in sodium, since obesity in itself contributes significantly to non-communicable diseases such as diabetes, cardiovascular disease and cancer.[15] Also noteworthy is that recent research has shown that sodium is stored in the skin and is linked to essential hypertension.[16] Within this dissertation these different elements relating to sodium and obesity were included and the findings bring attention to the matter of skin surface area and its potential relationship to sodium.[16, 17]

Interpretations and Summary of Key Findings

Hypothesis 1: There will be positive independent correlations between estimated 24hr urinary sodium excretion and body mass index, waist circumference, hip circumference, neck circumference, waist-to-height ratio, waist-to-hip ratio, body fat %, body surface area and leptin; as well as negative correlations with adiponectin and lean mass %. A consistent and robust positive independent relationship was found between body surface area (BSA) and 24hr urinary sodium. However, this relationship was not consistently present with any of the other markers of body composition. I therefore partially accept my hypothesis. With this said, a number of the markers of body composition were borderline-significant in fully adjusted models, such as body weight (p=0.0567). To the best of my knowledge there is no literature available on the relationship between BSA and sodium. However, numerous studies have suggested a relationship between other markers of body composition such as body mass index (BMI) – although the nature of this relationship remains a matter of debate.[5-12] Our findings indicate that, despite the relationship between dietary sodium and obesity present in other studies, in our young black and white adult South African population, after adjustment for additional confounders not considered in earlier studies, this relationship did not hold true. Additionally, in all models adjusted for objective total energy expenditure (TEE), an independent positive relationship between BMI and 24hr urinary sodium was not present, thereby suggesting a strong influence by TEE. TEE refers to the total amount of energy that is expended on a given day and is composed of resting energy expenditure, active energy expenditure and dietary induced thermogenesis.

While BSA is a valid measure of body composition, it is unclear as to whether it is a good measure of obesity. There is little available information on whether BSA can be used as a predictor or estimate of obesity. It is known that calculations of BSA using a height and weight formula result in underprediction of BSA in obese patients.[18] Additionally BSA may be inaccurate in subjects that are either very tall or very short.[19]

We found that BSA was positively and independently associated with 24hr sodium for every formula used, when using the Mosteller equation. With this said Sardinha *et al* (2006) suggested that BSA calculations are critical in the determination of body fat and a more accurate calculation is needed for both obese and non-obese subjects.[20] Therefore it is unclear whether the above mentioned finding provides information on the relationship between dietary sodium and obesity or adiposity, or rather the relationship between dietary sodium, body composition and sodium storage by the skin.

To further clarify the BSA-sodium link, we performed multiple regression analysis using different formulae for BSA to determine whether it has any effect on the relationship between BSA and 24hr urinary sodium (Table 1). We can deduct from the five different formulas used in Table 1 that the link with BSA remains robust, independent of which formula is used.

Table 1: Multiple regression analysis with varying body surface area formula as dependent variable and 24hr urinary sodium as independent variable.

		Body Surface Area Formulae				
		Mosteller[b]	**Du Bois[c]**	**Shuter and Aslani[d]**	**Haycock[e]**	**Gehan and George[f]**
Fully Adjusted Model[a]						
24 hr Urinary Sodium	R^2	**0.72**	**0.71**	**0.71**	**0.72**	**0.72**
	β ± SE	**0.05 ± 0.02**	**0.05 ± 0.03**	**0.05 ± 0.03**	**0.05 ± 0.02**	**0.05 ± 0.02**
	p	**0.039**	**0.032**	**0.032**	**0.037**	**0.036**

[a]Adjusted for: age, socio-economic score, total energy expenditure, dietary energy intake, cotinine, γ-glutamyltransferase , high density lipoprotein cholesterol, systolic ambulatory blood pressure, glucose, C-Reactive protein, aldosterone.

[b] Mosteller Formula[21]- BSA = $\sqrt{\frac{W*H}{3600}}$ = 0.016667 x $W^{0.5}$ x $H^{0.725}$

[c] Du Bois Formula[22]- BSA = 0.00184 x $W^{0.425}$ x $H^{0.725}$

[d] Shuter and Aslani[23]- BSA = 0.00949 x $W^{0.441}$ x $H^{0.655}$

[e] Haycock[24]- BSA = 0.02465 x $W^{0.5378}$ x $H^{0.3964}$

[f] Gehan and George[25]- BSA = 0.235 x $W^{0.51456}$ x $H^{0.42246}$

Hypothesis 2: The above correlations will be more prominent in black than white participants and more prominent in men than women. Based on interaction analysis the above hypothesis was not expected to hold true. Interaction terms were only present for the relationship between neck circumference and 24hr urinary sodium for ethnicity and for the relationship between BSA and 24hr urinary sodium for sex, indicating an overall weak influence of both sex and ethnicity. In the interest of thoroughness, we did split by sex and ethnicity. We found the above relationship between BSA and 24hr urinary sodium to be consistent only in the total group. When adjustments were applied, no other relationships between any of the additional markers of obesity were consistently present in either black or white men or women. I therefore reject my hypothesis.

Limitations and Confounding Factors

Due to this study being performed in a cross-sectional manner, it does not provide us with the opportunity to determine cause and effect but only that a relationship exists between BSA and 24hr urinary sodium. Additionally, it remains unclear what specific mechanisms are at play that result in the above-mentioned relationship as well as what role is played by skin or skeletal muscle sodium storage. This study was performed using apparently healthy young adults which afforded the opportunity to examine the underlying physiology without interference from pre-existing disease. This did however limit the ability to explore the relationship between body composition and 24hr urinary sodium in individuals with cardiovascular or renal disease that may have a direct effect on sodium handling.

In addition to the above, a more precise method, such as magnetic resonance imaging (MRI) scanning which provides a non-invasive method for accurate measurement of adipose tissue,[26] could have been used in the determination of adiposity. MRI scanning could have additionally been used to determine salt storage in the skin as was demonstrated by Jens Titze and his research group.[17] Also, 7-day urinary collection for the analysis of urinary sodium, may provide more accurate results than a single 24hr urinary collection as urinary sodium levels have been shown to fluctuate rhythmically.[27] A further limitation to this study is the use of dietary data collected using three 24hr dietary recall interviews which is often found to be inaccurate as participants underreport their intake.[28]

Recommendations for Future Studies

- The long-term effects of increased dietary sodium intake on body composition should be evaluated in longitudinal analyses.
- Research on the relationship between body composition and dietary sodium intake should additionally be conducted in the elderly, as well as in diseased populations (such as renal disease or diabetes) to ascertain the nature of the relationship in individuals with decreased renal function.

- Investigation into the specific relationship between sodium and BSA is needed as well as further research on sodium storage in the skin and its effect of sodium handling and body composition is required.
- Studies on the accuracy of BSA as a marker of obesity and skin volume are required.
- A study should be performed using MRI scanning to determine adiposity, skin sodium, as well as 7-day urine collection.

Conclusions and Perspectives

We found a consistent and robust positive relationship between BSA and 24hr urinary sodium in the total population independent of energy intake, expenditure and numerous potential confounders. The significance and nature of this relationship could be of important value in understanding the physiological link between diet, obesity and cardiovascular disease development, although at this point the nature of the relationship remains unclear. Future research is required regarding the relationship between BSA and skin volume, whether increased skin volume results in increased sodium storage as well as the mechanisms at play in the relationship directly between BSA and dietary sodium intake.

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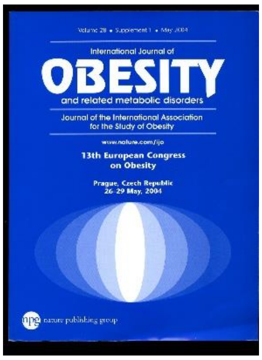
Appendix A

Author instructions

Submission Guidelines: International Journal of Obesity



ARTICLE TYPE SPECIFICATIONS ARTICLE DESCRIPTION	ABSTRACT	WORD LIMIT	TABLES/ FIGURES	REFERENCES
Original Articles Please see 'Preparation of Original Articles' below for further details	Structured abstract: Max 300 words	4,000 words max excluding abstract, references, figures and tables.	Max of 6	Max of 60

Abstract: Original Articles must be prepared with a structured abstract designed to summarise the essential features of the paper in a logical and concise sequence under the following mandatory headings:

- **Background/Objectives:** What was the main question or hypothesis tested?
- **Subjects/Methods:** How many subjects were recruited, how many dropped out? Was the study randomised, case-controlled etc? Interventions/methods used and duration of administration.
- **Results:** Indicate 95% confidence intervals and exact *P* value for effects.
- **Conclusions:** Answer (significant or not) to main question.

Introduction: The Introduction should assume that the reader is knowledgeable in the field and should therefore be as brief as possible but can include a short historical review where desirable.

Materials/Subjects and Methods: This section should contain sufficient detail, so that all experimental procedures can be reproduced, and include references. Methods, however, that have been published in detail elsewhere should not be described in detail. Authors should provide the name of the manufacturer and their location for any specifically named medical equipment and instruments, and all drugs should be identified by their pharmaceutical names, and by their trade name if relevant.

Results and Discussion: The Results section should briefly present the experimental data in text, tables or figures. Tables and figures should not be described extensively in the text, either. The discussion should focus on the interpretation and the significance of the findings with concise objective comments that describe their relation to other work in the area. It should not repeat information in the results. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Acknowledgements: These should be brief, and should include sources of support including sponsorship (e.g. university, charity, commercial organisation) and sources of material (e.g. novel drugs) not available commercially.

References: Only papers directly related to the article should be cited. Exhaustive lists should be avoided. References should follow the Vancouver format. In the text they should appear as numbers starting at one and at the end of the paper they should be listed (double-spaced) in numerical order corresponding to the order of citation in the text. Where a reference is to appear next to a number in the text, for example following an equation, chemical formula or biological acronym, citations should be written as (ref. X) and not as superscript. Example. "detectable levels of endogenous Bcl-2 (ref. 3), as confirmed by western blot"
All authors should be listed for papers with up to six authors; for papers with more than six authors, the first six only should be listed, followed by *et al.* Abstracts and letters must be identified as such. Papers in press may be included in the list of references. Personal communications must be allocated a number and included in the list of references in the usual way or simply referred to in the text; the authors may choose which method to use. In either case authors must obtain permission from the individual concerned to quote his/her unpublished work.
Examples:
Journal article, up to six authors: Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol* 2005; **6**: 353–360.
Journal article, e-pub ahead of print: Bonin M, Pursche S, Bergeman T, Leopold T, Illmer T, Ehninger G *et al*. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. *Bone Marrow Transplant* 2007; e-pub ahead of print 8 January 2007; doi: 10.1038/sj.bmt.1705565
Journal article, in press: Gallardo RL, Juneja HS, Gardner FH. Normal human marrow stromal cells induce clonal growth of human malignant T-lymphoblasts. *Int J Cell Cloning* (in press).
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Abstract: Syrjala KL, Abrams JR, Storer B, Heiman JR. Prospective risk factors for five-year sexuality late effects in men and women after haematopoietic cell transplantation. *Bone Marrow Transplant* 2006; **37**(Suppl 1): S4 (abstract 107).
Correspondence: Caocci G, Pisu S. Overcoming scientific barriers and human prudence [letter]. *Bone Marrow Transplant* 2006; **38**: 829–830.

FHouse Style

- Text should be double spaced with a wide margin.
- All pages and lines are to be numbered.
- Do not make rules thinner than 1pt (0.36mm).
- Spaces, not commas should be used to separate thousands.
- Statistical methods: For normally distributed data, mean (SD) is the preferred summary statistic. Relative risks should be expressed as odds ratios with 95% confidence interval. To compare two methods for measuring a variable the method of Bland & Altman (1986, Lancet 1, 307–310) should be used; for this, calculation of P only is not appropriate.
- Units**:** Use metric units (SI units) as fully as possible. Preferably give measurements of energy in kiloJoules or MegaJoules with kilocalories in parentheses (1 kcal = 4.186kJ). Use % throughout.

Appendix B

Approval from the Health Research Ethics Committee

NORTH-WEST UNIVERSITY ®
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Institutional Research Ethics Regulatory Committee
Tel: +27 18 299 4849
Email : Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)** on **31/05/2017** after being reviewed at the meeting held on **17/05/2017**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Dietary sodium intake and its relationship to adiposity in young black and white adults: The African-PREDICT study
Study Leader/Supervisor: Prof AE Schutte
Student: SH Crouch-27231569

Ethics number: N W U - 0 0 0 5 0 - 1 7 - A 1

Institution	Study Number	Year	Status
NWU	00050	17	A1

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single study
Commencement date: 2017-05-31 **Risk:** Minimal

Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *HREC can be contacted for further information or any report templates via Ethics-HRECApply@nwu.ac.za or 018 299 1206.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis
Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU, ou=Vaal Triangle Campus, email=linda.duplessis@nwu.ac.za, c=ZA
Date: 2017.07.31 13:04:55 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

Appendix C

Turn-it-in Report

SH Crouch Full Project TII Version1

ORIGINALITY REPORT

19%	11%	8%	11%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to North West University Student Paper	9%
2	dspace.nwu.ac.za Internet Source	1%
3	www.science.gov Internet Source	1%
4	Submitted to Nanyang Technological University, Singapore Student Paper	1%
5	"IUNS. 21st International Congress of Nutrition. Buenos Aires, Argentina, October 15-20, 2017: Abstracts", Annals of Nutrition and Metabolism, 2017 Publication	<1%
6	www.ajcn.org Internet Source	<1%
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Appendix D

Certificate of Language Editing

DECLARATION

I, C Vorster (ID: 710924 0034 084), Language editor and Translator and member of the South African Translators' Institute (SATI member number 1003172), herewith declare that I did the language editing of a thesis written by Ms SH Crouch from the North-West University (student number 27231569).

Title of the thesis: Dietary sodium intake and its relationship to adiposity in young black and white adults: The African-PREDICT study

17 November 2017

C Vorster

Date