EFFECT OF IODINE EXPOSURE ON BLOOD PRESSURE AMONG FEMALE CHILDREN AND WOMEN IN KATHONZWENI, MAKUENI, KENYA

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DECLARATION

This research project is my original work and has not been presented for a degree in any other University.

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DEDICATION STATEMENT

I dedicate this thesis first and foremost to God the Almighty and secondly to my husband Mr. Caleb G. Apungu and my two daughters Nicole A. Apungu and Natalie K. Apungu.

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ABBEVIATIONS

AGP	Alpha-1-acid glycoprotein
BMI	Body Mass Index
BP	Blood Pressure
CDC	Centre for Disease Control
CF	Correction Factor
CFR	Glomerular Filtration
COD	Coefficient of Dispersion
COV	Coefficient of Variation
CPHR	Centre for Public Health Research
CRP	C Reactive Protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic Blood Pressure
GLM	General Linear Modelling
HBP	High Blood Pressure
ICCIDD	International Council for Control of Iodine Deficiency Disorders
ID	Iodine Deficiency
IDD	Iodine Deficiency Disorders
IGN	Iodine Global Network
KEMRI	Kenya Medical Research Institute
KS:2009	Kenya Salt Standard 2009
LMIC	Low and Middle-Income Countries

MoH	Ministry of Health
NCDs	Non-Communicable Diseases
NHBPEP	National High Blood Pressure Education Programme
NIH	National Institute of Health
QA	Quality Assurance
QC	Quality Control
RCT	Randomized Controlled Trials
SAC	School Age Children
SACN	Scientific Advisory Committee on Nutrition
SBP	Systolic Blood Pressure
SD	Standard Deviation
SSA	Sub Saharan Africa
T4	Thyroxin
T3	Triiodothyronine
TGP	Total Goitre Prevalence
TSH	Thyroid Stimulating Hormone
UI	Urinary Iodine
UIC	Urinary Iodine Concentration
UNICEF	United Nation Children's Fund
UNaC	Urinary Sodium Concentration
USI	Universal Salt Iodization
WFP	World Food Programme
WHO	World Health Organization
WRA	Women of Reproductive Age

OPERATIONAL DEFINITION OF TERMS

Arm1-(H2L)	Study Group where the sequence starts with high iodine in salt and crossover to low iodine in salt after 3 weeks
Arm2-(L2H)	Study Group where the sequence starts with low iodine in salt and crossover to high iodine in salt after 3 weeks
High iodine	84mg/kg of Potassium Iodate (50mg/iodine within the Kenya Salt Iodization Standards)
Low Iodine	50mg/kg of Potassium Iodate (30mg/iodine within the Kenya Salt Iodization Standards)
Kenya Salt Standard: 2009	Salt iodization 50-84mg/kg potassium Iodate
School Age Children	Children aged between 8 years and 12 years

ABSTRACT

High or raised blood pressure (HBP) is a global public health issue and a major cardiovascular risk factor. While the Kenya Step*Wise* survey of 2015 confirmed that more than half (56%) of Kenyans have never been screened for BP, renewed interests in hypertension in children and adolescents has resulted from the recognition that its presence in adults often has its roots at a younger age. It is also still controversial whether mild thyroid dysfunction affects BP. To understand if iodine status affects blood pressure, A two- *arm* treatment; two periods' double-blind cluster randomized crossover study comparing the effect of low and high iodine in salt (within the iodization regulation levels) in women of reproductive age and school age girls (8-12years). Participants in clusters randomized to one of the two sequence groups: Arm1-(*H2L*) started with high iodine(50mg) in salt and changed to low iodine in salt after 3 weeks) while Arm2-(*L2H*) (started with low iodine (30mg) in salt and changed to high iodine in salt after 3 weeks – without wash out period). 171 women-girl pair participants were assessed for family history of chronic disease, age, level of education and anthropometrics. Urinary iodine, sodium, potassium, lithium and blood pressure were assessed weekly. Data were analysed using SPSS version 20.0. Student unpaired two sample t-test was used to compare the difference in BP levels while differences in the distribution of independent variables (low (30mg) and high (50mg) iodine intake) between groups was determined using Pearson's chi-square or Fisher's exact test for categorical variables. Coefficient of dispersion was used to understand the spread of lithium as a marker of intake while analysis of covariance using univariate General Linear Model was used to estimate the effect of the treatment adjusting for specific covariates identified to be significantly different between the two intervention arm sequences at baseline. In women, the total effect due to high dose iodine in salt was equivalent to -1.1 [95% CI: -4.0 to 1.9]; constant -2.7, (P=0.474) for Systolic Blood Pressure (SBP) and -3.7 [95% CI: -6.0 to -1.3] constant 1.03 (P =0.003) for Diastolic Blood Pressure (DBP). Regression analysis of treatment on BP showed a significant adjusted effect of high dose iodine in salt on DBP (P=0.001) but not SBP (P=0.474). The variability of the difference in SBP and DBP attributable to treatment was 0.4% and 8.1% respectively. In school age girls, the total effect due to high dose iodine in salt was equivalent to -1.68 [95% CI -4.49 to 1.12], (P=0.237) for SBP and -4.48 [95% CI -7.66 to -1.29] (P =0.006) for DBP. Univariate covariance analysis in General Linear Modelling showed statistically significant effect of treatment on DBP f=6.83, (P=0.010) but not for SBP f=1.38, (P=0.242) in the girls. General Linear regression on the net adjusted effect attributable to the treatment showed that the high dose of iodine was negatively associated with both SBP and DBP resulting in a positive role in lowering blood pressure in both girls and women. While salt is the main source of iodine, it is also known that sodium in the edible salt is also responsible for HBP worldwide. These findings highlight an inverse relationship between BP and Iodine intake. High iodine intake decreased BP. There is need for further in-depth research on the iodine and BP linkage and to understand whether restricting iodized salt intake may cause a decrease in iodine intake and could worsen HBP, rather than control it.

CHAPTER ONE: INTRODUCTION

1.1. Background

High or raised blood pressure (BP) also known as hypertension characterized by short-term fluctuations over 24-hour period (Magdás et al., 2015), has been identified as a public health concern and a major risk factor for cardiovascular disease, premature mortality (Hendriks et al., 2012, Sundar et al., 2013) and disability (WHO, 2013a). HBP Contributes to stroke, heart diseases and kidney failure (Muhamad et al., 2014) and has been ranked as a third cause of disability-adjusted life-years lost (Hendriks et al., 2012) and mainly affect health systems in low and middle income countries which are yet to be adequately strengthened (WHO, 2013a). It is also one of the most important and modifiable cardiovascular risk factors (Iqbal, Ahmad, Malik, & Mahmood, 2012). Globally, 80% Cardiovascular Disease (CVD) mortality has been reported to occur in low- and middle-income countries (LMIC) (Hendriks et al., 2012) where hypertension is the most important risk factor for its development.

It has been predicted that almost three quarters of the world's population with hypertension will be living in the developing countries by 2025, (Tibazarwa & Damasceno, 2014). The relationship between BP and cardiovascular risk has no evidence of a threshold, but is recommended to be maintained below 115/75 (Acelajado et al., 2012).

The 7th Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure shows that hypertension is linked to high morbidity. This link shows that an increment of 10mmHg in diastolic BP and 20mmHg in systolic pressure doubles

the risk of cardiovascular events (Narchi et al, 2011). Mild thyroid dysfunction, such as subclinical hypothyroidism and subclinical hyperthyroidism effects on BP remains controversial (Liu et al., 2010). A substantial proportion of the population is affected by thyroid abnormalities where CVD and adverse changes in blood lipids have been found to elevate overt hypothyroidism while increased CVD risk has been linked to subclinical hypothyroidism (Jung et al., 2003).

Elevated BP has been demonstrated in persons with hypothyroidism, where the there is significant plasma volume changes which create a volume dependent mechanism of elevating BP. Studies on the prevalence of hypertension in subjects with hypothyroidism have demonstrated elevated BP values. The possible link between hypothyroidism due to iodine deficiency and diastolic hypertension results from low cardiac output and increased peripheral vascular resistance ((Stabile, Papakatsika, & Kotsis, 2010)

1.2. Problem Statement

Every year, approximately 1 billion people globally suffer from hypertension at same time causing nealy 7.1 million deaths (Moinuddin et al., 2016). Western lifestyle highly associated with urbanization are vindicated as major cuases of Hypertension in Sub-Saharan Africa (SSA) . Early studies in SSA have reported low mean BP not affected by age and prevelance of hypertensition was low. (Hendriks et al., 2012). The Kenya Step*Wise* survey of 2015 confirmed that more 56% of Kenyans had never screened for BP. Only

22.3% previously screened and diagonosed to have elevated BP were on prescription treatment (WHO/MoH, 2015) at the time of the survey.

The recognition that presence of hypertension in adults has its roots at a younger age has renewed interest in hypertension in children and adolescents. Appropriate techniques compared with approved standards should be used to measure accurate BP in children so as to establish a diagnosis. An evaluation of possible primary causes is imperative. In young children, the causes are multiple and a thorough evaluation is critical (Narchi, 2011). Lack of age standardized data makes it difficult to compare results (Hendriks et al., 2012). Currently, Kenya too does not have data on school age children. The current practice in clinical care does not put emphasis on blood pressure measurement in children missing an opportunity for early detection and trajectory assessment. Furthermore, most people do not seek regular blood pressure checks except at point of illness where BP is measured as a routine indicator in seeking treatment for other ailments. As a result, the opportunity for early diagnosis is missed in both children and adults.

Effects of the thyroid hormones on the vasculature and heart result in characteristic CVD changes in thyroid disease, including increased BP (Spitzweg and Reincke, 2010). Data on iodine available for Kenya refers to the report of the National Micronutrient Survey conducted in 2011 (KNMS 2011). This report indicated that despite considerable advances towards the elimination of Iodine Deficinecy Disorders (IDD) in Kenya, there still exist deficiencies as well as excesses in the popupation (KNMS 2011). This study seeks to

understand the interactions or effects of iodine and blood pressure in women and school age girls.

1.3. Purpose of study

Recent Surveys have provided evidence on increase in hypertension especially in the adolescent that has gone undetected.

Since data on hypertension in the rural areas are also limited, the purpose of the present study was to assess the effect of low and high iodine from edible salt on blood pressure in school age girls and women of reproductive age in Makueni.

There is a direct association between BP and risks of several types of CVD. Disease risk and BP associations are continuous and an indication of sub-optimal BP values in the larger populations. To prevent complication resulting from hypertension, early screening and diagnosis is a critical strategy for its control (Sundar et al., 2013). Risks for development of thyroid disorders can be predicted from a measure of median UIC levels despite it not being a direct measure of the thyroid function. (Zimmermann & Boelaert, 2015).

1.4. Objectives of the study

1.4.1. General objective

The overall objective of this study was to compare the effect of low and high iodine from iodized salt on blood pressure levels among female school age children (8-12 years) and women of reproductive age (15-49 years) in Makueni County.

1.4.2. Specific objective

1. To assess the nutritional status of female school age children and women of reproductive age in the Makueni County
2. To compare the effect of low and high iodine intake from iodized salt on blood pressure and urinary sodium and potassium concentrations among female school age children and Women of Reproductive Age in the Makueni County.
3. To determine the time effect on blood pressure changes in female school age children and women of reproductive age in the Makueni County consuming high or low iodine from iodized salt.

1.5. Study hypothesis

Null hypothesis 1: There is no significant effect of iodine intake on blood pressure among female school age children consuming low and high iodine from iodized salt.

Null Hypothesis 2: There is no significant effect of iodine intake on blood pressure among women of reproductive age (WRA) consuming low and high iodine from iodized salt

1.6. Significance of the study

Iodination of salt as a universal strategy to eliminate iodine deficiency disorders (IDD) has been very successful in Kenya. At the same time, there is an increase in the incidences of HBP and, hence, CVD risk, in both rural and urban populations. The study will contribute to the scientific knowledge of the effect of iodine on BP, thus furthering understanding of CVD aetiology.

Findings of this study will add to public health efforts towards understanding that iodine may have a role in prevention of elevated BP. The identification of the inter relations between iodine, its role in the thyroid function and their effects on blood pressure will be useful in contribution on the blood pressure knowledge and more so in the aetiology of BP in school children who most often, do not have their blood pressure measured in regular hospital visits

1.7. Study delimitations

The study, a randomized double-blind crossover trial was carried out among two population groups, women of reproductive age and school age female children to assess the effects of iodine on blood pressure. The study was structured in three phases. Phase one, a pilot study to determine sample size for the interventions; phase two for the preparation of the lithium tagged iodized salt and phase three study implementation that included, sampling households and recruitment of participants and randomization of

households for the intervention, lastly, laboratory analysis and data management and reporting.

1.8. Study limitations

The study targeted a specific population; therefore, the findings cannot be generalised to the whole population. In the region of country.

The study collected reported (not tested) details on medical and family history and signs and symptoms of chronic diseases such hypertension, heart disease and diabetes. Information on personal health habits and patterns such as smoking, alcohol consumption and medication were also collected at baseline.

Loss to follow up

Loss to follow up especially dropout rates that are different between study groups or when the participants who drop out are different from those who do not drop out is common in experimental designs and can be a threat to validity (Schulz & Grimes, 2002) (Dettori, 2011). To address this all participants who were able to complete the study follow up were included in the statistical analysis to avoid creating any bias. In addition, conscious and unconscious bias is controlled through random sequence allocation. However, because this was a community-based study, the allocation was done by sub location.

Carryover and period effects

The other common challenges with these types of experimental designs is the carryover effects where the effects of one treatment crossover to the next treatment period (Velengtas

et al., 2012). This study used iodized salt as the treatment with two iodine levels for the treatment Arms and a crossover after 3 weeks of intervention. Since a washout period was excluded, a cross over effect when participants using salt A changed to Salt B and Vice Versa might be expected. However, in this particular trial, the key exposure factor is the dietary iodine supply, which is known to be reflected in the UIC within 24-48 hours of consumption. While this immediate response may not entirely mitigate against the likelihood of carry-over in an effect on blood pressure, we felt confident that the immediacy of the changes in dietary iodine supply justified the omission of a period for wash-out of the exposure factor.

Naturally, blood pressure fluctuates based on many factors, therefore the period of follow up too may have an effect on the condition under investigations and this may have affected the outcomes. (Sibbald et al., 1998)(Bakker et al., 2009,Welled & Blettner, 2012).

Study Strengths

Tracing intake using lithium as a marker technique

For a study on food intake differences, tracing the intake of participants to understand if they consumed study products improves validity of the study. Pure lithium has been used in trace amounts as a tracer of salt intake due to the body's ability to excrete up to 98% of lithium through urine.

CHAPTER TWO: LITERATURE REVIEW

2.1. Developments in iodine nutrition

Iodine is an important micronutrient needed for the synthesis of thyroid hormones which are essential for the normal functioning of the human body (Menon et al., 2011; Maia et al., 2011 and Zicker and Schoenerr., 2012). Production of the thyroid hormones thyroxine (T4) and triiodothyronine (T3), require iodine. these hormones regulate physiological processes (temperature, growth, body weight and more importantly, neurological development (Tayie & Jourdan, 2010). Iodine deficiency disorders (IDD) result from impaired thyroid hormone production resulting from iodine deficiency. Cognitive impairment is one of the most serious consequences of iodine deficiency (Zimmermann & Boelaert, 2015).

Before 1990, only a handful of countries were iodine sufficient (Iodine Global Network, 2015). Iodine deficiency prevention programmes introduced in the 1990s revolutionised the sustainabe iodine sufficiency in Africa. A resolution to eliminate IDDs globally was endorsed by the World Summit for Children in 1990 and a

strategy of universal salt iodization was promoted within the region through joint efforts by UNICEF, WHO, ICCIDD and all regional authorities.

In Kenya, recognition of chronic iodine deficiency dates back to 1927 when high prevalence of goitre was described among indigents in the central highlands The earliest recorded study on IDD in Kenya dates back close to 80 years. However, endemic iodine

deficiency (ID) in Kenya was only given attention in the 1970's following reports of high prevalence of goitre in the 1960's (KEMRI/UNICEF/MOH, 2004). Following a period of voluntary salt iodization, the Government of Kenya, through the Ministry of Health adopted Universal Salt Iodization (USI) in the prevention and control of IDD for slightly over three decades. During this periods salt iodate levels have been four times revised upwards from 33.7mg/kg in 1970 to 50.5mg/kg in 1973 to 168.5mg/kg in 1989 and finally to 50-84 mg/kg in 2009. The salt iodization levels' revisions were guided by the fact that iodine disorders had been controlled.

According to the Kenya IDD study of 2003/2004, corresponding improvements in urinary iodine (UI) between 1974 and 1994 and reductions of TGP in high risk districts was reported. Based on low urinary iodine (UI) levels that reflect short-term iodine intake inadequacy and high Total Goitre Prevalence (TGP) that reflect inadequate chronic iodine intake, the 1994 national IDD survey indicated that IDD continued to be a public health problem in many parts of the country (KEMRI/UNICEF/MOH, 2004).

Thyroid gland function (or, less often, over-stimulation of the thyroid) are suppressed by both inadequate and excess iodine (Stanbury et al., 2012). This makes the relation between iodine intake and thyroid disorders in populations U-shaped (Zimmermann & Boelaert, 2015). Globally, Iodine was found to be adequate in 69 countiries, more than adequate in 36 countiries, inadequate and excess inadequate in 32 and 11 countries respectively (Andersson et al., 2012).

2.2. Metabolism of iodine

Dietary iodine compounds are absorbed predominantly in the small intestiness in the form of iodide. Despite little dietary significance, Thyroxin can be absorbed intact in small amounts in form of organic iodide compounds (SACN, 2014). Dietary intake and systemic needs for iodine determine the bioavilability and absorptive efficiency of iodine. (SACN, 2014). Adult humans require about 150 to maximally 300 µg for normal thyroid function (Winkler, 2015).

2.3. Thyroid disorders

The thyroid gland has been nicknamed "Gland Central" because it influences almost every organ, tissue, and cell in the body. T4 is created from the iodine from food which is stored in the thyroid gland. If the gland excretes low T4, body temperature, appetite, sleep, weight, patterns, sex drive, mental capacity and emotional characteristics are affected (Fatourechi, 2009) Thyroid disorders are common worldwide (Okosieme, 2006) and identifed as the second most common endocrine condition while the leading is diabetes mellitus (Razvi et al., 2010).

2.3.1. Hyperthyroidism

An expansion of blood volume has been found to be associated with hyperthyroidism, due to its release of rennin and sodium resulting in systolic hypertension. Subclinical hyperthyroidism in the long-term has been found to contributes to CVD changes increasing heart rate irregular cardiac rythms (Watts, 2015). A high cardiac output, increases heart

rate where peripheral vascular resistance is reduced resulting in hyperdynamic circulation as a short term characterisitics of hyperthyroidism.(Biondi, 2012). Populations with optimal (100-299 Ug/L) or excessive iodine (above 300ug/L) intakes respectively tend to have lower prevalence of hyperthyroidsm than populations with mild-to-moderate (levels) iodine deficiency relative to iodine intake (Zimmermann & Boelaert, 2015).

2.3.2. Hypothyroidism

Increase in thyroid hormone in the perinatal stage determines the transition from the foetal to the adult phenotype heart. Moreover, to maintain normal cardiovascular function in adult life, there is need for normal thyroid hormone (Biondi, 2012). High blood pressure is affected by hypothyroidism when the cardiac muscle contracts and contributes to HBP as a result of increased stiffness of blood vessels and peripheral vascular resistance. Subclinical hypothyroidism may be an independent risk factor for the development of coronary artery disease, as well as congestive heart failure in older adults (Watts, 2015). Quality of life is impared when systolic and diastolic functions reduce during exercise and at rest (Biondi, 2012). Severe iodine deficiency in populations increases the prevalence of hypothyroidism when compared to areas of optimum iodine intake. (Zimmermann & Boelaert, 2015).

2.4. Sources of iodine

Iodine is very scarce in many parts of the world. Despite this scarcity the need for iodine-containing hormones remains important (Stanbury et al., 2012) . Iodine content of the soils

determines the iodine content of food grown in it. Seawater is a rich source of iodine as a result of leaching of soils that take away the upper crust of the earth into the sea (Stanbury & Hetzel, 1980). Japanese have access to reef fish which thrive on sea weed becoming a major source of iodine among the Japanese consuming between 2 and 3 mg/day. For most regions, mean native-source iodine intake varies from 20 to 80 µg/day increasing to as high as 500 µg/day in Canada, United States and sections of Europe(Becker et al., 2006) due to adventitious intake from sources such as supplements, sanitizers and food colorants. Effort has been devoted to increasing the iodine intake of populations through iodization after the realization that natural sources of ioidine are low in most parts of the world. Salt iodination has been adopted as an inexpensive source of stable iodine content, and is consumed widely (Pearce, 2008).

The International Council for the Control of Iodine Deficiency Disorders (ICCIDD) (currently Iodine Global Network), World Health Organization (WHO) and UNICEF recommend daily iodine intakes of 90 µg/d for preschool children and 150 µg/d for adults, reaching 250 µg/d for pregnant and lactating women (Ristic-medic et al., 2009). Deficiencies still exist albeit national and international efforts to improve ioidne intake through iodization. At the same time, thyroid disorders remain common worldwide despite successful efforts in many countries (Delange et al., 2001).

2.5. High blood pressure in populations

Hypertension or HBP is the commonest cardiovascular disorder (Perez & Chang, 2014)regarded as a major public health problem worldwide (Chataut et al., 2011 and Manimunda et al., 2011) because of its risk of cardiovascular and kidney disease (Chataut et al., 2011). Also known as a silent threat to health worldwide (Sundar et al., 2013), It has been attributed to cause many conditions including heart failure, stroke, renal disease glaucoma, peripheral vascular diseases among others (Tayie & Jourdan, 2010, Slinger and Villlers, 2009). In 2013, WHO reported that approximately 17 million deaths a year globally are attributed to cardiovascular disease (Wang, Tiwari, & Wang, 2014).

World Health Organization defines normal adult BP as a systolic blood pressure (SBP) of 120 mmHg or below and a diastolic blood pressure (DBP) of 80 mmHg or below (WHO, 2013a). HBP or hypertension on the other hand is a SBP threshold of 140 mmHg or above and DBP of 90mmHg or above and determines overall cardiovascular risk for initiation of treatment (Mensah, 2008). HBP occurs when the arterioles contract (become narrowed) for some reason, and the blood cannot easily pass through them. When this happens, the heart has to pump harder to force the blood through. When the pressure increases above normal and stays elevated, the result is HBP, the conventional risk factor for CVD (Misner, 2008).

Hypertension is a common disease associated with high morbidity and mortality (He et al., 2012). Its high prevalence in urban areas, and its frequent under-diagnosis severity of its complications has an immense economic importance in SSA (Slingers & De Villiers,

2009). More than 90% of patients with haemorrhagic stroke and more than half with ischaemic stroke are found to have HBP, increasing in low and middle-income countries (LMICs) (van de Vijver et al., 2013).

Research findings show that hypertensive individuals are currently unaware of their condition (Adeloye & Basquill, 2014). In Kenya, for example, according to the Step *Wise* Survey 2015, raised BP (defined as having SBP ≥140 mmHg and/or DBP ≥90 mmHg or on medication for raised BP pressure) was found in 23.8% of the respondents. Eight percent of the Kenyans have severe hypertension (defined as having SBP ≥160 mmHg and/or DBP ≥100 mmHg) and among this group 7% were not currently taking medication (WHO/MoH, 2015).

2.6. High Blood Pressure in Adults

Adult patients with Prehypertension (120– 139/80–89mmHg) have an increased risk of cardiovascular morbidity and mortality compared with patients who have normal BP (<120/80mmHg) (Zhang & Li, 2011). It's important to have accurate, reproducible BP measurement for comparisons and correct BP classification (Pickering et al., 2005). Opportunity to identify increased risk for kidney disease and vascular events is missed with incorrect classification of hypertensive patient as normotensive. Classification of normal BP as hypertensive results in unnecessary worry, time costs, medical costs and exposure to treatment-related harm (Frese et al., 2011).

2.7. Blood Pressure in Children

There is growing evidence that children and adolescents with mild BP elevations are much more common than it was thought in the past (Lurbe et al., 2009). HBP prevalence among children in western countries range from 7% to 19%, while limited data on children in developing countries is available . Consequently, prevention, detection and management of elevated BP at an early age may be an important means for limiting the disease burden due to hypertension (Abolfotouh et al., 2011). It is apparent that primary hypertension is detectable in the young and occurs commonly and long-term health risks substantial. Prediciton of essential adult hypertention can be predicted from childhood BP values (Merhi *et al*., 2011).

Hypertension, also known as a silent threat to the health of people all over the world, is commnly associated with morbidity and mortality. Unless specifically looked for during childhood, hypertension often goes undetected despite having its origin in childhood. To prevent complications of hypertension, early detection including identification of the precipitating or aggravating factors is important (Sundar et al., 2013). BP in children and adolescents is based on height, sex, age and reference tables available for interpretation. However BP is often overlooked in children and adolescents. The definitions of prehypertension and hypertension in children and adolescents are published in the National High Blood Pressure Education Program (NHBPEP).

2.8. Iodine status and blood pressure

Baroreflex function and autonomic control of arterial pressure and heart rate are inflenced by the thyroid status, where any thyroid status changes are associated with changes in the vascular and cardiac functions and in autonomic regulation of the cardiovascular system (Foley et al., 2011). Numerous experimental and clinical studies have demonstrated the relationship between thyroid hormone levels and the cardiovascular system. (Biondi, 2012). Differences in normal thyroid function may be associated with differences in future BP (Burki, 2013).

Hypertension can also occur in parasympathetic individuals who have subclinical or overt hypothyroidism as well as adrenal insufficiency (Watts, 2015). Sympathetic dominance is associated with an increase in thyroid and adrenal activity. Normally it is logical to assume that, HBP would be associated with an increased metabolic rate as seen in sympathetic dominant individuals(Watts, 2015).

Both hypo and hyperthyroidism are associated with exercise intolerance. Heart rate (HR), BP, and skeletal muscle blood flow responses to dynamic exercise suggest hypothyroid animals exhibit blunted activation of the sympathetic nervous system during dynamic exercise and an exaggerated sympathetic response in hyperthyroidism (Foley et al., 2011). Hypothyroidism is associated with a decreased cardiac output, cardiac contractility, left ventricular compliance, as well as an increased level of total peripheral vascular resistance and risks of hypertension and atherosclerosis (Kim et al., 2014).

2.9. High blood pressure risk factors

2.9.1. Non-modifiable risk factors (genetics, sex and age)

Factors that affect BP include: sex, age, and body maturation. Variations are also observed between populations according to ethnic and environmental factors (Merhi et al., 2011).

2.9.1.1. Genetics

Genetic factors account for 30% to 50% of inter-individual variability in BP. There is a possibility that menopause might provide the environmental trigger for the expression of certain genetic susceptibilities and a further understanding hypertension is likely a polygenetic disorder. Polymorphisms have been shown to affect a number of pathways involved in hypertension (Coylewrightet al., 2008).

2.9.1.2. Sex

In Africa, hypertension has predominantly affected more males than females. However, in a few countries there were higher levels of prevalence in women than men as evidenced by differences in Algeria 31.6% vs. 25.7% in 2003, Botswana 37.0 % vs. 28.8 % in 2006 and Mali 25.8% vs. 16.6% in 2007, for women and men, respectively (Van de Vijver et al., 2013).

2.9.1.3. Age

Increase in BP with age was first reported by Mahomed FA in 1879. In most industrialized countries hypertension affects the majority of the population over the age of 55 years and increases with age (Kosugi et al., 2009). Although not common in all societies, presumably, the pathogenesis of age-related hypertension is multifactorial. Loss of vascular pliability

due to stiffening of the arteries that can manifest as isolated systolic hypertension with widened pulse pressure is a likely mechanism in this hypertension pathogenesis. Decrease in glomerular filtration rate (GFR) coupled with progressive impairment in sodium excretion is associated with aging. (Kosugi et al., 2009).

2.9.2. Modifiable Risk Factors

2.9.2.1. Lifestyle (dietary, physical activity, smoking, dyslipidaemia, weight gain/obesity)

Hypertension has a stronger association and causal link with five particular behaviours: obesity, unhealthy diets (insufficient fruit and vegetable consumption and high salt intake) physical inactivity, tobacco use and excessive use of alcohol. Generally hypertension is associated with environmental and lifestyle factors rather than with genetics (Van de Vijver et al., 2013).

For the prevention of high BP, it is critical for all persons to adopt healthy lifestyles which is an indispensable component of hypertension management. For most overweight persons, a weight loss of as little as 10 lbs.' (4.5kg) reduces BP and/or prevents hypertension, although it is ideal to maintain normal body weight (Harsha & Bray, 2008). BP is also benefited by adoption of the Dietary Approaches to Stop Hypertension (DASH) eating plan which is a diet rich in fruits, vegetables, and low fat dairy products with a reduced content of dietary cholesterol as well as saturated and total fat (modification of whole diet) (Reddy & Katan, 2004, Iqubal et al., 2015).

Dietary interventions with reduced sodium and increased potassium, have demonstrated their ability to reduce blood pressure in humans. For example, the Dietary Approaches to Stop Hypertension (DASH) diet, a U.S.-based multicentre randomized controlled trial (RCT), showed that a high-potassium and high-calcium dietary intervention was associated with significantly reduced mean BP at low, intermediate, and high sodium intakes compared with the control (Perez & Chang, 2014). Many researches show that excess salt intake affects BP (Wang et al., 2014).

2.9.1.2. Weight gain, Obesity and hypertension

Primary hypertension is strongly correlated with overweight and obesity in children. Children who were breastfed have a reduced risk of hypertension while family history of hypertension or CVD, male sex, and maternal smoking during pregnancy are additional risk factors for hypertension (Riley & Bluhm, 2012). Obesity and especially visceral obesity is the main determinant of BP in the general population (Bouchi et al., 2016). Prevalence of elevated BP or hypertension with increasing obesity has progressively increased as shown by many epidemiological studies. (Chan & Woo, 2010). Weight gain is almost invariably associated with increased BP (Nagori & Muley, 2015).

The World Health Organization (WHO) defines "overweight" in adults as a BMI (Body Mass Index) equal to or more than 25, and "obesity" as a BMI equal to or more than 30. These cut-off points provide a benchmark for individual assessment, but there is evidence

that risk of chronic disease in populations increases progressively from a BMI of 21kg/m^2 (WHO, 2006).

In children, weight varies with sex and age, not only with height. BMI calculated as weight in kilograms divided by height in meters squared can be used to express weight adjusted for height. To account for variability by sex and age, BMI in children is compared with sex- and age-specific reference values (Ogden & Flegal, 2010). The risk for hypertension increases with the increase in BMI (Wang et al., 2014). Furthermore, studies have demonstrated that obesity is related to elevated SBP and DBP, dyslipidaemia and diabetes (Dua et al., 2014).

2.10. Salt and high blood pressure in hypertension

Salt is a common additive in most homes and industries that produce packaged food stuff. Evidence shows that there is a direct relation between dietary sodium intake and elevated BP, and implies that reducing dietary sodium intake lowers BP. A number of meta-analyses of large-scale clinical trials have shown that elevated BP increases the risk of cardiovascular events. Thus, restricted sodium intake is widely recommended for the management of hypertension (Teramoto et al., 2011). Compelling evidence shows that dietary salt intake is the major cause of the rise of BP with increasing age and that a reduction in the salt intake of a population from the current level of ≈ 9–12 g/day in most countries to the recommended level of < 5 g/day will prevent the rise of, and lower existing elevated BP.

A further reduction to 3–4 g/day has a greater effect and there needs to be ongoing consideration of lower targets for population salt intake (He et al., 2012). Population-based intervention studies have shown that when the salt intake is decreased population-wide, a reduction in population BP follows (He et al 2011). Many researches show that excess salt intake affects BP. This conclusion is proved by plenty of scientific experiments and is recognized worldwide (Wang et al., 2014).

For most of our evolution, humans consumed less than 0.25 g of salt per day. Currently use of salt in food seasoning and especially consumption of highly salted processed foods, has increased salt intake (Frisoli et al ., 2012). In individuals without hypertension, dietary changes reduce BP and prevent hypertension, thereby lowering the risk of BP-related complications (Appel, 2009). Depending on the baseline BP pressure and degree of salt intake reduction, SBP can be lowered by 4 to 8 mmHg. A greater decrease in BP is achieved when a reduced salt intake is combined with other lifestyle interventions, such as adherence to Dietary Approaches to Stop Hypertension (Frisoli et al., 2012).

Certain lifestyle modifications, including reduction of sodium intake and weight loss are efficacious in lowering BP, reducing progression of prehypertension to hypertension, and perhaps diminishing long-term risk of CV events (Hedayati et al., 2011). The concept of salt sensitivity versus salt resistance originated from studies demonstrating heterogeneous BP responses to changes in sodium intake. These changes were observed both in hypertensive and normotensive subjects. To date, there is no uniform definition of salt

sensitivity due to great variations in published studies regarding study protocols, techniques, duration, and magnitude of sodium intake and BP changes (Nguyen, et al., 2013).

2.11. Role of sodium and potassium in hypertensions

The relationship between sodium intake and BP changes has been a topic of discussion for decades (Nguyen et al., 2013). One meta-analysis reported that higher potassium consumption was associated with a reduction in BP in hypertensive populations. Two recently published meta-analyses reported that lower sodium intake resulted in lower levels of BP. Several mechanisms exist by which sodium and potassium can influence BP and evidence indicates that the interaction between these nutrients plays a dominant role in the development of primary hypertension (Perez & Chang, 2014).

Potassium is essential for maintenance of electrolyte and acid balance, total body fluid volume , and normal cell function. Potassium intake was very high, often exceeding 200 mmol/day in the pre-agricultural and post-agricultural diets of our human ancestors. These levels have markedly reduced in the modern society. A diet high in processed foods and low in fresh fruits and vegetables is often lacking in potassium (Aburto et al., 2013). An increased potassium intake has been shown to positively affect the vasculature by decreasing BP. This is thought to occur by hyperpolarization of the vascular smooth muscle cells, allowing them to relax and vasodilate (Allman, 2013). The sodium:potassium excretion ratio has become an important marker of increased CVD risk. Many large-scale

studies have utilized the ratio to quantify sodium and potassium intake and determine mortality risk (Allman, 2013) .

2.12. Tracing salt intake: lithium marker technique

Lithium has been used by Sanchez-Castillo et al for tracing salt consumption in order to monitor the domestic use of salt (Leclercq et al, 1990). The choice of lithium marker technique is based on the understanding that lithium is not taken up by the body and that there is none or relatively little interference from other sources (Melse-Boonstra et al., 1998;Melse-Boonstra et al, 1999). The idea of using lithium as a marker/tracer of intake was tested by Sanchez Castello in the late 1980 to early 1990s (Sanchez-Castillo, Branch, & James, 1987) In 1986, Sanchez- Castello validated the use of lithium as a tracer of salt intake to assess sodium excretion in urine. In his study, 5 volunteers were given lithium tagged salt for 44 days. The results showed that 93% of dietary lithium and sodium were excreted in urine an indication of suitability of lithium method as a tracer of salt intake. (Sanchez-castillo et al., 1987a, Sanchez-Castillo et al.,1987b, Sanchez-Castillo et al., 1987c). In the same year Sanchez-Castello et al investigated for its possible use as a marker for identifying the various sources of sodium chloride in the diet(Sanchez-Castillo, Seidell, & James, 1987)).

The Castello Sanchez study found that the rates of penetration into food for both elements were proportional to their concentration in the cooking water despite a sodium/lithium ratio of 50: l. bringing to the conclusion to suggest that lithium carbonate may be a useful marker

for the uptake of NaCl into cooked food. In addition, urinary lithium output is useful for quantifying the amount of sodium derived from specific foods (Sanchez-Castillo et al, 1987). Similar studies by the same author (Sanchez-Castillo and James 1994)(Sanchez-Castillo and James, 1995) were conducted in the subsequent years. Based on these studies' findings, the same methodology was used to include lithium as a marker of intake in the current study to trace intake of study salt.

2.13. Literature summary

Based on the literature reviewed, there is knowledge that thyroid function has a role in the cardiovascular system and BP control is one of the major risk factors for cardiovascular disease. Literature also shows that childhood blood pressure could be a determinant of adulthood hypertention. There seems to be a relationship between blood pressure and iodine status based on literature, however, there still remains gaps on iodine and blood pressure linkage, especially in the Kenyan population, in women of reproductive age and school age girls. The literature also shows that there are other risk factors for hypertension or elevated blood pressure including, genetics, lifestyle changes, increaased weight and as well increased consumption of sodium from salt. This study therefore seeks to assess the relationship between blood pressure and iodine.

CHAPTER THREE: METHODOLOGY

This was a randomized double blind controlled crossover trial to assess the effect of iodine on blood pressure. The study was conducted in three phases.

Phase One: Pilot study for sample size determination undertaken from in August 2012

Phase Two: To develop the lithium tagged low and high iodine salt for the intervention was done from April to September 2013

Phase Three: Field work which included recruitment, participant randomization, intervention and follow-up, laboratory and data analysis staggered between October 2013 and 2015

3.1. Phase One: Pilot study for sample size computation and lithium tagging

3.1.1. Sample size determination

In order to calculate an appropriate sample size for this study, a pilot study was undertaken between May and June 2012 during the implementation of the project '*Enhancing Household Nutritional and Health Outcomes through Innovation for Resilient Farming Systems and Food Security in the Semi-Arid Midlands of Kenya*'. This was an implementation project implemented between 2011 and 2014 by The Kenya Agricultural and Livestock Research Organization (formerly known as the Kenya Agricultural Research Institute), McGill University, Canada, Kenya Medical Research Institute, the Ministry of Agriculture and allied collaborators with support from the Canadian International Food Security Research Fund (CIFSRF).

3.2. Phase Two: Preparation of Lithium Tagged Iodized Household Salt

3.2.1. Premix Preparation

The preparation of lithium tagged sodium chloride (salt) took four and a half months from April to September 2013. A 100% pure Lithium Carbonate (LiCO3) was tested for its purity at the Kenya Bureau of Standards (KEBS) (appendix 3.1). A premix of lithium tagged salt was prepared according to Sanchez Castillo method (Sanchez-Castillo et al., 1987; Melse-Boonstra et al., 1994). 5.0kg of $LiCO_3$ and (20kg) raw non-iodized sodium chloride (NaCL) was molten in a muffle oven at 900^C for 2 hours and, after cooling, milled into the common grain size of edible salt. The fused product was sieved and two randomly collected samples analysed at KEBS to ascertain the ratio of Sodium vs. $LICO_3$ as well as determine the homogeneity of the premix (appendices 3.2 and 3.3). This was considered necessary because it was believed that after fusion of the product, there could have been some losses due to the moisture content of the non-iodized salt.

The premix (24.8kg) was then provided to Kensalt Ltd, one of the salt industries in Mombasa at the Kenyan Coast. The premix was mixed with the 425kg of non-iodized salt (Appendix 3.4 and 3.5). The 450kg lithium spiked salt available for this study was calculated to be sufficient to study salt consumption for 200 families before it was iodized with potassium iodate (KI). In Kenya, salt iodization is mandatory and regulated by an act of parliament. The regulation requires that all the edible and animal salt is iodized with 50mg/kg - 84mg/kg of potassium iodate (KS: 2009 edible salt standard). In line with the

project methodology, during production, the levels of iodine in the product were constantly analysed until homogeneity was achieved.

3.2.2. Iodization and lithium- tagging of the salt

Salt A (50mg KIO_3 (29.7mg iodine) per kg salt) was prepared by mixing 10.677g of potassium iodate into 213.5 kg of salt, while Salt B (84mg KIO_3 (49.6mg iodine) per kg salt) was prepared by mixing 17.937g of potassium iodate in 213.5kg of salt. 213.5kg of each batch (Salt A and Salt B) of the iodized salt mixtures was then mixed with 12.4kg of the premix (lithium spiked NaCL). Thus, 12.396kg of the premix was mixed with (213.5kg) of Salt A - low iodine (29.7mg/kg) while 12.432kg of the premix was added to (213.5 kg) Salt B - high iodine (49.6mg/kg). The targeted end-concentrations in both salt types (A and B) were 1.5mg Li per kg salt. To ensure homogeneity during mixing the premix was milled into coarser particles than the iodized salt. If too many fines would be formed in the premix a homogeneous distribution of lithium would have been difficult to obtain. The spread of lithium was checked by taking samples to KEBS to ascertain the ratio of $LiCO_3$ to sodium. The two samples showed a ratio lithium to sodium of 0.7: 32.6 and 0.7: 33.6 for samples 1 and 2, respectively.

3.2.3. Quality Assurance/Quality Control (QA/QC)

At Kensalt Industries, dosing of iodine is done by spray mixing before salt is graded to ensure all salts are iodized and monitored by the researcher while adhering to the standards and procedures using titration method to test the salt iodine levels (Appendix 3.6). QA

results were counter checked with KEBS in line with the regulations and industry procedures. At the factory as a requirement by the law, the industry has set up at the laboratory which has set its own standards for QA/QC which are monitored by KEBS on a monthly basis for quality assurance and control.

3.3. Phase Three: Study implementation

3.3.1. Research design

This was a 2 arm, double blind, cluster randomized 6 weeks (42 days) cross-over trial to compare the effects of iodine on blood pressure (BP). The study participants in Arm 1 were provided with household salt iodized with high dose iodine (50mg iodine content and Arm 2 with low dose iodine 30mg iodine content both within the Kenyan mandatory potassium iodate iodization levels of (50-84mg/kg) or iodine 30-50mg/kg (Kenya Salt Standard: 2009). The study targeted pairs of women of reproductive age (WRA) 15-49 years and school age girls (SAC) aged 8-12 years living and eating together as a household. Pairs were assigned to either of the study Arms. It's recommended that girls consume 90-120 μg iodine and WRA 150 μg per day

3.3.2. Study Variables

The primary outcome for this study was blood pressure in both school age girls and women of reproductive age. The blood pressure was interpreted based on systolic and

diastolic blood pressure measurements cut offs of 120/80mmHg. BP was the dependent variable while low and high iodine in salt was the independent variable.

3.3.3. Study Area

This study was undertaken in Kathonzweni Sub-county of Makueni County. Makueni is located in the southern part of Eastern Kenya and borders four counties with Kitui to the East, Taita Taveta to the South, and Kajiado to the West and Machakos to the North as shown in Appendix 3.7. It covers an area of 880.7 Km^2. Makueni County has a population of 884,527 (Male – 49%, Female – 51 %) with population density of 110.4 people per Km^2. Kathonzweni was purposively selected because of its rural population to facilitate the implementation of a randomized trial on salt-derived iodine intake with low risk of interference due to minimal access to processed/packaged foods which tend to be high in salt. In addition, an initial working relationship with the community on previous Canadian International Food Security Research Fund (CIFSRF) funded project created a more conducive working environment for this study.

3.3.4. Target population, Inclusion and exclusion criteria

Pairs of female children aged 8-12 years and non-pregnant, non-lactating, 15-49 years old women who volunteered and were willing to participate by consenting were included in the study. Children and adults not within the age range of interest; those who self-reported

known illnesses such as hypertension, diabetes and thyroid conditions excluded from the study.

3.3.5. Sampling Techniques

Two sub locations were randomly selected and allocated either of the two treatments. This was followed by recruitment of the respondents. from randomly selected villages in each sub location Using simple random technique, eligible respondents (School age children aged 8-12 years and a women of reproductive age 15-49 years residing in the same household) were randomly selected from a sampling frame of women of reproductive age and school age children that was developed specific for the study and invited to participate in the trial.

In each household, the criteria were to ensure a school age girl and a woman of reproductive age were recruited if they were eligible. 175 women and 176 children were allocated treatments and followed up. In households which had twin girls, they were both recruited to participate in the study. This is illustrated in figure 3.1.

Small non-overlapping units called Enumeration Areas (EAs) for each of the sub-locations were created within the selected sub-county and used as the sampling units. The EAs designed and created by the Kenya National Bureau of Standards have a household count of between 50 and 149 households with an average of 100 households. The EAs had specific maps showing boundaries, structures total households and population by sex. For a representative sample, the sampling frame was updated based on the national census

undertaken in 2008-2009 (KNBS 2009). A quick household count exercise that involved identifying the boundaries of the EAs using the available maps and then estimating the number of people in each household in the selected EAS was undertaken.

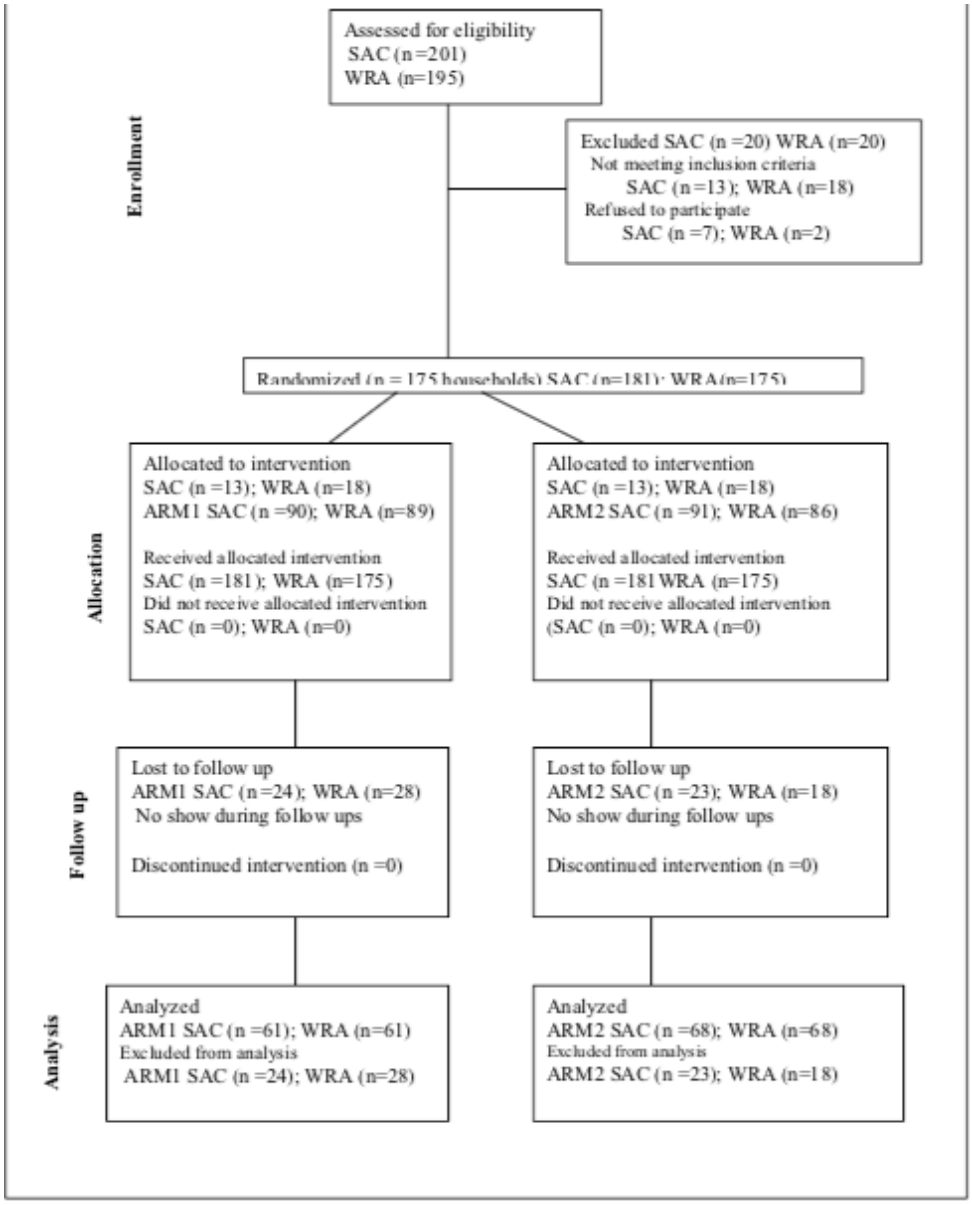

Figure 3.1. CONSORT diagram Recruitment, randomization, placement into study Arm

3.3.5. Sample Size

During the pilot study indicated on page 41, an assumed standard deviation of 12.7 was determined and used in the formula below. A sample size of 144 respondents was needed to detect a 5.0 unit difference in SBP with a 95% level of confidence and 90% power. The following formula was used for sample size computation according the formula

Formula

$$n = \frac{2\{Z_{1-\alpha/2} + Z_{1-\beta}\}^2 \delta^2}{(\mu_1 - \mu_2)^2} \quad n = \frac{2\{1.96 + 1.28\}^2 12.7^2}{(125.3 - 120.3)^2}$$ (Fisher, Laing, & Stoeckel, 1983)

Where:

N = Minimum sample size required per group.

α = Type I error / level of statistical significance (0.05).

β = Type II error (0.10).

$Z_{1-\alpha/2}$ = Standard normal deviate for α (1.96).

$Z_{1-\beta}$- = Standard normal deviate for β (1.28).

Ơ = Estimated standard deviation from the systolic BP score for subjects before intervention (12.7).

μ_1 = Estimated mean BP for patients after 28 days of follow-up with low intervention.

μ_2 = Estimated mean BP for patients after 28 days of follow-up with high intervention.

μ_1-μ_2 = Desired effect size (5 units).

Using the formula, the minimum sample size desired per group to achieve sufficient power for the primary outcome (BP) was 72. Allowing for 10% attrition and 10% refusal, the sample size was adjusted upwards to girl-woman pair per arm (87/ (1 – 0.2)). The total

sample size in both arms was therefore 174 girl-mother pairs. If the study sample size reached 174 (87 per group), the study would achieve 90% power.

A total of 79 female adults (15-49 year) were randomly selected for blood pressure measurements. Three measurements were taken on the right arm in sitting position after resting for 10 minutes and 3 minute intervals between measurements were observed. An Omron M6 Digital Blood pressure machine, (HEM-7211-E8 (v), Omron Healthcare UK Ltd) was used to measure BP. The systolic and diastolic BP (Mean ± SD) was 125.3 ± 12.7 mmHg and 74.5 ± 9.72 mmHg respectively. The variance of BP was calculated from the pilot study in the target area and used in the formula in section 3.3.4 to determine the study sample size of 144 Mother -child pairs

3.3.6. Randomization and Blinding

A random allocation of four-digit numbers to the lithium spiked iodized salts (Salt A-(high iodine) 84mg/kg and Salt B-(low iodine) 50mg/kg potassium iodate), was created and only known by the bio-statistician, independent of the study team. In order to facilitate the process of cross over, and avoid cross contamination of information, the population had been segmented into two clusters by sub-location that were far away from each other. The two sub-locations selected for the intervention were each randomly allocated to a study Arm. Each selected household in the sub location was provided either Salt A or B for 3 weeks. After 3 weeks of intervention a crossover of the salts across the sub-locations was done (figure 3.2).

To minimise use of usual salt at the households, all participants were requested to surrender their household salts before receiving the study lithium spiked iodized salts packed in 500g packets.

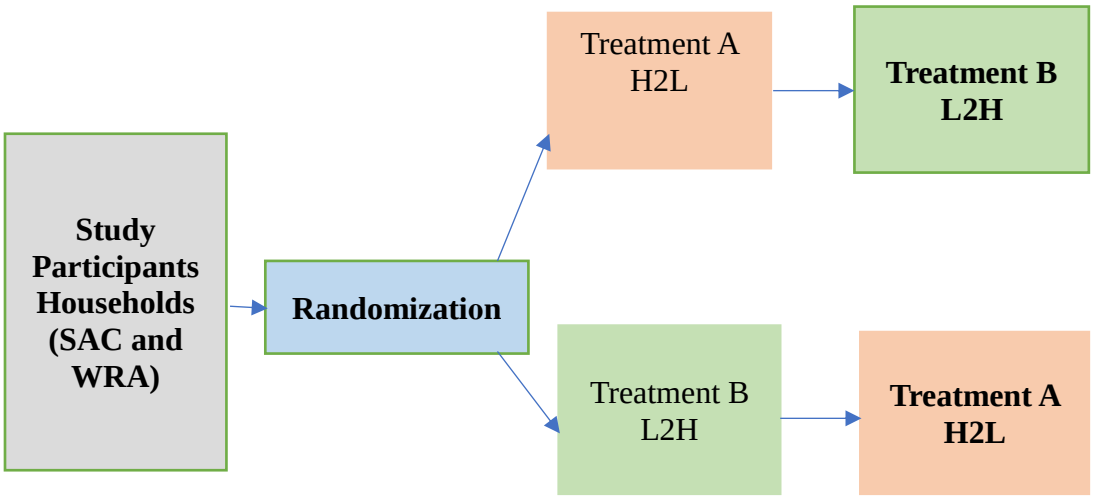

Figure 3.2: Illustration of the study design

Consumption of salt was not controlled, household were left to use the study-provided salt to their usual taste and practice in the household. To ensure compliance, the researchers continuously engaged with the households, ensuring sufficient salt supply for every household throughout the study period. Each household received a packet of their usual iodized salt after the 6 weeks follow up. This salt was to replace what had been withdrawn prior to the intervention.

3.3.7. Research Instruments

Structured questionnaires (appendix 3.7) were used to collect population description information on socio-demographics including, occupation, age, and income. Anthropometric, clinical and biochemical assessments were undertaken using nutrition assessment tools (weighing scales, height boards, and waist circumference tapes). Blood pressure was assessed using automated blood pressure machines. All the laboratory collected information was recorded on laboratory forms at different levels of biological sample collections (appendix 3.8).

3.3.8. Pretesting of study instruments

The pre-test of the tools was undertaken in Mavindini village in an adjacent sub location that was not included in the study sites

3.3.9. Validity and reliability

The study tools were pretested for and reliability and necessary adjustments undertaken before embarking on actual field work. A total of 17 questionnaires were administered and data analysed for reliability.3.3.10. Data collection Techniques

3.3.10.1. Anthropometric, clinical and biochemical assessments

Anthropometric assessments

Weight, height, waist and hip circumference were undertaken with minimal clothing (weight) and without shoes (height) in line with standard procedures (Appendix 3.9) while waist circumference was assessed using the World Food Programme circumference tapes (WFP WC model)

Clinical assessments

Information on family history and signs and symptoms of chronic diseases such as hypertension, heart disease and diabetes were collected through structured questionnaires (appendix 3.7). Two sitting BP and heart rate measurements were taken after 5 minutes rest interval using WHO approved OMRON M6, (HEM-7211-E8 (v), Omron Healthcare UK Ltd) compact upper arm BP machine with children arm cuffs for populations. Two consecutive measures were taken after 5 minutes rest. The measurements were taken through the day depending on availability of participants. The averages of the 1st and 2nd systolic and diastolic BP measurements were used in data analysis.

All efforts were made to minimize factors which might affect BP such as: anxiety, fear, stress, crying and laughing by ensuring the participants were not destructed. All blood pressure measurements were taken on the right upper arm (NHBPBP). Blood pressure in children is classified in percentiles as a function of age and height.

Biochemical Assessments

Spot urine sample collection: Spot casual urine samples were collected once a week from the study population for the assessment of UIC, Na, K and Li concentrations. Respondents were asked to pass about 20-30ml urine directly into a plastic cup with a tight-fitting lid. In the field, two aliquots of 10ml of urine samples for each respondent were prepared at the central field laboratory and stored in portable freezers at -20^{0}C before being transported to CPHR –KEMRI for storage at -30^{o}C awaiting analysis at the relevant laboratories.

Blood: Blood indicators for C-reactive protein, and α-1 acid-glycoprotein (AGP). Experienced phlebotomists collected blood from an arm by vene-puncture using an evacuated tube collection without additives. 5ml and 8ml of blood were collected at baseline, midpoint (after 3 weeks) and end line (after 6 weeks) into red top vacutainer with separate gel from SAC and WRA respectively. The blood was centrifuged in a central field lab site and serum aliquoted into appropriately labeled cryovials. The vacutainers, and cryovials were labelled with appropriate respondents' study numbers temporary at -20^{o}C in portable freezers before being stored in liquid nitrogen for transportation to the KEMRI laboratories for storage before further analysis

3.3.10.2. Transportation from Nairobi to International Laboratories

Urine samples (for the analysis of iodine) and serum samples were shipped on dry ice to respective international laboratories for analyses. UIC analysis was undertaken at Tanzania Food and Nutrition Centre (TFNC). AGP and CRP were shipped to VitMin Laboratories

Kastanienweg 5, 77731 Willstaett in Germany (http://www.nutrisurvey.de/blood_samples) for analysis.

3.3.10.3. Analysis of Sodium, Potassium and Lithium in Urine

The sodium, potassium and lithium concentration in urine were analysed using Microprocessor Flame photometer (LT-471) at CPHR KEMRI. Diluted urine solutions were passed through the atomizer where air, sample and fuel are mixed together in the mixing chamber. The mixture is then sprayed as a very fine mist in flame. The colour change of the flame depends upon the concentration of elements present in the analysis procedure as described in Appendix 3.10.

3.3.10.4. Analysis of Urinary Iodine

Urinary iodine concentration was analysed using the *Sandell Kolthof* reaction that uses ammonium persulfate (Pino et al., 1996) (Dunn et al., 1993) for pre-digestion of interfering substances. The World Health Organization recommends using the median iodine concentration in urine for population-based surveys as a reflection of a group's recent intake, while there is some variation at the individual level.

During the analysis, both external and internally checked quality control (QC) materials were used covering low, medium and high iodine concentration run within and between the assays (Appendix 3.11 and 3.12)

Test samples

A total of 2158 urine samples were analysed at the Tanzania Food and Nutrition Centre micronutrient laboratory. The overall median urinary iodine was 246.0 ug/L (range 0-3142 ug/L).

Out of the 2158 urine samples received, 10% were assessed on a separate day for repeatability after completion of the analysis. The statistical analysis showed that there was no significant difference of samples results obtained in run 1 and run 2, *Pearson's correlation* =0.99. (Figure 3. 3).

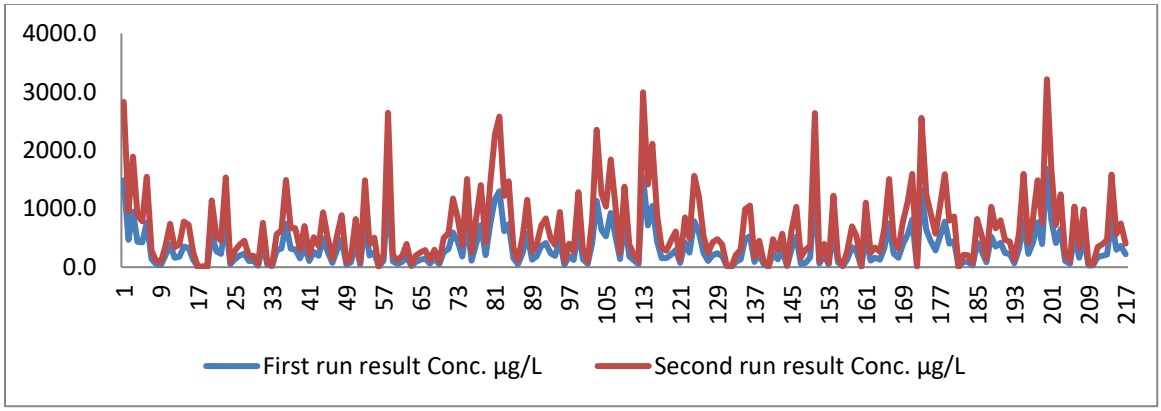

Figure 3.3: Test for Precision/repeatability run

3.3.10.5. Analysis of Acute Phase Proteins (CRP, AGP)

CRP and AGP, was analysed using the Enzyme Linked Immunosorbent Assay (ELISA) technique (WHO, 2014). Antibodies (anti-CRP and anti AGP) were diluted with coating buffer and 25 µl of diluted antibodies were added to a 384-well plate. The plate was covered and incubated overnight in refrigerator. The plate was then washed 3 times with wash buffer and 25 µl of diluted serum sample and standard samples added to the wells. The

plate was incubated for 2 hr at 37°C in a shaking water bath and washed as above. A total of 25 µl of diluted HRP (horseradish peroxidase) coupled antibodies in coating buffer were added to the wells and the plate again incubated for 45 min at 37° in a shaking water bath and washed as above. The color reagent, TMB (trimethyl benzidine) solution was prepared and 25 µl of the solution added into each well. Within 5-10 min, the reaction was stopped by addition of 100 µl /well of 1mol/L sulphuric acid. The color intensity was measured at 450 nm with the reference wavelength set at 650 nm.

3.3.10.6. . Quality control

Quality control was carried out by sampling sub samples and the values represent the mean of an independent double measurement. Measurements with a high CV were repeated and obvious outliers removed. Calibration curves were adjusted with a control sample which was measured in 10 wells and Biorad Liquickeck controls in 3 different concentrations with 6 wells on each plate. Coefficient of Variation of the different indicators on the 384-plates (n = 26) was calculated and compared with calibration curves from certified QC samples from the CDC/Atlanta and Biorad Liquicheck controls) (Appendix 3.13).

Based on concentrations of serum C-Reactive Protein (CRP) and Acid-Glycoprotein (AGP), four inflammation groups were determined, namely: 1-Normal (non-elevated) or reference group (CRP ≤5 and AGP ≤1); 2- Incubation (CRP >5 and AGP ≤1); 3- Early convalescence (CRP >5 and AGP >1); and 4- Late convalescence (CRP ≤5 and AGP >1).

The correction factor (CF) for each indicator was defined as the ratio of the median value of the indicator for the reference group to those in groups 2, 3 and 4. Adjusted corrected concentrations of the indicators were calculated by multiplying the individual values by their group-specific CF. these adjusted concentration indicators were used determine presence of acute and chronic infection.

3.3.11. Data Analysis and Presentation

3.3.11.1. Calculation of Variables

Sex- and age-specific BMI-for-age Z-scores (BAZ) were calculated using WHO AnthroPlus for children aged 8-12 years (WHO, 2009) while BMI for adults and Standard WC and HC were calculated in reference to the WHO standards (WHO, 2000) (NHS, 2009).

Using the guidelines provided by the 4th report of the Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents (NIH 2005), the BP percentiles were computed using the formula described in Appendix 3.14 in reference to the regression coefficients from blood pressure regression models (Appendix 3.15), Blood pressure reference chart (Appendix 3.14). Blood pressure was then classified in reference to corresponding percentiles using the guidelines provided in Appendix 3.16.

3.3.11.2. Statistical Analysis

All the data collected were checked and corrected at the end of each day. Data were entered into Microsoft Access before being transferred to the Statistical Package for Social Sciences (SPSS) for analysis after cleaning and validation. Data analysis was undertaken using SPSS statistical software version 20.0. Data for children was analysed separately from that of the adult women. However, a comparison of the BP patterns between SAC and WRA were undertaken to understand the link between childhood and adulthood BP of participants from the same household. Distribution of the outcome variable (blood pressure) was tested for normality using Kolmogorov-Smirnov test. Student unpaired two sample t-test was used to compare the difference in BP levels between those consuming low or high iodine from salt. Differences in the distribution of independent variables (low and high iodine intake) between groups was determined using Pearson's chi-square or Fisher's exact test for categorical variables. Means of changes in BP between the two arms was measured.

Pearson's Chi-square test was used to compare the distribution of categorical variables (age, income categories, and UIC, UNac and BP classifications) between treatment sequences at baseline. Independent t-test was used to compare mean differences of continuous variables (BP, age, UIC, UNaC, Potassium,) between treatment sequences at baseline. The effect of High (H) versus Low (L) iodine in salt is measured by comparing the cumulative net differences accrued at the ends of period 1 and period 2 between the two sequences. Individual differences accrued at the end of period 1 were realized by

subtracting individual endpoint measurements values (outcome) at midline (at week 4) from the baseline. Similarly, individual differences accrued at the end of period 2 were realized by subtracting individual endpoint measurements values (outcome) at end line (at week 7) from the midline (at week 4).

Cumulative net differences accrued at period 1 and period 2 were halved because the differences are doubled by virtue of having the two periods. Mean cumulative halved net differences accrued at period 1 and period 2 were then compared between the two sequences using independent t-test. The difference in the mean of differences was the estimator of the effect of High (H) versus Low (L) iodine in salt. The effect was equally demonstrated by performing a linear regression on the halved net differences accrued at period 1 and period 2, with the treatment sequence. Analysis of covariance using univariate General Linear Model was used to estimate the effect of the treatment adjusting for specific covariates identified to be significantly different between the two sequences at baseline. Coefficient of dispersion was used to understand the dispersion of lithium which was introduced as a marker of intake. This was achieved by calculating the ratios of UIC to urinary lithium and urinary sodium to urinary lithium. Level of statistical significance was fixed at 0.05 ($p<0.05$) and 95% confidence intervals computed as appropriate.

3.3.12. Ethical Considerations

The proposal was submitted to KEMRI/National Scientific and Ethical Review Unit for scientific and ethics review (Appendix 3.17) and other relevant entities including County

of Makueni Health Committee and permission sought from the County administration (Appendix 3.18) to implement the study. Participants were given details of the study and expectations for participating including biological samples' collection, the right to participate and to withdraw at will without prejudice. Information regarding the product was also detailed prior to consenting and questions arising addressed before the participants were recruited. Written information about the study was provided in English, Kiswahili and Kamba. WRA participants signed informed consent documents (appendix 3.19 guardians/parents signed enrolled consents (appendix 3.20) for the children while the SAC signed assent documents appendix 3.21) before participating in the study. Confidentiality was maintained at all times through anonymisation of participants' details. The participants were also informed of the follow up process.

3.3.13. Discussion on Methodology

A randomized double-blind time dependent cross over study design was used to assess the effect of iodine consumption on BP among women and school age girls living in the same households. The choice of the crossover is based on the understanding that crossover design allows participants to act as their own controls, enabling comparisons between and within groups (Velengtas et al, 2012) and the interventions are controlled to compare the effects in the outcomes.

The benefits of randomized trials eliminate bias in treatment through facilitated blinding to reduce bias from the investigators and participants as well as allow the use of probability

such that the likelihood of any difference in outcomes would be by chance. In addition to the randomization, the study's crossover component allows for within subject assessments removing between participant variations. Like many other study designs, these types of studies too have threats to validity. The significant differences at baseline were addressed by controlling for these differences using difference in difference analysis.

CHAPTER FOUR: FINDINGS

4.1. Introduction

This randomized, double blind crossover trail was undertaken to assess the effects of iodine on blood pressure in women of reproductive age and school age girls.

The results are presented in three sections:

Section one: Describes the study participants recruited and assessed at baseline before the intervention.

Section two: Presents the baseline characteristics of the study population that completed all follow ups taking note that this study's main dropouts were at baseline, thereafter the subsequent follow ups had less than 5 dropout not sufficient numbers to compare those that completed verses those that did not complete.

Section three: Details the changes after the interventions of the study populations that completed follow ups as described in section two. All respondents who started with lithium spiked salt with high iodine (Salt A) were referred to as Arm 1-*(H2L).* Those with lithium spiked salt with low iodine (Salt B) were referred to as Arm 2-*(L2H*).

Response Rate

The response rate was computed from the minimum sample size required to estimate blood pressure without adjustment for non-response (Table 4.1a.)

Table 4.1a: Response rate of study participants at recruitment at baseline

Description	Respondents	Targeted Households	Response (%)
Women of reproductive age at recruitment	175	174	100.0
Arm 1-(*H2L*)	89	88	100.0
Arm 2-(*L2H*)	90	86	100.0
School Age girls at recruitment	175	174	100.0
Arm 1-(*H2L*)	85	88	100.0
Arm 2-(*L2H*)	91	86	100.0

The response rate at recruitment was 100% for both women and children. While for the follow ups, 89.4% and 77.5% SAC and WRA in Arm 1 and 68.8% and 87.1% SAC and WRA in Arm 2 came for at least one to seven follow ups (table 4.1b). The completion rate was defined seven follow ups completed for urinary iodine and blood pressure. The completion rate therefore 71.8% and 68.5% SAC and WRA in Arm in Arm 1 respectively and 67.0 and 80% SAC and WRA in Arm two respectively.

Table 4.1b: Response rate of participants during follow ups

Timeline	Women		Children	
	Arm1-(H2L)	Arm2-(L2H)	Arm1-(H2L)	Arm2-(L2H)
A then B				
Baseline	89	86	85	91
Week 1	68	74	78	84
Week 2	64	75	70	89
Week 3	66	72	72	81
B then A				
Week 4	61	75	75	87
Week 5	65	76	75	84
Week 6	61	77	75	87
Week 7	62	74	76	79
Analysed	61	68	61	68
Excluded	28	18	24	23
Final Analysis	68.5%	79%	71.2%	75%

4.2. Baseline Characteristics

4.2.1. Baseline Characteristics of Women of Reproductive Age

One hundred and seventy-five (175) WRA were recruited from 174 households at baseline to participate in the study. All the women selected had a relation to the SAC recruited and they resided in the same households. The mean (SD) age of the WRA was 35.3 (7.5) years, the youngest being 15 years and the oldest 49 years. Close to three quarters (74.3%) of the women were in the age category of 31-49 years compared to 25.7% aged between 15-30 years. Table 4.2 presents characteristics on level of education and household income of the women recruited in this study.

Most women had primary (74.3%) or secondary (18.6%) (Table 4.2) school level of education. Close to half (52.1%) of the households indicated an average monthly income of KES 2,000/= to 5,000/= with 29.9% reporting less than 2,000/= per month. Arm1-(*H2L)* had more (73.5%) women with primary level education compared to Arm2-*(H2L)* (62.4%). While more women in Arm2-(*L2H*) had secondary level of education 27.1% compared to Arm1-(*H2L)* (14.5%) these differences were not statistically significant ($P > 0.05$). A positive correlation was found in the WRA between both SBP (r=0.173 P =0.026) and DBP (r=0.212 P=0.006) and age. No statistically significant difference (P>0.05) in level of education and income between the two Arms.

Table 4.2: Distribution of level of education and income among WRA by study Arm

Level of Education	N=167	Total %	Arm1-(*H2L*). %	Arm2-(*L2H*) %	P value
Primary 1-4	7	4.2	42.9	57.1	
primary 5-8	117	70.1	47.0	53.0	
Secondary	31	18.6	35.5	64.5	0.194
Tertiary	10	6.0	70.0	30.0	
University	2	1.2	100.0	0.0	
Level Income					
<2000	50	29.9	56.0	44.0	
2001-5000	87	52.1	46.0	54.0	
5001-10000	25	15.0	32.0	68.0	0.386
10001-20000	3	1.8	33.3	66.7	
20001-30000	2	1.2	50.0	50.0	

4.2.2. Family History of Chronic Conditions

High blood pressure (HBP) or hypertension in the family was reported by 21.0% of the women as shown in Table 4.3.

Chest related pains (angina) were reported in 25.7% of the women with 46.7% in Arm1-(*H2L*) and 53.3% in Arm 2-(*L2H*) while diabetes was reported in 12.6% of the families, 53.3% in Arm 2-(*L2H*) and 46.7% in Arm1-(*H2L*). Asked whether the women had ever been diagnosed for any of the conditions, only 6.5% had ever been diagnosed with HBP), 9.4% in Arm 2-(*L2H*) and 3.6 in Arm1-(*H2L*), while back related complaints in the family were reported in 15.0% of the women, 52.0% in Arm1-(*H2L*) and 48.0% in Arm 2-(*L2H*).

Table 4.3: Distribution of reported family history of chronic diseases by study Arm

Description	**N=167**	**Total %**	**Arm1-(*H2L*). %**	**Arm2-(*L2H*) %**	**P value**
Family history of Heart Attack					
No	161	96.4	47.2	52.8	0.405
Yes	6	3.6	33.3	66.7	
Family history Angina					
No	124	74.3	39.5	60.5	0.18
Yes	43	25.7	46.7	53.3	
Family history HB Pressure					
No	132	79.0	22.9	77.1	**0.001**
Yes	35	21.0	46.7	53.3	
Family history Diabetes					
No	146	87.4	52.4	47.6	0.372
Yes	21	12.6	46.7	53.3	
Family history Back pain					
No	142	85.0	45.8	54.2	0.359
Yes	25	15.0	52.0	48.0	

4.2.3. Nutrition Status among WRA at Baseline

Table 4.4 provides anthropometric and BP characteristics of the WRA at baseline. The mean (SD) BMI among the women was 24.0 (4.4) kg/m^2; no statistically significant difference (p>0.05) was observed between Arm1-(*H2L*) (24.2 (4.5) kg/m2) and Arm2-(*L2H*) (24.2(4.3) kg/m^2). The mean (SD) waist circumference among the WRA was 81.3 (10.6) cm, 82.44 (11.3) cms in Arm1-(*H2L*) and 80.2 (10.1) cm in Arm2-(*L2H*). This is an indication of low metabolic disease risk according to the cut off set by World Health Organization (WHO) (<88cms) (NHS, 2009).

Table 4.4: Mean distribution of baseline anthropometric characteristics of WRA by study Arm

	Total					**Arm1-(*H2L*)**			**Arm2-(*L2H*)**			
	N	**Mean**	**SD**	**95% CI**		**N**	**Mean**	**SD**	**n**	**Mean**	**SD**	**P value**
Age (yrs.)	166	35.5	6.4	34.5	36.5	78	35.44	7.3	88	35.6	5.6	.862
Wt (kg)	175	60.1	11.4	58.4	61.8	85	59.0	10.5	90	61.1	12.2	.224
Ht (cms)	175	157.7	6.3	156.7	158.6	85	15.8	6.0	90	157.4	6.5	.568
WC(cms)	174	81.2	9.8	79.8	82.7	85	81.2	9.8	89	81.3	9.8	.943
HC (cm)	175	96.6	8.8	953	97.9	85	95.5	8.0	90	97.6	9.5	.117
BMI (kg/m2)	175	24.2	4.3	23.5	24.8	85	23.6	4.0	90	24.6	4.5	.125

Difference between arms =P< 0.05

Wt-Weight, Ht-Height, WC-Waist circumference, HC- Hip Circumference

Table 4.5 describes the nutritional status of women classified according to World Health Organization Guidelines where BMI below 18.5 kg/m^2 is regarded as underweight while BMI between 18.5 and 24.9 and 25-29.9 and above 30kg/m^2 are regarded as normal, overweight and obese respectively. Waist circumference above 88cm in women is regarded as risk for cardiovascular disease.

Table 4.5: Percent distribution of nutritional status among WRA by study Arm

	Total		**Arm1-(*H2L*)**		**Arm2-(*L2H*)**		**P values**
BMI	**n**	**%**	**N**	**%**	**N**	**%**	
Underweight	11	6.4	5	5.6	6	7.1	0.773
Normal	95	54.9	52	58.4	43	51.2	
Overweight and Obese	67	38.7	32	36.0	35	41.7	
Total	173	100.0	89	100.0	84	100.0	
WC							
Normal (<80cms)	85	49.4	41	46.1	44	53.0	0.946
Increased risk (>80cms)	48	27.9	24	27.0	24	28.9	
Substantially Increased risk (>88cms)	39	22.7	24	27.0	15	18.1	
Total	172	100.0	89	100.0	83	100.0	

Despite a normal average body mass index (BMI), overweight and obesity were observed in 38.7% of the women higher in Arm2-(*L2H*) (41.7%) than in Arm1-(*H2L*) (36%). Increased risk and substantially increased risk as determined by the waist circumference were 27.9% and 22.7%, respectively. Women in Arm1-(*H2L*) with increased risk and substantially increased risk was 27% compared to Arm2-(*L2H*) where more women with increased risk (24.0%) were observed compared to substantially increased risk (18.1%). There was an association between BMI and Waist circumference ($\chi 2$=86.342 *P=0.001*) where 52.1% of the women with increased risk and 89.1% with substantially increased risk were overweight and obese

4.2.4. Blood Pressure Measurements among WRA at Baseline

The mean (SD) systolic blood pressure (SBP) was 124. 5 (12.4) mmHg, 124.3 (12.2) mmHg in Arm1-(*H2L*) and 125.0 (12.6) mmHg in Arm2-(*L2H*) while mean (SD) DBP was 77.1 (8.7) mmHg; 77.0 (8.7) mmHg in Arm1-(*H2L*) and 77.2 (8.6) mmHg in Arm2-(*L2H*). The mean (SD) pulse rate was 81.4(11.6): 82.1(10.2) in Arm1-(*H2L*) and 80.8 (12.7) in Arm2-(*L2H*).

An average of two BP measurements on the right arm showed that only 40.8% of the women would be classified as having optimal SBP measurements. Nearly similar patterns are seen in Arm1-(H2L) and Arm2-(L2H), while 46.6% and 13.2% would be classified as pre-hypertensive and hypertensive respectively. DBP showed a higher proportion (63.2%) of the women had optimal BP, compared to SBP; more women in Arm1-(H2L) had elevated DBP compared to Arm2-(L2H). Bearing in mind that one BP measure does not define one as hypertensive, it was noted that when both measurements were used to classify BP; close to half (46.0%) and 16.1% of the women presented with pre-hypertension and hypertension respectively.

The mean (SD) SBP and DBP increased with increasing BMI (Pearson's correlation coefficient (r=0.173 P=0.023) and (r=0.163 P=0.033) respectively. At baseline, a negative correlation was observed between DBP and UIC r=0.032 but was not statistically significant (P=0.686*)*, while a positive correlation is observed between SBP and UIC (r=0.039 P= 0.626*)*, also not statistically significant.

4.2.5. Urinary Iodine Concentration in WRA at Baseline

Table 4.6 presents baseline characteristics of biochemical parameters of women participants at recruitment. The median spot UIC levels were 435µg/L (IQR 256-814µg/L), higher in Arm 2-(*L2H*) (510 µg/L (IQR 298-979 µg/L) compared to Arm 1-(*H2L*) (355 µg/L (IQR 202.19-793µgL)). Spot urine showed more than half of the women (52.5%) had excess UIC levels (>=500µg/L), also higher (56.3 %) in Arm2-(*L2H*).

Table 4.6: Median distribution of baseline urinary Iodine Concentration, lithium, sodium and potassium among WRA by study Arm

	Total				**Arm1-(*H2L*)**				**Arm2-(*L2H*)**				
	N	**Median**	**IQR**		**n**	**Median**	**IQR**		**n**	**Median**	**IQR**		**P value**
UIC µg/L	166	435	256	814	79	355	202	793	87	510	298	979	0.008*
ULi (mg/L)	173	6.8	4.5	8.5	83	6.5	4.8	7.5	90	6.0	5.0	8.0	0.338
UNaC (mg/L)	141	4144	2712	6302	72	4132	2413	6212	69	4524	2694	6417	0.820

4.2.6. Urinary lithium concentration in WRA at baseline

At baseline, the median urinary lithium concentration (ULiC) levels were 6.8mg/L (IQR 4.5-8.5) 6.5mg/L higher in Arm1-(*H2L*) (IQR 4.8-7.5) and 6.0 in Arm2-(*L2H*) (IQR 5.0-8.0).

4.2.7. Urinary sodium concentration in WRA at baseline

Median baseline spot urinary sodium concentrations (UNaC) levels were 4144mg/L (IQR 2,712 to 6,302), higher in Arm2-(*L2H*) 4524 mg/L (IQR 2694. to 6417) compared to Arm1-(*H2L*) 4,132 mg/L (IQR 2413 to 6212). According to the World League of Hypertension UNaC levels of 2000-4000mg/L and 6000mg/L is an indication of very high and excess sodium consumption respectively. The data in table 4.7 shows very high UNaC at baseline in both arms. Only 6.4% women had optimal UNaC as shown in table 4.7.

Table 4.7: Classification of urinary sodium of WRA by study Arm

		Total	**Arm1-(*H2L*)**		**Arm2-(*L2H)***		**P values**
	n	**%**	**N**	**%**	**N**	**%**	
Sodium							
< 1333mg/L-Optimal	9	6.4	4	5.3	5	7.7	0.155
>=1333-2667 -High	24	17.1	14	18.7	10	15.4	
> 2667 Very high	107	76.4	57	76.0	50	76.9	
Total	140	100.0	75	100.0	65	100.0	

Using Pearson's Chi Square for normally distributed data and Spearman's rho for non-normally distributed data, an analysis of associations was done between various indicators. No association was observed between UIC and SBP (χ^2=5.585 P=0.471); UIC and DBP (χ2 = 4.236 P=0.645); or SBP and BMI (P=0.377) at baseline. Statistically significant associations were, however, found between spot UIC and UNaC (χ^2=57.368 P=0.001)

4.2.8. Baseline Characteristics of School Age Girls

At baseline, 176 school age girls (SAC) between the ages of 8 and 12 years were included to participate in the study because they resided in the same households with the WRA. The mean (SD) age (Table 4.8) of the girls in the study was 10.0 (1.4) years (95% CI 9.8-10.3). The proportion of the girls by age was lowest in the 10-year olds at 12.5% compared to 8-year olds (19.9%), 9-year olds (21.6%), 11-year olds (25.6 %) and 12-year olds (20.5%). Close to two thirds of the school age girls (SAC) (61.4%) were in lower primary (standard 1-4) while 38.1% were in upper primary (standards 5-8). No child reported to suffer from hypertension or diabetes. Disability was reported in one child who could not speak well. Most of the children (95.5%) reported participating in various physical activities in school.

4.2.9. Nutritional Status of School Age Girls

The mean Body Mass Index for Age Z-score (BAZ) (Table 4.8) was -0.9 (0.8). Mean BAZ for treatment ARM1-(*H2L*) and Arm2-(*L2H*) were (-0.7 (0.7) and -1.0 (0.8) respectively, all within the normal Z Score cut off of -2 to <1 SD. A statistically significant difference (*P=0.017*) was observed in mean BAZ between study Arms. No observable difference between arms

Results show that the majority (93.1%) of the girls had a normal BMI for Age, with no difference (P<0.05) between Arms.

Table 4.8: Baseline anthropometric characteristics of school age children by treatment Arm

	Total			Arm1-(*H2L*)			Arm2-(*L2H*)			
Variable	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**n**	**Mean**	**SD**	**P-value**
Age (yrs.)	175	10.0	1.4	85	10.2	1.5	90	9.9	1.4	.093
Weight(kg)	174	27.6	6.3	84	28.7	6.7	90	26.6	5.7	.023
Height(cm)	175	133.4	10.2	85	134.3	10.2	90	132.5	10.1	.243
Waist Circ(cm)	175	57.2	5.4	85	58.2	5.0	90	55.9	5.5	.001
Hip Circ (cm)	175	67.0	6.2	85	68.1	6.5	90	66.0	5.7	.034
Pulse rate (R)	175	90.8	12.7	85	91.4	13.3	90	90.2	12.2	.557
BAZ Z-score	174	-0.9	0.8	84	-0.74	0.73	90	-1.02	0.79	.017*

*P <0.05

4.2.10. Baseline Blood Pressure among School Age Girls

Mean blood pressure and percentile measurements among the SAC are shown in Table 4.9. The mean (SD) SBP Percentile score of 82.2 (18.9) and DBP Percentile score of 80.9 (14.4) were within the normal percentile levels according to World Health Organization (WHO) and the National High Blood Pressure Education Program (NHBPEP). Arm1-(*H2L*) had a mean SBP and DBP of 117.3 (10.1) mmHg and 72.6(9.4) mmHg and a mean SBP and DBP Percentile score of 84.5 (17.3) and 81.0 (14.8) respectively. Arm2-(*L2H*) on the other hand, had slightly lower mean SBP of 114.0 (10.1) mmHg, mean SBP percentile score of 79.9 (20.2) and mean DBP of 71.1(7.3) and mean percentile of 81.1 (14.2). BP in children was

classified in percentiles as a function of age and height using standard formulas as described in Chapter 3 and Appendix 3.6.

Table 4.9: Baseline blood pressure among school age children by treatment Arm

	Total			**Arm1-(*H2L*)**			**Arm2-(*L2H*)**			
Variable	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**n**	**Mean**	**SD**	**P-value**
SBP(mmHg)	175	115.7	10.2	85	117.2	10.2	90	114.3	10.1	.056
DBP(mmHg)	175	71.8	8.4	85	72.6	9.6	90	71.1	7.2	.228
SBP Percentile	174	82.2	18.9	84	84.1	17.4	90	80.4	20.1	.198
DBP Percentile	175	80.9	14.4	85	80.9	14.9	90	81.0	14.0	.965
Pulse rate (R)	175	90.8	12.7	85	91.4	13.3	90	90.2	12.2	.557

$P < 0.05$

A single moderately elevated measurement does not indicate hypertension. There must be repeated evaluation under normal conditions, over time. WHO recommends that at least 3 measurements at different times if consistently elevated, would classify the children as hypertensive. No significant difference was observed in both SBP (*P=0.141*) and DBP (*p=0.580*) in both ARMs at baseline.

Table 4.10: Median distribution of UIC, ULithium, and USodium of SAC by treatment Arm

	Total				**Arm1-(*H2L*)**				**Arm2-(*L2H*)**				
		Median	**IQR**		**n**	**Median**	**IQR**		**n**	**Median**	**IQR**		**P - value**
UIC µg/L	168	414.5	228.7	643.6	80	408.2	186.7	619.6	88	424.5	266.1	686.1	.197
Urinary Li	158	6.8	4.9	9.0	74	7.3	5.0	9.0	84	6.0	4.5	8.4	.123
Urinary Na	167	5014.0	2783.0	7452.0	82	4945.0	2991.2	7187.5	85	5014.0	2645.0	7498.0	.909
Urinary K	167	172.0	93.8	289.3	82	187.7	109.5	305.0	85	164.2	82.1	273.7	.442

4.2.11. Status of Urinary Lithium, Sodium and Potassium Concentration in School Age Girls at Baseline

The median urinary lithium, sodium (Na+) and potassium (K+) were 6.8 (IQR 4.9-9.0); 5014 (IQR 2783-7452) and 172.0 (IQR 93.8-289.3) mg/l, respectively. Median lithium was slightly higher in Arm1-(*H2L*) (7.3 (IQR 5.0-9.0) than in Arm2-(*L2H*) (6.0 (IQR 4.5-8.4). Median sodium (table 4.10) intake at baseline was very high (>4000-6000 mg/L) according to the World Hypertension League classification of urinary sodium. Arm1-(*H2L*) had a lower median Na+ (4945 (IQR 2991-7188) mg/L and K+ 187.7 (IQR 109.5-305.0)

4.2.12. Urinary Iodine Concentration in School Age Girls at Baseline

The median Urinary Iodine Concentration (UIC) for school age girl population were above optimal UIC levels as shown in Table 4.10 above. According to WHO, optimal median UIC levels should be between 100-299 µg/L. The median UIC was of 414 (IQR 229-644) µg/L, 408 (IQR 187-620) µg/L in Arm1-(*H2L*) and 425 (IQR 266-686) µg/L in Arm2-(*L2H*). The distribution of UIC levels shows that in 41.7% of the girls the UIC was excessive, 13.1% insufficient, 22.0 % above requirements and 23.2% in the adequate range. No significant difference (*P=0.471*) was observed between the two arms (Table 4.11).

About 64% of the SAC have UIC above recommended values and 13% have insufficient values.

Table 4.11: Distribution of UIC among SAC at baseline

Baseline UIC	**Total**		**Arm1-(*H2L*)**		**ARM2-(L2H)**		
	n	**%**	**n**	**%**	**N**	**%**	**P value**
Insufficient (0-99µg/L)	22	13.1	13	15.7	9	10.6	*0.471*
Adequate (100-299 µg/L)	39	23.2	18	21.7	21	24.7	
Above requirements (300-499 µg/L)	37	22.0	21	25.3	16	18.8	
Excess (>500µg/L)	70	41.7	31	37.3	39	45.9	
Total	168	100	83	100	85	100	

- *Based on League of World Hypertension classification

Urinary sodium was partitioned as indicated in Table 4.12 in reference to the League of World Hypertension classifications. About forty six percent (45.8%) of the girls had excess urinary sodium, 20.1% very high and 22.9% high, while just about 11.1 % had optimal levels. Spearman's rho correlation found a negative correlation between SBP and UNaC (r= -0.005 *P=0.951),* DBP (r=0.075 *P=0.373)* and DBP with UIC (r=-0.001 *P=0.990)*

Table 4.12: Distribution of urinary sodium in school age girls at baseline

	Total		**Arm1-(*H2L*)**		**Arm2-(*L2H*)**		
Baseline Sodium Partitioning	**n**	**%**	**n**	**%**	**N**	**%**	
Optimal (<2000mg/L)	16	11.1	5	5.8	11	13.2	0.139
High (>=2000-4000mg/L)	33	22.9	15	29.0	18	25.0	
Very High (>4000-6000mg/L)	29	20.1	11	15.9	18	23.7	
Excess (> 6000mg/L)	66	45.8	38	49.3	28	38.2	
Total	144	100	69	100	75	100	

P<0.05

While a positive correlation was found between SBP and age that was statistically significant (r=0.169 P=0.025) but not with DBP (r=0.073 P=0.338).

4.3. Section Two: Results of Respondents with Complete Follow Ups

After the baseline description of the study population characteristics, the data was evaluated for respondents with all follow up data across all seven-time points with respect to the dependent variable, BP, and independent variable UIC.

4.3.1. Evaluation of Lithium Intake as a Marker /Tracer of Intake

Before the participants consumed the lithium tagged salt, the mean lithium in Arm1-(H2L) was 6.2 (2.9) mg/L in the WRA and 6.8 (3.0) mg/L in the SACs. This concentration increased to 10.9 (6.2) mg/L and 14.0 (7.2) mg/L in the SAC after one week of consuming lithium marked salt. This pattern was similar to Arm2 where at baseline Lithium was lower 6.2 (2.2) mg/L in the WRA and 6.5 (3.0) mg/L in the SACs. This concentration increased to 10.5 (5.4) mg/L and 13.5 (7.1) mg/L in the SAC after one week. This was an indication that they were consuming the study salt. Table 4.13 presents the mean levels on lithium across the 7-time points with all showing an upward trend from the baseline based on the mean (SD) levels.

Table 4.13: Mean Lithium levels in school age girls and WRA across 7-time points

			Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
WRA	Arm1	Mean(mg/L)	6.2	10.9	11.1	9.4	11.3	8.9	9.2
		SD	2.9	6.2	6.8	6.9	8.5	4.6	5.4
	Arm2	Mean(mg/L)	6.2	10.5	11.3	9.9	8.2	10.3	10.0
		SD	2.2	5.4	5.8	5.6	5.2	6.1	6.9
SAC	Arm1	Mean (mg/L)	6.8	14.0	14.7	12.4	11.3	10.2	12.0
		SD	3.7	7.2	7.0	7.4	6.8	5.8	7.8
	Arm2	Mean (mg/L)	6.5	13.5	14.9	11.2	10.2	10.8	11.6
		SD	3.0	7.1	6.5	7.3	5.3	5.8	7.5

The resulting COD and COV per follow up are indicated in table 4.14.

Table 4.14: Ratio of UIC to urinary lithium over the study weeks

	Total			**Arm 1(*H2L*)**			**Arm 2(*L2H*)**		
	n	COD	COV	n	COD	COV	n	COD	COV
Baseline	121	0.561	62.5	55	0.567	71.2	66	0.505	53.4
Week 1	105	0.819	100.3	54	0.93	110.7	51	0.658	78
Week 2	110	0.712	94.3	46	0.786	91.1	64	0.688	95.7
Week 3-MP	114	0.756	95.4	57	0.768	89.5	57	0.775	92.7
Week 4	114	1.075	154	57	0.946	103.7	57	1.269	208.5
Week 5	116	0.418	56.8	57	0.501	67.2	59	0.361	45.9
End-line	119	0.865	121	59	1.172	152.7	60	0.623	70.6

MP-Midpoint

Table 4.15 presents the ratio between urinary sodium and lithium through the follow up periods. ANOVA was used to estimate the effect of the treatment adjusting for specific covariates identified to be significantly different between the two sequences at baselines. The results showed that the observed covariance matrices of the dependent variable were equal across the two study Arms, Assuming Mauchly's test of Sphericity. The week to week variations in urinary lithium concentration within subjects was not statistically significant (P=0.443) and homogeneity of variance was not violated.

Table 4.15: Ratio of urinary sodium to lithium

	Total			Arm 1(H2L)			Arm 2(L2H)		
	n	**COD**	**COV**	**n**	**COD**	**COV**	**n**	**COD**	**COV**
Baseline	106	0.436	54.6	50	0.454	58.7	56	0.394	49
Week 1	109	1.161	148.2	56	1.48	166.5	53	0.948	113.8
Week 2	112	0.848	103.2	51	0.671	87.3	61	1.057	110.8
Week 3-MP	114	0.754	100.8	57	0.662	84.1	57	0.994	99.7
Week 4	115	0.56	82.6	57	0.592	79.4	58	0.604	86.3
Week 5	114	0.403	47.8	57	0.335	44.1	57	0.466	49.8
End-line	118	0.391	49.6	59	0.426	55.9	59	0.375	43.6

*MP=Midpoint

These findings indicate that the participants consumed the study provided salt and the dispersion was mostly consistent between urinary lithium and urinary sodium compared to urinary lithium and UIC.

4.3.2. Linking Child Blood Pressure to Woman Blood Pressure

The women and school age girls who participated in this study were sampled as pairs from the same households. The demographic findings showed that over 90% of the girls were daughters of the women recruited to participate. As part of understanding the effect of iodine on BP, and whether there is a difference between the children and the women, a correlation analysis between women's and the girls' BP were undertaken and illustrated in scatter plots (Figure 4.1-4.14)

Relationship between WRA SBP and SAC SBP

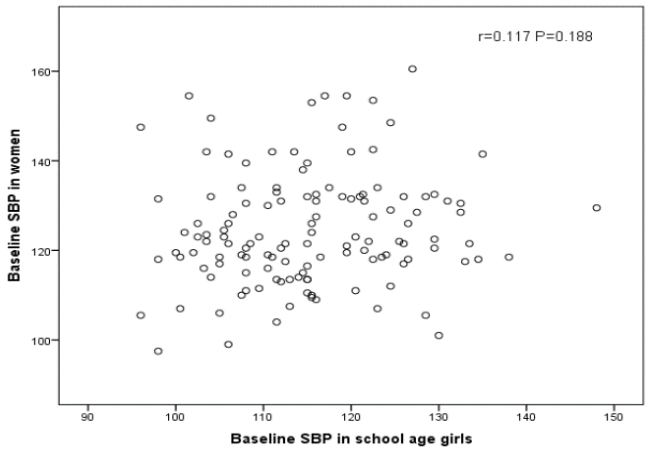

Figure 4.1: SBP in WRA vs SBP in SAC baseline

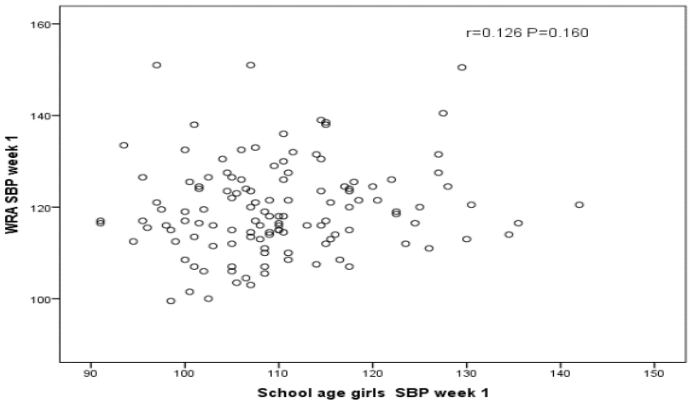

Figure 4.2: SBP in WRA vs SBP in SAC week1

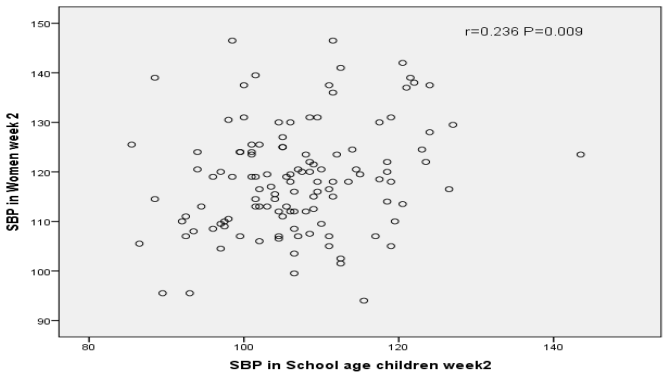

Figure 4.3: SBP in WRA vs SBP in SAC week 2

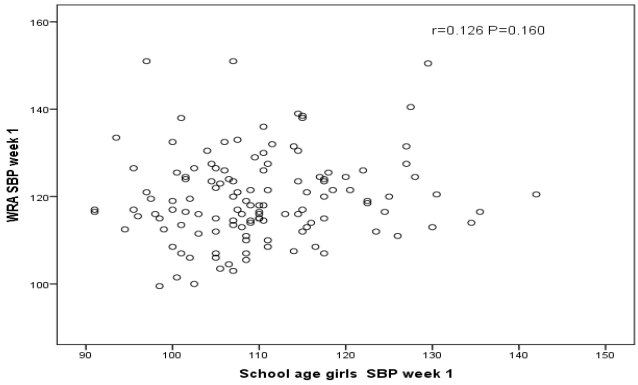

Figure 4.4: SBP in WRA vs SBP in SAC midpoint

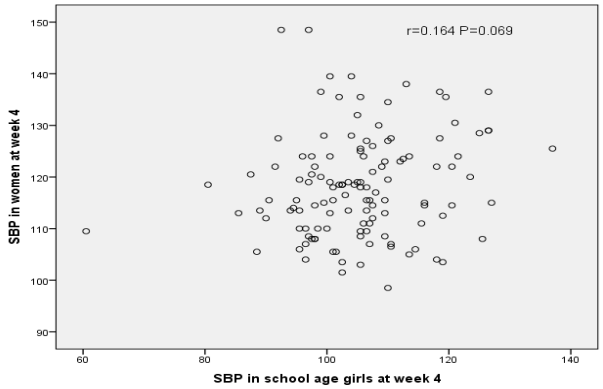

Figure 4.5: SBP in WRA vs SBP in SAC week 4

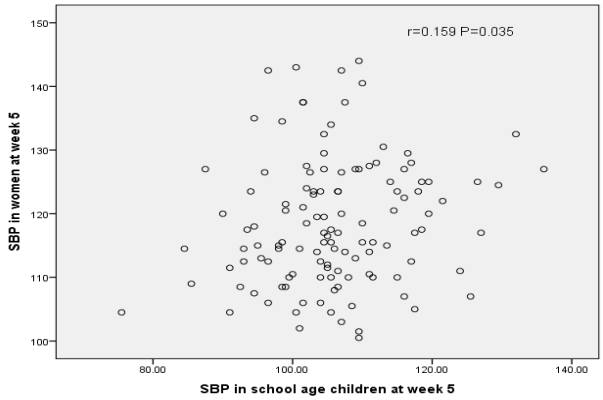

Figure 4.6: SBP in WRA vs SBP in SAC week 5

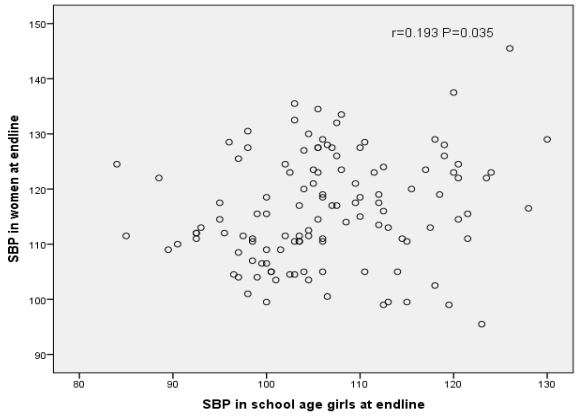

Figure 4.7: SBP in WRA vs SBP in SAC end line

The findings show no correlation at baseline for both SBP and DBP but a statistically significant correlation between SBP of the women and the girls at week 2, 3 and week 6 while a significant correlation is only observed for DBP at 3 weeks.

Relationship between WRA DBP and SAC DBP

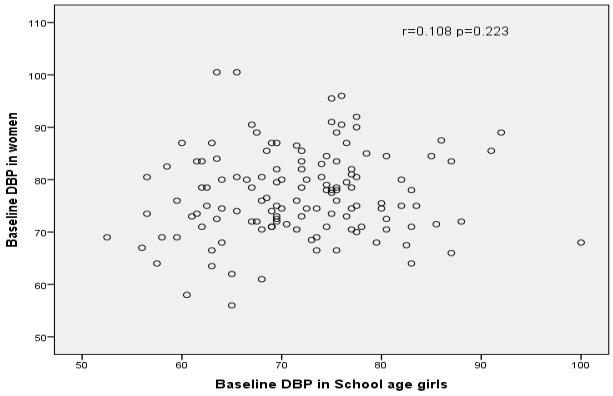

Figure 4.8: DBP in WRA vs DBP in SAC Baseline

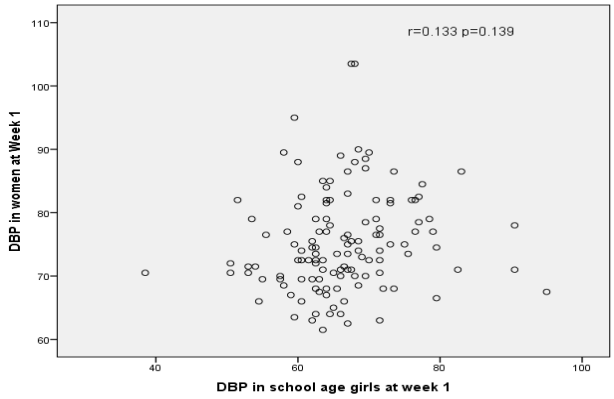

Figure 4.9: DBP in WRA vs DBP in SAC week 1

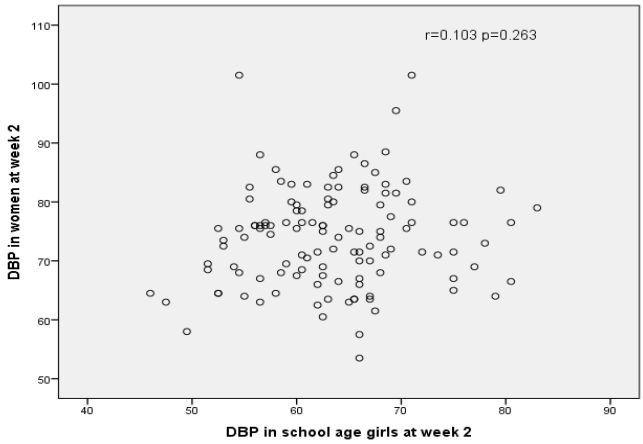

Figure 4.10: DBP in WRA vs DBP in SAC week 2

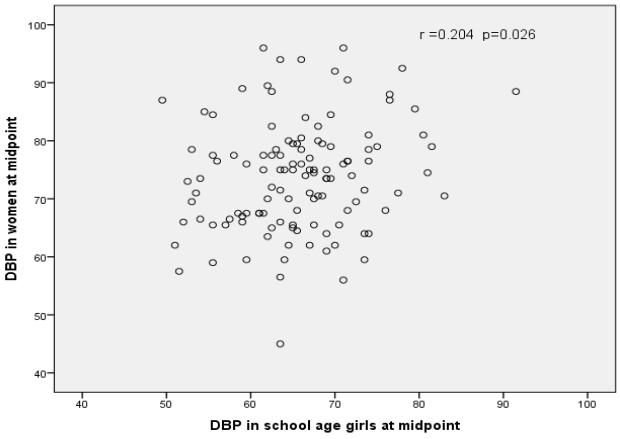

Figure 4.11: DBP in WRA vs DBP in SAC midpoint

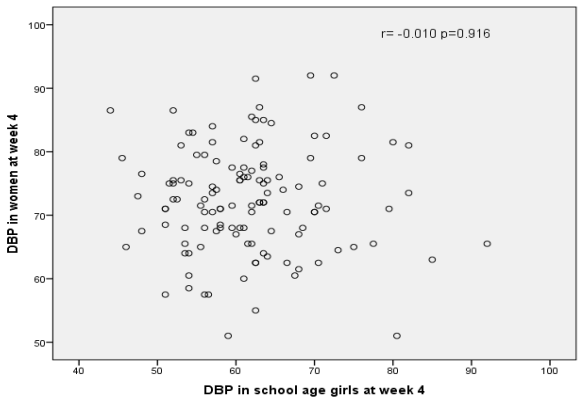

Figure 4.12: DBP in WRA vs DBP in SAC week 4

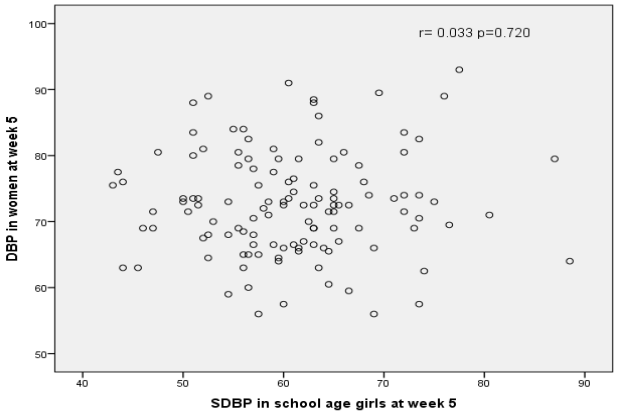

Figure 4.13: DBP in WRA vs DBP in SAC week 5

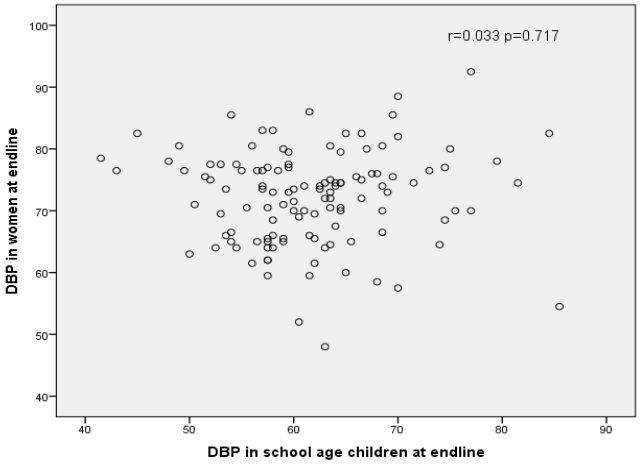

Figure 4.14: DBP in WRA vs DBP in SAC end line

The next section presents results with complete follow ups to assess the effect of the treatment over the follow up period and determine a pattern. In this study, loss to follow up was majorly at the baseline, thereafter the loss to follow up was minimal to affect the outcome of the findings.

The section has been divided into two sub sections;

1. Sub Section 1: Baseline characteristics of women of reproductive age and for school age girl respondents with complete follow ups.
2. Sub Section 2: Explores the effect of the treatment between the study arms and within the two study populations.

4.4. Sub-Section One: Characteristics of Women and SAC with Complete Follow Up

4.4.1. Demographic Characteristics

Most (68.3%) of the women had primary level education as shown in table 4.16. No statistical difference (*P= 0.150*) was observed between the arms. Slightly more than half (52.8%) of the respondents had monthly income of between 2000-5000 KES with about a third (30.9%) having a monthly income of less than KES 2000. Similarly, no statistical difference (*P= 0.640*) was observed between Arms. Most respondents were in the age groups of 25-36yr and 37-49yrs. When compared to the baseline

characteristics of participants recruited at baseline, the characteristics with regards to income were similar

Table 4.16: Socio demographics among WRA at baseline by treatment

	Total		**Arm1-(*H2L*)**		**Arm2-(*L2H*)**		**P value**
	N	**%**	**n**	**%**	**n**	**%**	
Level of education							
Primary 1-4	6	4.9	3	5.5	3	4.4	0.150
primary5-8	84	68.3	37	67.3	47	69.1	
secondary	24	19.5	8	14.5	16	23.5	
Tertiary	9	7.3	7	12.7	2	2.9	
Total	123	100.0	55	100.0	68	100.0	
Level of income							
<2000	38	30.9	21	38.2	17	25.0	0.640
2001-5000	65	52.8	29	52.7	36	52.9	
5001-10000	19	15.4	4	7.3	15	22.1	
10001-20000	1	0.8	1	1.8	0	0.0	
	123	100.0	55	100.0	68	100.0	
Age groups							
15-24years	4	3.3	4	7.3	0	0.0	0.570
25-36 years	55	45.1	26	47.3	29	43.3	
37-49 years	63	51.6	25	45.5	38	56.7	
Total	122	100.0	55	100.0	67	100.0	

This section presents results of school age girls who were assessed for the complete study period and have results for BP for the follow up period. No statistically significant difference was found in age nor level of education of the girls between the study Arms as shown in table 4.17, similar characteristics to all study participants at baseline.

Table 4.17: Baseline age and class distribution in the school age girls by study Arm

			Arm1-(*H2L*)		Arm2-(*L2H*)		
Age			**n**	**%**	**n**	**%**	**P value**
8 years	22	18.6	10	18.2	12	19.0	0.279
9 years	22	18.6	6	10.9	16	25.4	
10 years	16	13.6	7	12.7	9	14.3	
11 years	31	26.3	17	30.9	14	22.2	
12 years	27	22.9	15	27.3	12	19.0	
Total	118	100.0	55	100.0	63	100.0	
Class							
primary (1-4)	71	60.2	36	65.5	35	55.6	0.346
Primary (5-8)	47	39.8	19	34.5	28	44.4	
Total	118	100.0	55	100.0	63	100.0	

4.4.2. Anthropometric Assessment of School Age Girls

Nutritional status of children above 5 years of age was assessed based on weight, height and age as BMI for age and classified in Z-scores. The mean BAZ Z-score was -0.85 (0.80) [95% CI -0.99 to -0.71], -0.64(0.7) in Arm1-(*H2L*) and -1.04(0.80) in Arm2-(*L2H*) a small proportion of the girls (7.6%) were found to be thin.

Nutritional status (Table 4.18) in school age children was assessed for weight for height based on age and sex and reported as BMI for age. The proportion of thin children was 7.6% compared to 92.4% who had normal BMI for age. No significant difference between the two Arms.

Table 4.18: Mean distribution of age and anthropometric values among school age girls by study Arm

	Total				**Arm1-(*H2L*)**			**Arm2-(*L2H*)**			
	Mean	**SD**	**95% CI**		**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**F**	**P value**
Age in Years	10.1	1.5	9.9	10.4	55	10.4	1.5	63	10.1	1.4	.019	0.158
Weight	28.0	6.7	26.7	29.2	55	29.2	7.2	63	26.9	6.0	.667	0.061
Height	133.8	10.7	131.9	135.7	55	134.5	10.5	63	133.2	10.9	.342	0.483
Waist Circ	57.4	6	56.3	58.6	55	59.1	5.4	63	55.9	6.1	.257	**0.003***
Hip Circ	67.4	6.5	66.2	68.5	55	68.6	6.9	63	66.3	6.00	.353	0.053
BMI for Age	-0.9	0.8	-1.0	-0.7	55	-0.6	0.7	63	-1.0	0.8	.563	0.005

*Statistical significance at P<0.05

4.4.3. Baseline Blood Pressure and Blood Pressure Percentiles

The mean SBP and DBP was 115.4 mmHg and 71.8 mmHg, while the mean SBP and DBP percentiles among the girls was 81.6 (19.2) [95% CI 78.2-83.3] and 80.8(14.1) [95% CI 78.2-83.3] respectively. These means were similar for both arms and no statistically significant difference (P>0.05) between arms was observed.

BP in children is classified in percentiles based on age, sex and height P <0.05. Table 4.19. Presents the distribution of percentiles based on WHO classifications. More children were within the < 90^{th} percentile diastolic BP (68.6%) compared to SBP percentiles (52.1%), while after combining SBP and DBP percentiles the proportion reduces to 44.1%. Of these girls 14.4% had elevated blood pressure classifying them in the >99^{th} percentile which essentially means hypertensive.

4.4.4. Baseline Urinary Iodine Concentration

Table 4.20 presents results of UIC, urinary Sodium and Lithium. The median UIC in SAC at baseline was 419.3 [IQR 216.2-638.5] µg/L, higher in Arm2-(*L2H*) 490.0[IQR 261.6-670.7] compared to Arm1-(*H2L*) 400.4 [IQR 207.6-624.5]. Insufficient UIC was present in 10.4 % of the girls while 24.3 % had adequate UIC levels, equally distributed between the Arms. More girls (42.6%) had excess UIC while 22.6% had levels above requirements.

Table 4.19: Percent distribution of blood pressure percentiles

	Total		**Arm1-(*H2L*))**		**Arm2-(*L2H*)**		
	N	**%**	**n**	**%**	**n**	**%**	**P-Value**
SBP Percentiles							
< 90th Percentile	61	52.1	27	44.3	34	55.7	0.496
90th to < 95th Percentile	12	10.3	8	66.7	4	33.3	
95th to < 99th percentile	29	24.8	12	41.4	17	58.6	
> 99th Percentile	15	12.8	7	46.7	8	53.3	
Total	117	100.0	54	46.2	63	53.8	
DBP percentiles							
< 90th Percentile	81	68.6	38	46.9	43	53.1	0.091
90th to < 95th Percentile	19	16.1	5	26.3	14	73.7	
Table 4.19continued							
95th to < 99th percentile	10	8.5	6	60.0	4	40.0	
> 99th Percentile	8	6.8	6	75.0	2	25.0	
Total	118	100.0	55	46.6	63	53.4	
SBP and DBP Percentiles							
< 90th Percentile	52	44.1	26	50.0	26	50.0	0.872
90th to < 95th Percentile	17	14.4	8	47.1	9	52.9	
95th to < 99th percentile	32	27.1	13	40.6	19	59.4	
> 99th Percentile	17	14.4	8	47.1	9	52.9	

P<0.05

4.4.5. Urinary Sodium and Potassium Levels among the Girls

Table 4.20 presents results on biochemical data. The median urinary sodium was 5290 [IQR 3014-7613] slightly higher in Arm1-(*H2L*) 5474 [IQR 3138-7590] compared to Arm2-(*L2H*) 5267[2591 -7831]. These levels are much higher than the optimal levels of less than 2000mg/l. These levels classify the girls as having very high average (>=4000-

6000mg/l) UNaC levels. Table 4.21 classifies the girls based on the UNaC levels. Similar to the UIC levels, most (43.9%) of the girls had excess UNaC levels, while only 13.2% had optimal levels.

Table 4.20: Baseline UIC, Urinary Sodium and Lithium among school age girls by study Arm

	Total				**Arm1-(*H2L*)**				**Arm2-(*L2H*)**				
	n	**Median**	**IQR**		**N**	**Median**	**IQR**		**n**	**Median**	**IQR**		**P value**
UIC µg/L	115	419	219	638	53	400	208	625	62	490	262	671	0.759
ULiC mg/L	109	6.5	4.5	8.8	50	6.8	4.5	9.0	59	6.5	4.5	8.5	0.707
UNaC mg /L	114	5290	3014	7613	54	5474	3184	7590.	60	5267	2591	7831	0.982

Table 4.21: Distribution of Urinary sodium concentrations among school age girls by study Arm

	Total		**Arm1-(*H2L*)**		**Arm2-(*L2H*)**		
	N	**%**	**n**	**%**	**n**	**%**	**P value**
Urinary Sodium Concentration							
Optimal	15	13.2	4	26.7	11	73.3	0.137
high	29	25.4	18	62.1	11	37.9	
very high	20	17.5	8	40.0	12	60.0	
Excess (2333)	50	43.9	24	48.0	26	52.0	
Total	114	100.0	54	47.4	60	52.6	

The baseline characteristics showed statistically significant Spearman's Rho positive correlation coefficients between UIC and lithium (r=0.611; *P<0.001*); UIC and UNaC (r =0.445; *P=0.001*); and UNaC and ULi (r=0.327; *P=0.020*) and between UNaC and urinary potassium (r= 0.426; *P= 0.001*) but no significant correlation between UIC and potassium (r=0.231; *P=0.096*) for respondents in Arm 1(*H2L*). A similar pattern was observed in Arm 2 for UIC and lithium (r=0.587; P<*0.001*); UIC and UNaC (r =0.422; P=0.001); and UNaC and ULi (r=0.341; P=*0.009*) and between UNaC and urinary potassium (r= 0.308; *P= 0.017*), but not between UIC and potassium (r=0.231; *P=0.068*).

4.4.6. Nutritional Status among Women of Reproductive Age with Complete Follow Ups

The mean age was 35.7(6.4) years [95% CI 34.6-36.8], 35.00(7.7) years in Arm1-(*H2L*) and 36.3 (4.9) years in Arm2-(*L2H*). The mean (SD) weight was 60.6(11.6) [95% CI 58.6-62.6] kg/m^2, height (157.7(6.3) [156.6-158.8] cm, waist circumference (81.8 (10.1) [95% CI 80.0-83.6] cm and hip circumference (97.3(9.0) [95% CI 95.8-98.9] cm. No statistically significant (*P>0.05*) differences are observed between arms as shown in Table 4.22.

Distribution of the study population's nutritional status and blood pressure at baseline is shown in Table 4.23. More women (40.3%) were overweight/obese, 55.7% in Arm1-(*H2L*) and 52.9% for Arm2-(*L2H*). Close to half (48.4%) the women had a normal waist circumference, while 28.1% and 23.4% had increased and substantially increased risk to metabolic condition, respectively. No statistically significant (p>0.05) differences were observed

Table 4.22: Mean distribution of anthropometric measurements among Women by study Arm

	Total					**Arm1-(*H2L*)**			**Arm2-(*L2H*)**			
Variable	**N**	**Mean**	**SD**	**95% CI**		**N**	**Mean**	**SD**	**n**	**Mean**	**SD**	**P value**
Age(years)	122	35.7	6.4	34.6	36.8	55	35.0	7.7	67	36.3	4.9	.290
Weight(kg)	129	60.6	11.6	58.6	62.6	61	59.3	11.2	68	61.7	12.0	.247
Height(cm)	129	157.7	6.3	156.6	158.8	61	157.5	6.1	68	158.0	6.4	.642
WC (cms)	128	81.8	10.1	80.0	83.6	61	81.7	10.6	67	81.9	9.7	.887
HC (cms)	129	97.3	9.0	95.8	98.9	61	96.4	8.4	68	98.1	9.5	.284
BMI (kg/M2)	129	24.4	4.6	23.6	25.2	61	23.9	4.4	68	24.8	4.7	.311

P<0.05 No significant difference between arms

Table 4.23: Percent distribution of baseline characteristics, anthropometrics and BP in WRA

	Total		Arm1-(*H2L*)		Arm2-(*L2H*)		
	n		**n**		**n**		**P value**
BMI							
Underweight	7	5.4	4	6.6	3	4.4	0.773
Normal	70	54.3	34	55.7	36	52.9	
Overweight/Obese	52	40.3	23	37.7	29	42.6	
Total	129	100.0	61	100.0	68	100.0	
WC							
Normal (<80cms)	62	48.4	29	47.5	33	49.3	0.946
Increased risk (>80cms)	36	28.1	18	29.5	18	26.9	
Substantially Increased risk (>88cms)	30	23.4	14	23.0	16	23.9	
Total	128	100.0	61	100.0	67	100.0	

4.4.7. Blood Pressure Measurements

Mean right arm SBP and DBP were 24.6(12.6) [95%CI 122.4-126.8] mmHg and 77.2(8.3) [95%CI 75.7-78.6] mmHg, respectively. Both the SBP and DBP were within recommended optimal levels. No statistical differences (P>0.05) were observed between the arms. SBP (Arm 1-(*H2L*) was 123.1(11.9) vs. Arm 2-(*L2H*) 125.9 (13.2) while DBP was 76.9 (8.1) in Arm 1(*H2L*) vs. 78.1 (8.4) in Arm 2 – (*L2H*)

Blood pressure taken on the right arm was categorized (Table 4.24) to identify those with elevated BP at baseline: 45.0% and 30.2% of the women presented with SBP Prehypertension (121-139 mmHg) and DBP Prehypertension (81-89 mmHg), respectively. Hypertension (≥140 mmHg) was present in 14.7% based on SBP and 7.0% based on DBP (>90mmHg). When SBP and DBP were combined to classify the BP levels, the proportion of prehypertension (121/81-139/89 mmHg) and hypertension (≥140/90 mmHg) was found to be 45.0% and 17.8%, respectively. A statistically significant difference (P=0.049) at baseline between the study arms was only observed in hypertension prevalence based on DBP only.

4.4.8. Baseline Urinary Iodine Concentration

Table 4.25 presents baseline median Urinary Iodine Concentration (UIC) and Inter-quartile ranges (IQR) among the participants who completed the follow ups. Just like the entire population recruited into the study, the median spot UIC among the WRA with complete follow-ups was high 446 (IQR 224-760), and much higher in Arm2-(*L2H*) (522 (IQR 384

-860) compared to Arm1-(*H2L*) (369(IQR 165-639). These difference between arms was statistically significant (P=0.020).

Table 4.24: Percent distribution of baseline SBP and DBP among women

	Total		**Arm1-(*H2L*)**		**Arm2-(*L2H*)**		
	n	**%**	**N**	**%**	**N**	**%**	**P value**
SBP right Arm							0.524
Optimal	52	40.3	27	44.3	25	36.8	
Prehypertension	58	45.0	27	44.3	31	45.6	
Hypertension	19	14.7	7	11.5	12	17.6	
Total	129	100.0	61	100.0	68	100.0	
DBP right Arm							
Optimal	81	62.8	38	62.3	43	63.2	0.049
Prehypertension	39	30.2	22	36.1	17	25.0	
Hypertension	9	7.0	1	1.6	8	11.8	
Total	129	100.0	61	100.0	68	100.0	
SBP & DBP combined right arm							
Optimal	48	37.2	25	41.0	23	33.8	0.198
Prehypertension	58	45.0	29	47.5	29	42.6	
Hypertension	23	17.8	7	11.5	16	23.5	
Total	129	100.0	61	100.0	68	100.0	

Close to half (46.0%) the women had high UIC levels while 23.4% had excess UIC levels with a higher proportion in Arm2-(*L2H*) (high 23.9% vs. excess 56.7%) compared to Arm1-(*H2L*) (high 22.8% vs. Excess 33.3%). Those with below optimal UIC concentration were 10.5%, all in Arm1-(*H2L*). These differences between Arms were statistically significant (*P=0.020*)

4.4.9. Baseline Urinary Lithium, Sodium and Potassium Concentration

Median urinary lithium, sodium and potassium at baseline was 6.0 (IQR 4.5-8.0), 3928.40 [IQR 2729.0-6348.0] and 148.60 [IQR 101.70 – 213.08], respectively (table 4.28). Statistically significant difference (*P=0.021*) was observed in urinary sodium between study Arms. Arm2-(*L2H*) having higher median levels 4595.4 [IQR 3207.35-7705.0] than Arm1-(*H2L*) 3071 [IQR 2564 -5244]. Most (76.4%) of the women had high UNaC levels (above 2667mg/l) while only 7.5% had optimal UNaC levels and 16.0% had high levels (Table 4.25). The distribution across the Arms was not statistically different (P=0.155).

Positive Spearman's Rho correlation were found at baseline in WRA with complete follow-ups between UIC and UNaC (r=0.544 *P=0.01*); UIC and U-Lithium (r=0.640 *P=0.01*) and between UNaC and U-Lithium (r= -0.458 *P=0.01*). No correlation was found between UIC and SBP (r=0.70 *P=0.444*) or between UNaC and SBP (r=0.117 *P=0.232*).

4.4.10. Mean Differences in Blood Pressure

As shown in table 4.26, the one sample t-tests showed that the sample SBP and DBP means were different from the hypothesized value of Systolic BP of 120 mmHg and DBP of 80 mmHg for both study Arms

Table 4.25: Baseline UIC urinary lithium, sodium and potassium among women by study Arm

	Total				**Arm1-(*H2L*)**				**Arm2-(*L2H*)**				
		Median	**IQR**			**Median**	**IQR**			**Median**	**IQR**		**P values**
	n		**25**	**75**	**N**		**25**	**75**	**n**		**25**	**75**	
SpotUIC µg/L	123	446	224	758	57	369	165	639	66	522	384	860	**.020**
UrinaryLi mg/L	127	6.0	4.5	8.0	59	6.0	3.5	8.5	68	6.00	5.00	8.00	.880
Urinary Na Mg/L	106	3928	2729	6348	50	3070	2563	5244	56	4595.40	3207.35	7705.00	**.021**
Urinary K Mg/L	106	148.6	101.7	213.1	50	152.5	89.90	217.0	56	144.70	109.50	215.08	.744

P<0.05 Significant difference in Urinary Na and Spot IUC between arms

Table 4.26: SBP and DBP means compared to the recommended 120 and 80 respectively

		n	**Mean**	**SD**	**Mean difference with recommended BP values**	**95%CI of the differences**	**t test**	**P value**
SBP	Total	129	124.61	12.62	4.612	2.413 to 6.811	4.150	0.005
	Arm1-(H2L)	61	123.13	11.93	3.131	0.763 to 6.186	2.050	0.045
	Arm 2-(L2H)	68	125.94	13.16	5.941	2.756 to 9.127	3.723	0.005
DBP	Total	128	77.17	8.27	-2.833	-4274 to -1.392	-3.891	0.005
	Arm1-(H2L)	61	76.18	8.05	-3.819	-5.882 to -1.757	-42.493	0.005
	Arm 2-(L2H)	68	78.05	8.42	-1.949	-3.986 to 0.089	-1.909	0.061

Arm1-(H2L) –High iodine treatment; Arm2-(L2H) – Low iodine treatment

4.5. **Sub-Section 2: Effect of Treatment on blood pressure in women of** reproductive age and school age girls

This section presents the analyses of the effect of the treatment with consideration of the treatment period and sequence of treatment. In brief, the study design (detailed in chapter 3) can be described as a two treatment: two period's crossover comparing the effect of high and low iodine in salt (within the iodization regulation levels) where two populations, women of reproductive age (WRA) and school age girls (SAC) were randomized to one of the two sequence groups, Arm1-(*H2L*)-started with high iodine in salt and changed to low iodine in salt after 3 weeks while Arm2-(*L2H*)-started with low iodine in salt and changed to high iodine in salt after 3 weeks. The effect of high (H) versus low (L) iodine in salt on BP outcome indicators was measured by comparing the cumulative net differences accrued at the end of period 1 and of period 2 between the two treatment sequences.

Using difference in difference, Individual differences accrued at the end of period 1 are realized by subtracting individual endpoint measurements values (outcome) at midpoint (at week 4) from the baseline. Similarly, individual differences accrued at the end of period 2 are realized by subtracting individual endpoint measurements values (outcome) at end line (at week 7) from the midpoint (at week 4). Cumulative net differences accrued during periods 1 and 2 are halved because the differences are doubled by virtue of having the two sequences. Mean cumulative halved net differences, further referred to as "mean cumulative changes", accrued at the end of periods 1 and 2 are then compared between the two sequences. The difference between the mean accumulated changes is the estimator of

the effect of high (H) versus low (L) iodine in salt. The effect is equally demonstrated by performing a linear regression of the halved net differences accrued during periods 1 and 2 on the treatments.

4.5.1. Mean SBP and DBP differences in Arm1-(*H2L*) study arm

Table 4.27 gives results of the mean SBP and DBP among the WRAs. The mean (SD) SBP and DBP for Arm1-(*H2L*) started with high dose iodine in salt in period 1 and subsequently changed to low dose iodine in salt in period 2. They started at a higher mean (SD); SBP 123.1 (11.9) mmHg and DBP (76.2 (8.1) mmHg in period 1 compared to period 2 SBP 118.5 (9.1) mmHg and DBP (72.6 (9.0). The mean of differences after high dose iodine in salt was -7.2 (10.3) for SBP and -4.8 (6.6) for DBP at the end of period 1. However, the mean of the differences after low dose iodine in salt was 0.3 (9.1) for SBP and 0.5 (6.1) for DBP at the end of period 2.

4.5.2. Mean SBP and DBP differences in Arm2-(*L2H*) study arm

Results of mean SBP and DBP among WRAs are presented in table 4.27. The mean (SD) SBP and DBP for Arm2-(*L2H)* also started at a higher mean (SD) SBP 125.9 (13.2) mmHg and DBP (78.1(8.4) mmHg in period 1 compared to period 2 (SBP 118.4 (10.6) mmHg and DBP 72.3 (7.5)). The mean of differences after low dose iodine in salt followed by high dose iodine in salt was -7.6 (11.4) for SBP and -1.9 (8.9) for DBP at the end of period 1 whereas mean of differences after high dose iodine in salt for SBP was -2.2 (10.2) and -4.0 (9.0) for DBP at the end of period 2.

4.5.3. Mean SBP and DBP differences in women

The mean of differences at week 4 for SBP and DBP is the measurement for period 1 while mean of differences at week 7 are the measurements for period 2.

Table 4.27: Mean SBP and DBP among Women

			Period 1					Period 2			
			SBP		DBP			SBP		DBP	
Sequence/period	Time point	n	Mean	SD	Mean	SD	Time point	Mean	SD	Mean	SD
High dose iodine in salt/ Low dose iodine in salt	Baseline	61	123.1	11.9	76.2	8.1					
	Week 2	61	120.0	10.3	75.1	7.9	Week 5	118.5	9.1	72.6	9.0
	Week 3	61	117.7	10.9	72.7	8.1	Week 6	117.9	10.1	71.0	9.2
	Week 4	61	116.0	11.2	71.4	9.4	Week 7	116.3	10.4	71.9	8.9
Difference after High dose iodine in salt/ Low dose iodine in salt		61	-7.2	10.3	-4.8	6.6		0.3	9.1	0.5	6.1
Low dose iodine in salt/ High dose iodine in salt	Baseline	68	125.9	13.2	78.1	8.4					
	Week 2	68	119.3	10.0	75.4	7.9	Week 5	118.4	10.6	72.3	7.5
	Week 3	68	119.4	10.8	75.1	8.3	Week 6	118.8	10.0	73.5	6.7
	Week 4	68	118.4	10.8	76.1	8.6	Week 7	116.2	9.8	72.2	6.2
Difference after Low dose iodine in salt/ High dose iodine in salt		68	-7.6	11.4	-1.9	8.9		-2.2	10.2	-4.0	9.0

P<0.05

Results of the mean of differences in SBP and DBP between treatment sequences among WRA are presented in Table 4.28. Presents, which, in essence, estimates the net effect attributable to the different treatments. The total effect due to high dose iodine in salt is equivalent to -1.1 [95% CI: -4.0 to 1.9]; constant -2.7, (P=0.474) for SBP and -3.7 [95% CI: -6.0 to -1.3] constant 1.03 (P =0.003) for DBP. This indicates that high iodine dose salt is negatively associated with both systolic and diastolic BP in WRA (significantly ($p<0.05$) for DBP) and suggests that high iodine levels in salt could have a role in lowering DBP levels.

Table 4.28: Mean of differences in SBP and DBP among Women between the treatment sequences

		SDP		DBP	
Difference between the sequence	**N**	**Mean**	**SD**	**Mean**	**SD**
½ (Difference after High dose iodine in salt – Difference after Low dose iodine in salt)	61	-3.73	7.88	-2.63	5.12
½ (Difference after Low dose iodine in salt – Difference after High dose iodine in salt)	68	-2.67	8.79	1.03	8.06
Total effect due to high dose iodine in salt		-1.06		-3.66	

The treatment effect is also demonstrated by performing a linear regression of the halved net differences accrued during periods 1 and 2 on the treatment sequence (Table 4.29). Similar as for the examination of mean accumulated change, the regression analysis of treatment on BP shows a significant effect of high dose iodine in salt on DBP (P=0.003) but not SBP (P=0.474).

Table 4.29: Effect of High dose iodine in salt on SBP and DBP Right Arm among women

	SBP			
		95% CI		
Model	**B**	**Lower**	**Upper**	**p value**
(Constant)	-2.67	-4.68	-0.66	0.010
Treatment arm: *High dose iodine in salt*	-1.06	-3.98	1.86	0.474
	DBP			
		95% CI		
Model	**B**	**Lower**	**Upper**	**p value**
(Constant)	1.03	-0.61	2.67	1.03
Treatment arm: *High dose iodine in salt*	-3.66	-6.04	-1.28	-3.66

When the effect of treatment on BP was controlled for UIC in WRA at baseline in an Analysis of Covariance using univariate General Linear Model, the results were as presented in Table 4.30. The adjusted effect of the treatment on SBP was not statistically significant (P=0.491), meaning that the effect of the high dose iodine in salt treatment on SBP has a 49.1% chance that it occurred by random error. The variability of the difference in SBP attributable to treatment was 0.4%. However, for DBP the adjusted effect of high dose iodine in salt treatment was statistically significant (P=0.001) and had a 0.1% chance that it occurred by random error. The variability of the difference in DBP attributable to treatment was 8.1%.

Table 4.30: Treatment effect on SBP and DBP among WRA, corrected for baseline UIC

	SBP					
Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**p value**	**Partial Eta Squared**
Corrected Model	116.24	2	58.12	0.85	0.430	0.014
Intercept	790.98	1	790.98	11.55	0.001	0.088
UIC Spot urine at Baseline	59.91	1	59.91	0.88	0.351	0.007
Treatment arm	32.63	1	32.63	0.48	0.491	0.004
Error	8216.86	120	68.47			
Total	9678.19	123				
Corrected Total	8333.10	122				
	DBP					
Source	**Sum of Squares**	**Df**	**Mean Square**	**F**	**p value**	**Partial Eta Squared**
Corrected Model	504.63	2	252.32	5.32	0.006	0.081
Intercept	10.95	1	10.95	0.23	0.632	0.002
UIC Spot urine at Baseline	9.35	1	9.35	0.20	0.658	0.002
Treatment arm	501.79	1	501.79	10.58	0.001	0.081
Error	5689.04	120	47.41			
Total	6261.00	123				
Corrected Total	6193.68	122				

Table 4.31 presents Mean of differences in SBP and DBP between the sequences adjusting for UIC spot urine at baseline. After adjusting for baseline differences in spot UIC, the mean accumulated change in SBP and DBP accrued at the end of periods 1 and 2 in the high to low dose iodine sequence was -3.87 [95% CI -6.07 to -1.68] for SBP and -2.96 [95%CI -4.79 to -1.14] for DBP, while for the low iodine to high iodine sequence this change was -2.82 [95% -4.86 to 0.78] and 1.18 [95% CI -0.52 to 2.88] for SBP and DBP,

respectively. The net adjusted effect attributable to the high dose iodine in salt treatment was -1.05mm Hg for SBP and -4.14mm Hg for DBP.

Table 4.31: Mean of differences in Right Arm SBP and DBP between the sequences adjusting for spot UIC at Baseline

		SBP		
			95% CI	
Difference between the sequence	n	**Mean**	**Lower**	**Upper**
High dose iodine in salt - Low dose iodine in salt	57	-3.87	-6.07	-1.68
Low dose iodine in salt - High dose iodine in salt	66	-2.82	-4.86	-0.78
Total effect due to high dose iodine in salt		-1.05		
		DBP		
			95% CI	
Difference between the sequence	n	**Mean**	**Lower**	**Upper**
High dose iodine in salt - Low dose iodine in salt	57	-2.96	-4.79	-1.14
Low dose iodine in salt - High dose iodine in salt	66	1.18	-0.52	2.88
Total effect due to high dose iodine in salt		-4.14		

4.5.4. Mean SBP and DBP differences in H2L study arm among SAC

Table 4.32 presents results of the mean SBP and DBP among the SAC by treatment sequence at the different time points. Changes in mean SBP and DBP within the H2L sequence i.e. High dose iodine in salt in period 1 and subsequently changed to Low dose iodine in salt in period 2 was as follows; at baseline before treatment mean (SD) SBP was 116.3 (9.8) mmHg and DBP was 72.3 (9.2) mmHg. After treatment with High dose iodine in salt, mean (SD) SBP reduced to 107.2 (9.5) mmHg while DBP reduced to 64.3 (8.2) mmHg. The difference in means after high iodine salt was -9.1 (10.0) for SBP and -8.0

(11.5) for DBP. Upon switching to treatment with Low dose iodine in salt, mean (SD) SBP increased slightly to 108.7 (9.6) mmHg while DBP reduced marginally to 64.0 (8.7) mmHg. The mean difference after the switch from high to low iodine salt was 1.5 (10.4) for SBP and -0.3 (11.6) for DBP.

4.5.5. Mean SBP and DBP differences in L2H study arm among SAC

Considering changes in mean SBP and DBP within the L2H sequence i.e. Low dose iodine in salt in period 1 and subsequently changed to High dose iodine in salt in period 2, the changes were as follows (Table 4.32); at baseline before treatment mean (SD) SBP was 114.6 (9.9) mmHg and DBP was 71.4 (7.1) mmHg. After treatment with low dose iodine in salt, mean (SD) SBP reduced to 106.4 (9.1) mmHg while for DBP reduced to 69.9 (7.8) mmHg. The difference in means after high iodine salt was -8.2 (9.4) for SBP and -4.5 (8.6) for DBP. Upon switching to treatment with high dose iodine in salt, mean (SD) SBP reduce slightly to 105.4 (9.3) mmHg while for DBP reduced further to 61.2 (7.5) mmHg. The difference in means after the switch from low to high iodine salt was -1.0 (8.8) for SBP and -5.7 (9.5) for DBP.

Table 4.32: Mean SBP and DBP by treatment Arm among SAC

Sequence/period			Period 1					Period 1			
			SBP		DBP			SBP		DBP	
	Time point	n	Mean	SD	Mean	SD	Time point	Mean	SD	Mean	SD
	Baseline	55	116.3	9.8	72.3	9.2					
High dose iodine in salt/ Low dose iodine in salt	Week 2	55	111.0	10.1	66.3	7.6	Week 5	107.9	11.1	62.6	8.9
	Week 3	55	108.4	11.0	64.2	9.2	Week 6	108.8	9.3	62.8	8.4
	Week 4	55	107.2	9.5	64.3	8.2	Week 7	108.7	9.6	64.0	8.7
Difference after High dose iodine in salt/ Low dose iodine in salt		55	-9.1	10.0	-8.0	11.5		1.5	10.4	-0.3	11.6
	Baseline	63	114.6	9.9	71.4	7.1					
Low dose iodine in salt/ High dose iodine in salt	Week 2	63	109.0	10.1	65.8	8.3	Week 5	103.4	10.3	60.3	8.6
	Week 3	63	106.9	8.0	63.0	5.5	Week 6	104.0	10.7	60.2	7.4
	Week 4	63	106.4	9.1	66.9	7.8	Week 7	105.4	9.3	61.2	7.5
Difference after Low dose iodine in salt/ High dose iodine in salt		63	-8.2	9.4	-4.5	8.6		-1.0	8.8	-5.7	9.5

4.5.6. Mean Changes in SBP and DBP among SAC by Treatment Sequence

Table 4.33 and 4.34 provide the mean differences in SBP and DBP between treatment sequences among SAC modelled using General Linear regression. In essence, the tables provide the net effect attributable to the different treatments. The total effect due to high dose iodine in salt was equivalent to -1.68 [95% CI -4.49 to 1.12], (P=0.237) for SBP and -4.48 [95% CI -7.66 to -1.29] (P =0.006) for DBP. Similar to the WRA, this indicates that high dose salt is negatively associated with both systolic and diastolic BP. This effect from high done iodine in salt was statistically significant for DBP.

Table 4.33: Mean of differences in SBP and DBP percentiles among SAC

		SDP		DBP	
Difference between the sequence	n	Mean	SD	Mean	SD
½ (Difference after High dose iodine in salt	55	-5.26	8.12		
– Difference after Low dose iodine in salt)				-3.88	9.75
½ (Difference after Low dose iodine in salt	63				
– Difference after High dose iodine in salt)		-3.58	7.25	0.60	7.70
Total effect due to high dose iodine in salt		-1.68		-4.48	

Waist circumference and BMI for age Z-score at baseline was controlled for using univariate covariance analysis in General Linear Modeling (table 4.35), the effect of the treatment on SBP was not statistically significant f=1.38, (P=0.242). The effect of the treatment had a 24.2% chance that it occurred by random error and was explained by a

1.2% effect size. However, it was different in DBP where the effect of the treatment on DBP statistically significant was=6.83, (P=0.010).

Table 4.34: Effect of High dose iodine in salt on SBP and DBP percentiles among SAC

	SBP				DBP			
		95% CI				95% CI		p
Model	**B**	**Lower**	**Upper**	**p value**	**B**	Lower	Upper	**value**
(Constant)	-3.58	-5.49	-1.66	<0.001	0.60	-1.58	2.77	0.589
Treatment arm: *High dose iodine in salt*	-1.68	-4.49	1.12	0.237	-4.48	-7.66	-1.29	0.006

The effect of the treatment had a 1.0% chance that it occurred by random error and was explained by a 5.7% effect size. The effect of high dose iodine in salt resulted to significant net reduction in DBP.

Table 4.35: Effect on SBP and DBP among SAC, corrected for baseline waist circumference

	SBP						DBP					
Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**p value**	**Partial Eta Squared**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p value**	**Partial Eta Squared**
Corrected Model	95.20	3	31.73	0.53	0.662	0.014	590.61	3	196.87	2.55	0.059	0.063
Intercept	56.70	1	56.70	0.95	0.332	0.008	6.78	1	6.78	0.09	0.768	0.001
Waist circumference at baseline	11.43	1	11.43	0.19	0.663	0.002	0.98	1	0.98	0.01	0.911	<0.001
BMI for age Z-score at baseline	4.53	1	4.53	0.08	0.784	0.001	1.81	1	1.81	0.02	0.879	<0.001
Treatment arm	82.54	1	82.54	1.38	0.242	0.012	527.84	1	527.84	6.83	0.010	0.057
Error	6812.12	114	59.76				8810.76	114	77.29			
Total	9153.03	118					9663.88	118				
Corrected Total	6907.32	117					9401.37	117				

After adjusting for baseline differences (Table 4.36) in waist circumference, and BMI Z-scores, the mean difference in SBP and DBP between sequences (period treatment) was as follows SBP H2L: -5.31 [95% CI -7.43 to -3.18], SBP L2H: -3.54 [95% CI -5.52 to -1.56]. DBP H2L: -3.87 [95% CI -6.29 to -1.46], DBP L2H: 0.59 [95% CI -1.66 to 2.84]. The total net adjusted effect due to high dose iodine in salt was -1.77 for SBP and -4.46 for DBP.

Table 4.36: Mean of differences in SBP and DBP between the sequences

Difference between the sequence	n	SBP			DBP		
			95% CI			95% CI	
		Mean	Lower	Upper	Mean	Lower	Upper
High dose iodine in salt - Low dose iodine in salt	55	-5.31	-7.43	-3.18	-3.87	-6.29	-1.46
Low dose iodine in salt - High dose iodine in salt	63	-3.54	-5.52	-1.56	0.59	-1.66	2.84
Total effect due to high dose iodine in salt		-1.77			-4.46		

* **adjusted for SBP/DBP percentiles at baseline, Waist circumference at baseline and BMI for age Z-scores at Baseline**

CHAPTER FIVE: DISCUSSION OF FINDINGS

This study was designed to assess the effect of high and low iodine on blood pressure in female children of school going age and women of reproductive age. High blood pressure as a risk factor for cardiovascular disease is well documented, however, little is known about the effect of iodine in blood pressure in Kenya. The study results have provided an insight into the possible role of iodine in blood pressure in both women and children of school age. The discussion and findings provide a general overview of the study population before the interventions and the changes after the intervention.

5.1. Baseline Characteristics

The baseline characteristics include social demographic, nutritional status, family history of chronic diseases, and BP among WRA and SAC, in addition to urinary iodine, and sodium and potassium concentrations. This section briefly discusses the entire study population at recruitment and details on the population subset that completed follow ups.

5.1.1. Level of Educations and income in Women

As is common in rural communities in Kenya, most of the women who participated in this study had primary level education, the Kenya Demographic Health Survey (KDHS) of 2014, indicates that close to 60% of women in Kenya live in rural areas. In this study over 70% of the women had up to primary level education, much higher (74.0 % vs. 50.3%) than the national statistics of primary education (both incomplete and complete). The KDHS also confirms that there were more rural women with primary

level education compared to the urban women population (KDHS, 2014). Close to three quarters of the women in the study population were aged above 30 years. These findings are in agreement with the national statistic of women in this age category 30-49ys (KDHS, 2014). Averagely, the population's income was low and typical of a rural setting where most income was irregular and depended on peasant farming and trading. Furthermore, from the self-reported medical history, majority of the women did not have history of chronic diseases such as diabetes and hypertension.

5.1.2. Nutritional Status of Women and School Age Children

The general characterization with regards to nutrition status of the women and girls showed a population with normal mean Body Mass Index (BMI) and BMI for Age Z score. These similar characteristics are observed in the study population with complete follow ups. However, when BMI was classified according to WHO classification, more than a third of the women were either overweight or obese. Compared to the national statistics, overweight and obesity were slightly higher, while underweight was lower than the national and county statistics for overweight or obesity (>25.0 kg/m^2) and underweight (<18.5kg/m^2) (KDHS, 2014).WHO identifies waist circumference (WC) as a risk factor for CVD and metabolic disease risk is increased with WC above 88cms in women (WHO, 2000). The WC measures in this study showed that there was low disease risk in this population based on waist circumference measures. Increased risk and substantially increased risk were observed in less than a quarter of the population with a strong association between BMI and WC found in the women.

5.1.3. Blood Pressure in Women

The study women population's mean BP showed a population without elevated BP (<120/80 mmHg) where both mean SBP and DBP were within the normal limits.

According to WHO systolic and diastolic BP should be maintained below 120 mmHg and 80mmHg, respectively. Despite the high urinary levels of sodium, they did not seem to affect the blood pressure in this study population. High sodium resulting from all sources including from edible salt has always been associated with elevated blood pressure. This was not the case for this population. .

5.1.4. Blood Pressure, BMI and Age

Systolic and diastolic BP were found to be positively but weakly correlated with BMI and age in the women. This agrees with other studies that found similar relationships. In a study by Du et al., 2010 a positive correlation was found between all the anthropometric indicators and both SBP and DBP. Isolated hypertension increases with increasing age more so in the elderly from 60 years (Dequattro, 2001), although our study population was less than 60 years.

A descriptive study done in urban slums of Kibra Nairobi found that hypertension in their study population was influenced by increasing age and BMI (Ongeti et al., 2013). The association between BMI and elevated BP has mixed findings, a study in 2007 which was looking at association between BP and BMI found that over time there was a decreasing association between BMI and BP (Danon-hersch et al., 2007) indicating possibilities of change in trends of BMI and BP over time that could be attributed to more control of BP though treatment. Nirmala (2001) found that age influences SBP more strongly than DBP. In Ghana a descriptive study found that SBP increased with increasing BMI, and age (Kunutsor & Powles, 2009).

5.1.5. Urinary Iodine Concentration in Women and School Age Girls

In this study, children and women had both inadequate and excessive iodine intake based on urinary iodine excretion. Possible contributing factors for inadequate intake could be attributed to low consumption of iodized salt or consumption of non-iodized salt. For excessive intake, besides high consumption of iodized salt, there are regions in the world that tend to have higher iodine concentration in soils and water. According to the Makueni First County Integrated Development plan 2013-2017, in the study area residents highly depend on surface and sub-surface water due to water scarcity in the region.

It is noted that the ground water resources are low and saline because of the basement rock systems (Makueni, 2013), a likely possible explanatory factor for the high UIC levels in this study. Excess iodine is of concern due to its adverse effects including autoimmune thyroid disease, subclinical hyperthyroidism (Gao et al., 2014). In certain populations such as China, high UIC levels have been associated with iodine concentrations in drinking water (Gao et al., 2014; Zhao et al., 2000).

It is universally accepted that urinary iodine concentration (UIC) is useful in assessing recent iodine intake across populations but not for assessing thyroid function (Karakochuk et al., 2016; Shakya et al., 2015; Vejbjerg, et al., 2009; Wong & Gavin, 2009). Median UIC levels in a group below 100 µg/L or above 300 µg/L are an indication if inadequate or excessive iodine intake respectively based on WHO, 2013b guidelines.

In addition, Iodine sources vary in different regions even within the same country. This is not unique to this study, a study done in in Eastern Nepal by (Shakya et al., 2015) in

school children found a similar outcome of presence of both inadequate and excess UIC levels. Important to note is that UIC varies for individuals within the same day and therefore this is not unusual in this study population (Zimmermann & Andersson, 2012).

5.1.6. Urinary Iodine Concentration and Blood Pressure at Baseline

Although not statistically significant, Spearman's Rho correlation coefficient showed UIC to be weakly associated with DBP in women and children and SBP in children in children but not in women. SBP in women was positively correlated with UIC before the intervention was commenced among the women. The weak correlations between BP and iodine status differs from a study done in India among women older than 35 years which found that iodine levels had significant negative correlation with age and systolic BP. The Indian study also found new and known hypertensive to have significantly high iodine deficiency (Menon et al., 2011). However, in this study, we targeted women without hypertension at the time of the study and this is a possible cause for the difference in the outcome. Just like in this study, the study by Tayie and Jourdan (Tayie & Jourdan, 2010) on iodine and blood pressure from the years 2001–2002 and 2003–2004 of the United States National Health and Nutrition Examination Survey (NHANES 2001–2004) found that neither history of hypertension nor current hypertension had any significant association with iodine nutritional status in both women and men (Tayie & Jourdan, 2010).

5.1.7. Urinary Sodium and Potassium Concentration in Women and School Age Girls

Many studies have proposed the use of casual (spot) urine collections as low-burden alternatives to the 24 hour urine collection for the assessment of sodium and potassium (Elliott & Brown, 2006; Mizehoun-Adissoda et al., 2016). High urinary sodium concentration (UNaC) were observed in both women and children in our study. The World League of Hypertension suggests that UNaC levels above 1333 mg/L in women and 2000mg/L in children is suggestive of high sodium intake (Campbell et al., 2015) while acknowledging the day-to-day variations. The role of sodium and potassium is to regulate and maintain the cellular homeostasis in our bodies. Both nutrients are needed for most metabolic processes and are only needed to be consumed in small amounts (Pohl et al., 2013).

In every population, there are various sources of sodium among them dietary salt, packed ready to eat foods and ground water whose mineral composition is determined by the underlying rocks. This study did not assess sodium levels in water, however, ground surface water is a possible source of sodium in this population due to the region underground rocks. The findings show very high sodium intake as measured through UNaC and low potassium levels. Not much is available on systematic review to determine if the sodium-to-potassium ratio is more strongly associated with BP and related risk factors for CVD than either sodium or potassium alone (Perez & Chang, 2014).

5.1.8. Urinary Sodium Concentration and Blood Pressure

Despite high mean urinary sodium concentration (UNaC) levels, the mean BP in this population was within normal ranges of below 120/80 mmHg in adults. A positive not statistically significant correlation was found between UNaC and SBP in both women and children. Studies have shown that high sodium intake has been directly linked to cardiovascular risks including elevated BP or hypertension. It is however noted that although in most populations individual sodium intake exceeds 100 mmol per day, most people remain normotensive. DBP on the other hand was positively correlated with UNaC in women and negatively correlated in the girls although not statistically significant. In comparison, The International Study of Salt and Blood Pressure (INTERSALT) that assessed populations in 32 countries found a positive correlation between sodium intake and BP after adjustment potentially confounding variables (Adrogué et al 2007).

5.2. Evaluation of Lithium Intake as a Marker /Tracer of Intake

The evidence of respondents consuming lithium spiked salt is shown by an increase in mean urinary lithium between baseline and the subsequent follow up weeks. To ascertain the dispersion of lithium in the urinary sodium and iodine concentration, the coefficient of variation (COV) showed even distribution of lithium with the exception of week one for UNaC and lithium and in week four for UIC and lithium. All the other follow up measurements had COV of less than 1 an indication of even or over dispersion. These findings indicate that the participants consumed the study provided salt in similar amounts over time, as the dispersion was mostly consistent between urinary lithium and urinary sodium compared to urinary lithium and UIC.

5.3. Effect of Iodine Treatment on Blood Pressure in Women and School Age Children

In the study Arm that started with high dose iodine Arm1-*(H2L*) both SBP and DBP decreased and when they crossed to low iodine salt, the BP increased slightly. This compared to study Arm2-(*L2H*) that started with low iodine in salt, again both SBP and DBP reduced and reduced further upon crossover to high dose iodine in salt. The total effect due to high dose iodine was higher for DBP than for SBP. These findings show that high iodine in salt is negatively correlated with both SBP and DBP, however, only significant for DBP elucidating an effect of iodine on BP.

Since Kenya like other countries use salt as a vehicle for iodine fortification and sodium content in salt is a known risk factor for high blood pressure, the restriction of salt due to its effect on BP could be worsening the BP control due to limited intake of iodine as a result of reduced salt intake. The findings point out to the need of larger studies that can confirm this linkage in order to inform policy appropriately.

Compared to other studies, according to Streeten et al (1988) hypothyroidism has been associated with diastolic hypertension (Prissant et al., 2006). Hyperthyroidism on the other hand is associated with reduction in DBP and sometimes with SBP (Fletcher & Weetman, 1998). Literature shows that Iodine stimulates the production of thyroid hormones and, therefore if all other causes of hyperthyroidism are held constant, and it is assumed that excessive intake of iodine is the cause of hyperthyroidism we can conclude that the high levels in UIC in this population are contributory to the low BP levels in this randomly selected study population.

General Linear regression on the net adjusted effect attributable to the treatment showed that the high dose of iodine was negatively associated with both SBP and DBP indicative that high iodine levels could have a positive role in lowering BP in both girls and women. The effect was statistically significant for DBP but not for SBP. This study findings agree with the findings of a study in India which showed that iodine levels had statistically significant negative correlation with SBP in females above 35 years (Menon et al., 2011). The study undertaken in India found that iodine deficiency was significantly higher in subjects with known hypertension. It is likely that the relationship in this Makueni study was weaker because we assessed normotensive women.

Another study done in school children in Santiago, Chile, showed that higher levels of UIC corrected for urinary sodium excretion correlated with lower SBP, possibly representing a cardiovascular protective effect (Grob et al., 2015). Further, an analysis of covariance using univariate General Linear Modelling in this study showed that the effect of treatment was statistically significant for DBP in both girls and women.

Likely assumptions of the observed outcomes in the study point to the fact that Iodine is the chief component of the thyroid gland (Chung, 2014; Lee et al., 2016) which produces two hormones tetraiodothyronine (thyroxine or T4) and triiodothyronine (T3). The thyroid gland is influenced by two hormones, the Thyrotropin Stimulating Hormone (TRH) produced by the hypothalamus and Thyroid Stimulating Hormone (TSH) produced by the pituitary gland. From literature, a study by (Asvold et al., 2007) found a linear relationship between TSH and BP. Considering how iodine levels and

TSH behave under normal circumstances, when the iodine levels are low, T3 and T4 decline prompting the stimulation of hypothalamus to release TRH which in turn stimulates the Pituitary gland to release TSH. An increase in iodine intake lowers BP because there is the likelihood that an increase in Iodine lowers TSH which is inversely related to BP (Asvold et al., 2007). These are possible interplays in the current study however, the study did not measure thyroid status.

Another study by Ittermann et al (2015) in Germany found a similar relationship in children and adolescents. The Germany study found a positive relationship between serum TSH levels and hypertension in children concluding that subclinical hypothyroidism is associated with an increased risk of hypertension (Ittermann et al., 2015).

5.4. Linking Blood Pressure in Children to Adulthood Blood Pressure

Data from diverse populations shows that the tracking of BP from childhood into adulthood is very strong (Raj et al, 2010). This study, found statistically significant correlations between women SBP and school age girls' SBPs at week 1, week 2 and week three into the study. Several studies have shown that adulthood hypertension may be linked to childhood elevated BP.

Theodore et al (2015), found that right from childhood, it is possible to identify harmful BP trajectories that are able to predict adult CDV risk. Early detection can allow for early intervention to reduce the effects of elevated BP (Theodore et al., 2015). This is further supported by Magnusen and Smith (2016) who reviewed 25 papers that assessed childhood BP and adult structural, functional, and mechanical markers of preclinical CV health, found that although most hypertension is seen as a risk factor established

and treated in adulthood, it usually persists from childhood. (Magnussen & Smith, 2016).

The study findings point to the need to monitor childhood blood pressure over time to enable monitor any trajectories towards hypertension in adulthood and create timely interventions to address this. There is also need for policy guidelines to ensure blood pressure is assessed and monitored from childhood.

5.5. Associations in Demographics in Children

The findings showed that age in children was significantly ($P<0.05$) associated with SBP. Studies undertaken in children to assess BP, have found that BP rises gradually by approximately 1mmHg per year in children 2-5yrs and by 1.5mmHg in children 7-11 years (Wong and Gavin, 2009). This is because normal BP values for children and adolescents are based on age, sex, and height (Riley & Bluhm, 2012). Approximately 40% of variability in BP in children is related to weight, arm circumference, height and skinfold thickness (Wong and Gavin, 2009). Since arm circumference and skinfold thickness was not measured, it's not possible to estimate variability in blood pressure in this population.

CHAPTER SIX: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary

The overall objective of this study was to compare the effect of low and high iodine intake from iodized salt on BP levels among female school age children (8-12years) and WRA (15-49 years) in Makueni County. The human body requires iodine for the production of thyroid hormones which are regulated by the thyroid gland. A number of studies have shown that the thyroid status has an association with the cardiac and vascular function and in the regulation of the cardiovascular system.

Since BP control is regulated by the cardiovascular system, any differences in normal thyroid function could be associated with changes in BP. In this study two populations were of interest, WRA and school age girls aged between 8 and 12 years. The cohort was chosen specifically to understand if there were any similar characteristic changes in effects of iodine on blood pressure between the two groups with the intention to understand if there are any possibilities that HBP risks start at an early age in women. The findings discussed in chapter four support achievement of the specific objectives of this study.

The findings show a study population with education characteristics within the normal national ranges where a high proportion of women had primary level education. Furthermore, both school age children and the women had normal nutrition status as assessed by BMI and BMI for age respectively. The blood pressure too was within the globally accepted normal ranges. This confirms that the study was undertaken in a relatively healthy population based on the parameters of interest.

Findings in this study undertaken in normotensive girls and women has shown that, when both populations were exposed to an increased level of iodine in salt, their DBP declined and their DBP increased when exposed to a lower salt iodine level. This finding is in conformity with knowledge that iodine is an important nutrient in the function of the cardiovascular system. This brings to the fore the importance for consideration of role of iodine in blood pressure control and the need for further in-depth studies to elucidate these findings further and Importantly note an inverse relationship between BP and iodine status.

It's evident from this study that time has effect on how blood pressure responds to iodine exposure. As shown in the findings, at week 2, 3 and 5 time points of the follow up, the BP of the school age girls correlated significantly with BP of the pair women in the household where in over 90% of the girl-mother pairs were daughter and mother.

6.2. Conclusion

In conclusion, the demographic, socio-economic and nutritional status presentation of the study population is not different from the general population in the county and in Kenya in general. Similarly, the level of education was also comparable with the national statistics. Most of the participants had normal nutritional status.

The study findings have shown the relationship between iodine and BP are inversely related. The findings point towards protective effects of iodine in BP in both school age girls and women of reproductive age.

These effects seem to further improve over a longer period of time. In essence and as is always with blood pressure, there are variations within a 24-hour cycle and over longer periods of time. As the participants continued on the intervention the effect of iodine on BP increased an indication that with longer exposure to higher levels of iodine the protective effect is increased.

Iodine is an important micro element whose major function in human development and mental growth is undisputed and well documented. Research has also shown a role of iodine in functions of the cardiovascular system. This study was undertaken in a small population in one region of Kenya, with assumed minimal exposure to processed foods. It is however important to note that, like most countries in the world, Kenya provides additional dietary iodine to the population with the salt iodization strategy. While salt is the main source of iodine, it is also known that sodium in the edible salt used for iodination is also responsible for HBP not only in Kenya, but many other populations worldwide.

These findings highlight the need for further in-depth understanding on the iodine BP linkage and to understand whether indeed salt restriction causing a decrease in iodine intake could worsen HBP, rather than control it through longitudinal studies that would require more follow up period of at least 12 to 24 weeks or more. Despite high urinary levels of sodium, this population did not exhibit HBP levels. Childhood blood pressure patterns could be a good predictor of adulthood HBP risks if the populations are followed up.

Further studies should consider different age categories, and more so those who are newly diagnosed hypertensive to understand the effect of iodine on BP levels over a longer period of time.

6.3. Recommendations

6.3.1. Recommendations for Policy

Routine measurement of blood pressure in children has been recommended for over a decade now. It's therefore important that blood pressure measurements are incorporated in routine care of children and adolescents.

The study findings of this illustrate the role of iodine on BP in both school girls and women. An inverse relationship between iodine intake and BP was observed for in both the girls and the women. It is therefore important for policy makers and the public to be made aware of the importance of consuming iodized salts despite the current need for efforts to reduce the consumption of sodium mainly from edible salt. However, sensitization strategies to reduction of sodium must also be a point of interest and focus.

6.3.2. Recommendations for Further Research

There are limited longitudinal studies that directly link blood pressure in childhood to cardiovascular events in adults. It is generally accepted that hypertension and prehypertension in childhood commonly lead to hypertension in young adulthood (Berendes et al., 2013; Falkner, Let al., 2010). Further research with different population groups, different age cohorts, including children, adult males is needed to understand further the iodine and BP relationship elucidated in this study including those with hypertension.

Longitudinal studies especially in children would add value to build up of evidence on the BP trajectory to adulthood HBP and CV risks' patterns and especially on the question how properly ensured iodine intake may be an ameliorating factor for this time trajectory.

Studies to understand the levels of salt vs. iodine and their effects on BP would equally be useful in building knowledge on how much salt intake is sufficient enough to provide adequate iodine while at the same time keep BP at acceptable levels.

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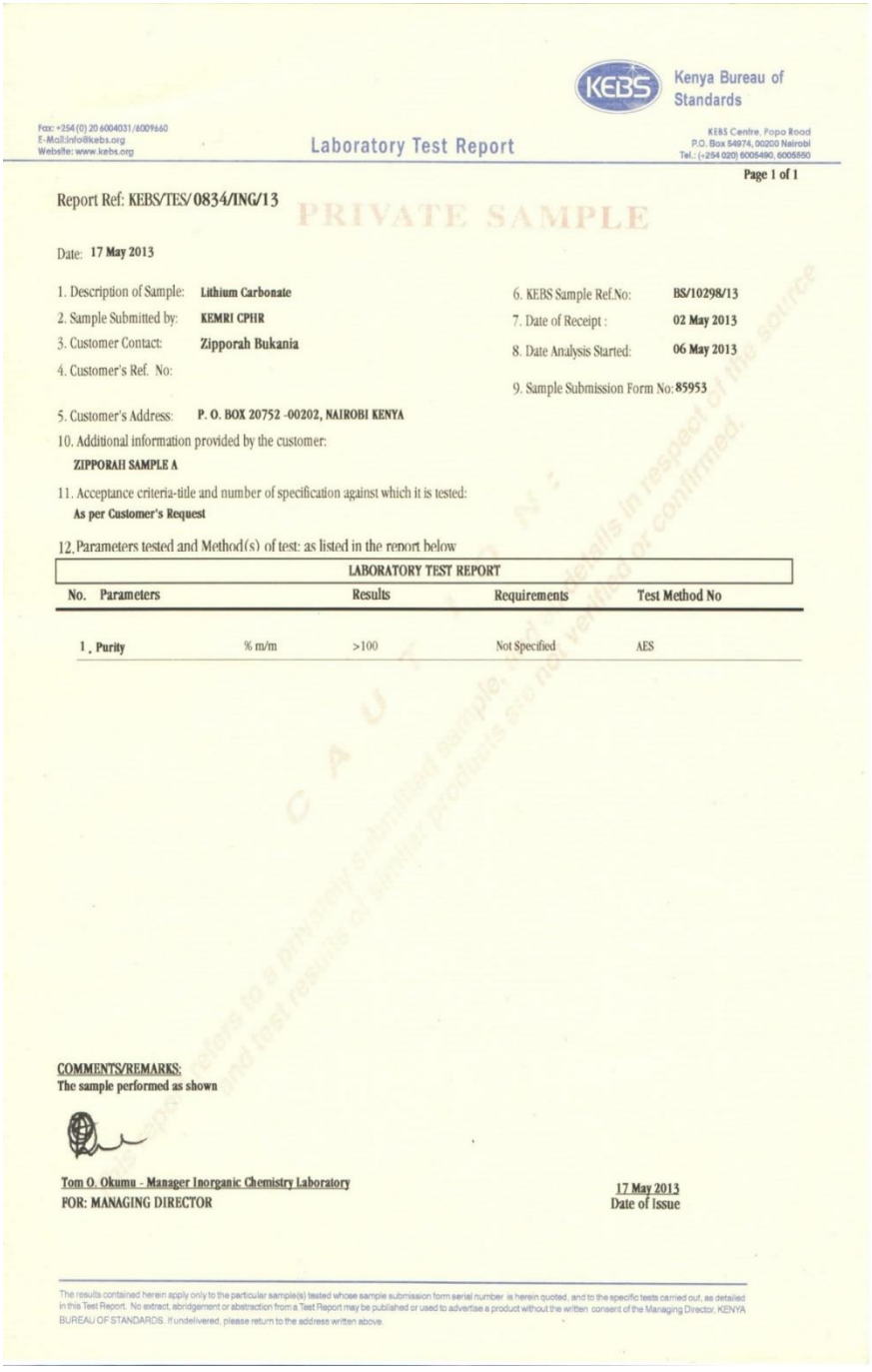
APPENDICES

APPENDIX 3.1: RESULTS ON ANALYSIS OF PURITY OF LITHIUM CARBONATE



Kenya Bureau of Standards

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Laboratory Test Report

Page 1 of 1

Report Ref: KEBS/TES/0834/ING/13

PRIVATE SAMPLE

Date: **17 May 2013**

1. Description of Sample: **Lithium Carbonate**
2. Sample Submitted by: **KEMRI CPHR**
3. Customer Contact: **Zipporah Bukania**
4. Customer's Ref. No:
5. Customer's Address: **P. O. BOX 20752 -00202, NAIROBI KENYA**
6. KEBS Sample Ref.No: **BS/10298/13**
7. Date of Receipt : **02 May 2013**
8. Date Analysis Started: **06 May 2013**
9. Sample Submission Form No: **85953**
10. Additional information provided by the customer:
ZIPPORAH SAMPLE A
11. Acceptance criteria-title and number of specification against which it is tested:
As per Customer's Request
12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1	**Purity**	% m/m	>100	Not Specified	AES

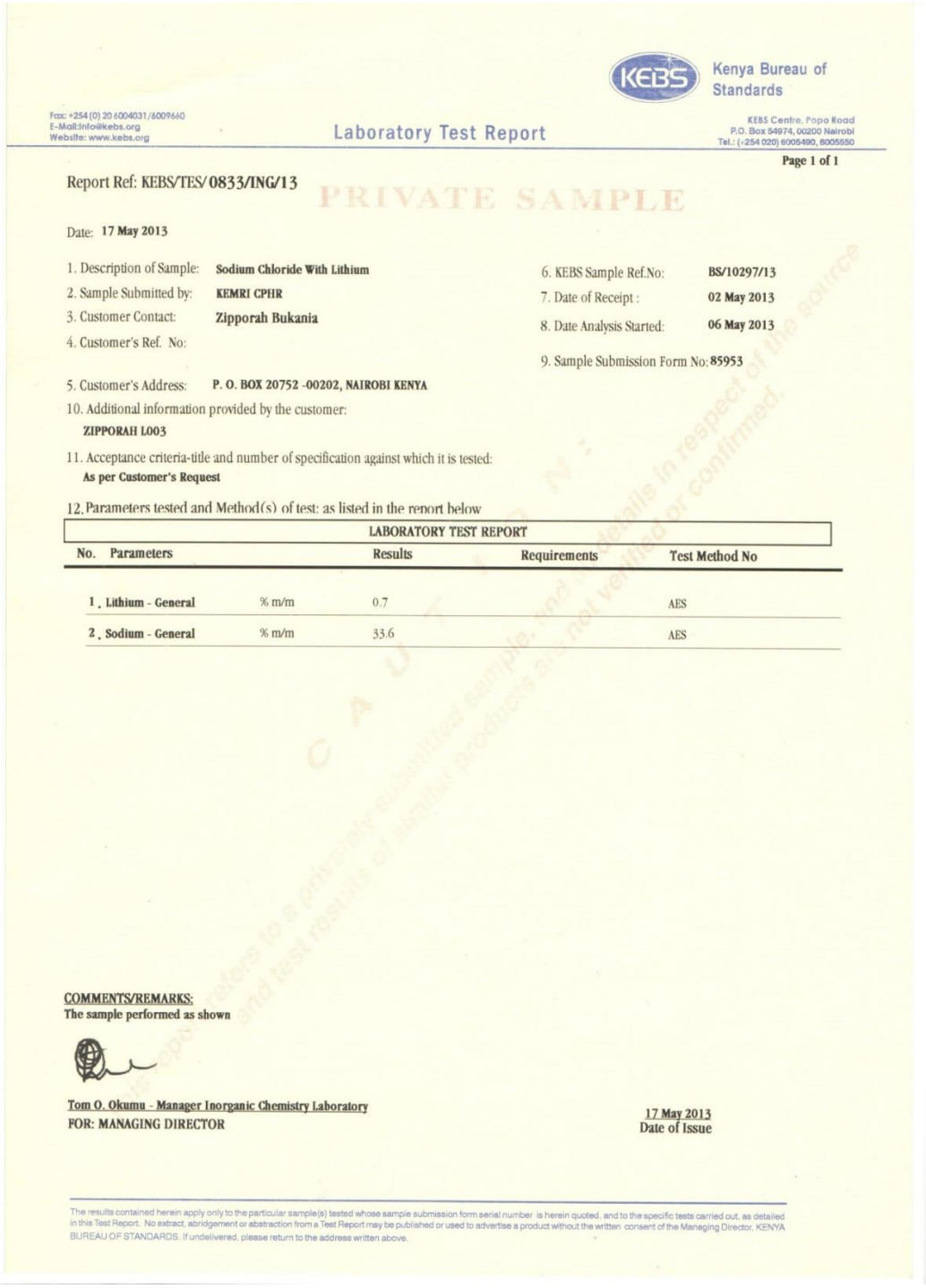
COMMENTS/REMARKS:
The sample performed as shown

Tom O. Okumu - Manager Inorganic Chemistry Laboratory
FOR: MANAGING DIRECTOR

17 May 2013
Date of Issue

The results contained herein apply only to the particular sample(s) tested whose sample submission form serial number is herein quoted, and to the specific tests carried out, as detailed in this Test Report. No extract, abridgement or abstraction from a Test Report may be published or used to advertise a product without the written consent of the Managing Director, KENYA BUREAU OF STANDARDS. If undelivered, please return to the address written above.

APPENDIX 3.2: SODIUM CHLORIDE AND LITHIUM CARBONATE RATIO-SAMPLE1



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Tel.: (+254 020) 6005490, 6005550

Laboratory Test Report

Page 1 of 1

Report Ref: KEBS/TES/0833/ING/13

PRIVATE SAMPLE

Date: 17 May 2013

1. Description of Sample: **Sodium Chloride With Lithium**
2. Sample Submitted by: **KEMRI CPHR**
3. Customer Contact: **Zipporah Bukania**
4. Customer's Ref. No:
5. Customer's Address: **P. O. BOX 20752 -00202, NAIROBI KENYA**
6. KEBS Sample Ref.No: **BS/10297/13**
7. Date of Receipt : **02 May 2013**
8. Date Analysis Started: **06 May 2013**
9. Sample Submission Form No: **85953**
10. Additional information provided by the customer:
ZIPPORAH L003
11. Acceptance criteria-title and number of specification against which it is tested:
As per Customer's Request
12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1.	Lithium - General	% m/m	0.7		AES
2.	Sodium - General	% m/m	33.6		AES

COMMENTS/REMARKS:
The sample performed as shown

Tom O. Okumu - Manager Inorganic Chemistry Laboratory
FOR: MANAGING DIRECTOR

17 May 2013
Date of Issue

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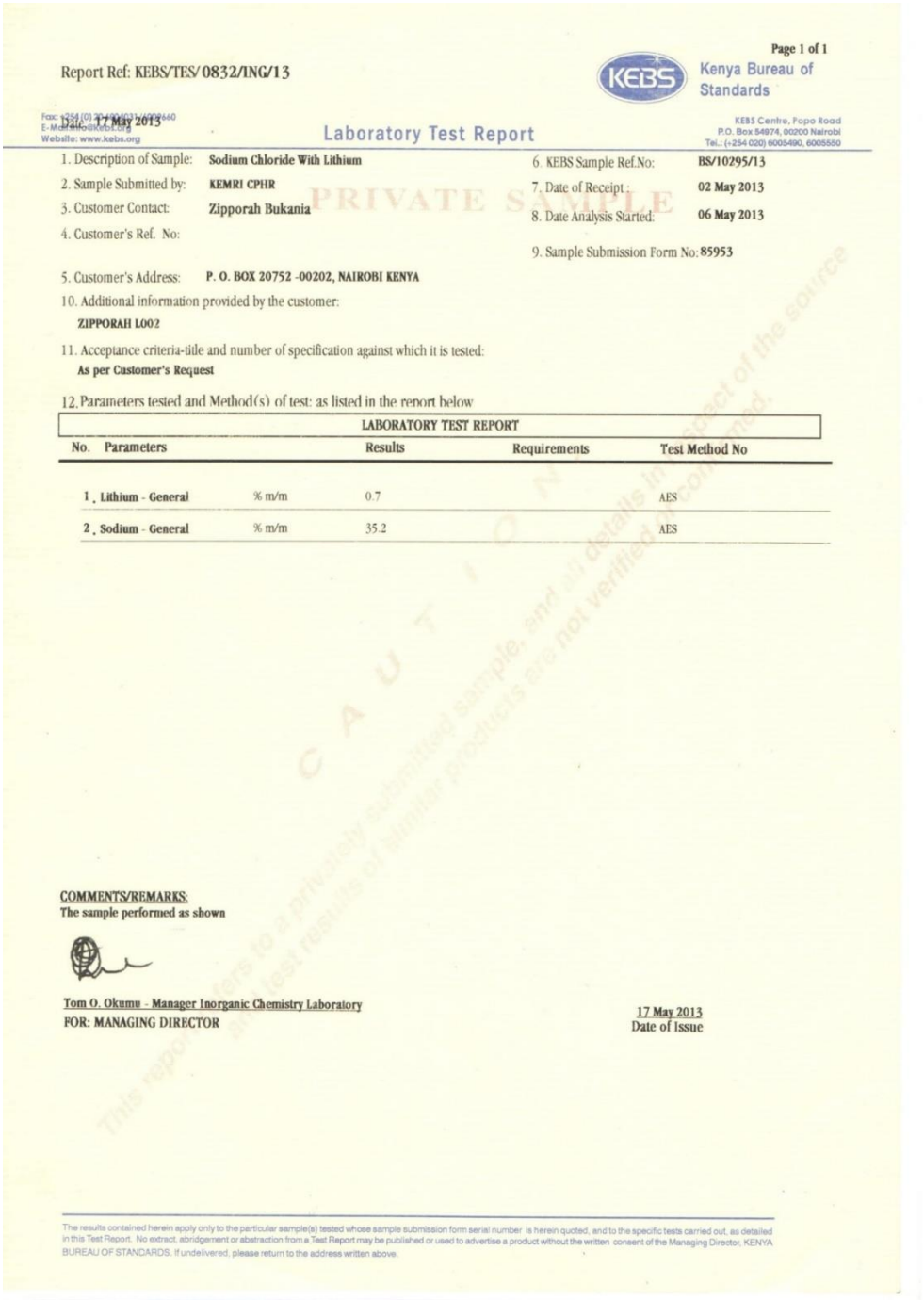
APPENDIX 3.3: SODIUM CHLORIDE AND LITHIUM CARBONATE RATIO-SAMPLE 2



Page 1 of 1

Report Ref: KEBS/TES/0832/ING/13

Kenya Bureau of Standards

Date: 17 May 2013

Laboratory Test Report

KEBS Centre, Popo Road
P.O. Box 54974, 00200 Nairobi
Tel.: (+254 020) 6005490, 6005550

Website: www.kebs.org

1. Description of Sample: **Sodium Chloride With Lithium**
2. Sample Submitted by: **KEMRI CPHR**
3. Customer Contact: **Zipporah Bukania**
4. Customer's Ref. No:
5. Customer's Address: **P. O. BOX 20752 -00202, NAIROBI KENYA**
6. KEBS Sample Ref.No: **BS/10295/13**
7. Date of Receipt: **02 May 2013**
8. Date Analysis Started: **06 May 2013**
9. Sample Submission Form No: **85953**

PRIVATE SAMPLE

10. Additional information provided by the customer:
ZIPPORAH L002

11. Acceptance criteria-title and number of specification against which it is tested:
As per Customer's Request

12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No
1	Lithium - General	% m/m	0.7		AES
2	Sodium - General	% m/m	35.2		AES

COMMENTS/REMARKS:
The sample performed as shown

Tom O. Okumu - Manager Inorganic Chemistry Laboratory
FOR: MANAGING DIRECTOR

17 May 2013
Date of Issue

The results contained herein apply only to the particular sample(s) tested whose sample submission form serial number is herein quoted, and to the specific tests carried out, as detailed in this Test Report. No extract, abridgement or abstraction from a Test Report may be published or used to advertise a product without the written consent of the Managing Director, KENYA BUREAU OF STANDARDS. If undelivered, please return to the address written above.

APPENDIX 3.4: KENSALT LTD PACKAGING MACHINES

NB: One of the Machines was isolated and cleaned to pack the lithium spiked salt.

APPENDIX 3.5: IODIZED SALT PACKED IN 500GM PACKETS AND SEALED

APPENDIX 3.6: SALT IODINE TITRATION METHOD

The reference method for measuring the iodine content of salt is iodometric titration with the thiosulfate-starch reaction as the external indicator. Reagents for testing iodate salt include 0.005 M sodium thiosulfate ($Na_2S_2O_3$), 2 N sulfuric acid (H_2SO_4), 10% (w/v) KI, and the starch indicator solution. For salt fortified with KI, additional reagents comprise methyl orange indicator, bromine water, sodium sulfite solution, and phenol solution.

Salt iodized with KIO_3:

1. Ten grams of the iodate salt sample were dissolved with 50 mL water in a 250-mL Erlenmeyer flask.
2. One milliliter of 2 N sulfuric acid was added to release the free iodine from the iodate.
3. Five milliliters of 10% KI was then added, which, in the presence of iodine, turns the solution pale yellow.
4. A stopper is placed in the flask; the flask is placed in a dark space for 10 minutes.
5. Upon removal of the flask from the dark space, sodium thiosulfate from the titration burette is added until the solution turns pale yellow.
6. Approximately 2 mL of the starch indicator solution is then added, turning the solution dark purple.
7. Titration is continued until the solution becomes colorless.
8. The burette reading indicates the total amount of thiosulfate added to the solution, which is converted to mg/kg of iodine in salt:
   "x" mL titrated sodium thiosulfate * 0.1058 * 100 = Iodine mg/kg.

Salt iodized with KI:

1. Salt fortified with iodide requires a slightly different procedure.
2. After dissolving 10 g of salt in 50 mL water, a few drops of methyl orange indicator are added until the solution turns pale orange.
3. Next, 1 drop of the 2N sulfuric acid is added, which results in a color change to pink. Bromine water (500 μl) is then added, changing the solution's color to yellow.
4. Drops of sodium sulfite solution are added to the solution until it turns to pale yellow.
5. Three drops of phenol solution are added turning the solution clear.
6. After 1 mL H_2SO_4 and 5 mL KI solution are added, titration with sodium thiosulfate is started.
7. Once the solution turns pale yellow, 1 mL of the starch indicator solution is added, turning the solution dark purple.
8. Titration is resumed until the solution is colorless. The burette reading is recorded and converted to mg/kg of iodine in salt.

Reagents:

1. Methyl Orange Indicator - Dissolve 0.01 g methyl orange in 100 mL water.
2. Bromine Water - Place 5mL in a small flask, (keep in fume hood due to Dangerous fumes).
3. Sodium Sulfite Solution - Dissolve 1g sodium sulfite in 100mL water.
4. Phenol Solution - Dissolve 5g phenol in 100mL water.
5. Starch Solution - Weigh 1 g soluble starch into a 100 mL beaker, add 10 mL water, heat to dissolve. Add saturated NaCl solution to the hot starch solution to make up to 100 mL.

APPENDIX: 3.7: DATA COLLECTION TOOLS

WOMEN OF REPRODUCTIVE AGE (15-49 YRS)
Effects of Iodine Exposure on Blood Pressure Among Female School Children and Women in Makueni , Kenya
Randomized Crossover Trial
BASELINE SURVEY

IDENTIFICATION		
HH01. CLUSTER (EA) NAME……	HH02. CLUSTER NUMBER: ____ ____ ____ ____	
HH03. HOUSEHOLD NUMBER: ____ ____ ____	HH04. SUB-LOCATION . ……. ____ 1=THAVU 2: ITUKA	
HH05.CLUSTERS: 1. KIUUMONI 2. KATHAMBANGI 3. MATHEMBA 4.MWAANI 5.YEEMULWA 6.KIVANI 7. 8. KOSYA 9. KIVANI 10. KITHAYONI 11. KILAANI/NGUTHUNI 12.KIUSINI 13. ITUKA 14. MASAKU NDOGO		
HH07. INTERVIEWER _____ : ____ ____ NAME CODE	HH08. TEAM LEADER _____ : ____ ____ NAME CODE	
_____ /_____ /_____ DD MM YY	_____ /_____ /_____ DD MM YY	

INTERVIEWER VISITS		
HH10. VISIT 1	**HH11. Follow up visit**	
DATE _____ /_____ /_____ DD MM YY TIME: START: ____:_____ STOP: ____:_____ **RESULT..... ____	DATE _____ /_____ /_____ DD MM YY TIME: START: ____:_____ STOP: ____:_____ **RESULT ..... ____	

2. SOCIAL DEMOGRAPHIC DATA

Now I would like to ask you some questions about yourself.

No.	QUESTION	CODING CATEGORIES	SKIP
2.1	Age (Years)		☐☐
2.2	Date of Birth	_____ /_____ /_____ DD MM YY	
2.3	Woman's Name (three names)		
2.4	Woman's Household Number (ID)	☐☐☐☐☐☐	
2.5	Identification No. of Girl selected in the household	☐☐☐☐☐☐☐	
2.6	Relationship of respondent to the SAC	Mother..... 1 ..... 2 Sister..... 3	

		Auntie (sister to father)..... 4 5 Auntie (Sister to Mother)..... 6 Cousin..... ..... Other	
2.7	Sex	1= Female	☐

2.8	What is your level of Education?	None……..…… 1 …..….. 2 Primary (1-4) 3 ……. 4 Primary (5- 5 8)…….. 6 Secondary ……. Tertiary ……... University ...……	
2.9	What is your households level of income in KES	<2000…… 0 …….. 1 2001- 2 5000…… 3 50001- 4 10000…….. 5 10001- 6 20000…….. 20000- 30000…….. 30001- 40000…….. >40000…… ……	
2.10	What is the average expenditure on food per day (Give actual figure in KES)	_____	
2.11	In the past(4 weeks/30days) was there ever no food to eat of any kind in your house because of lack of resources to get food	No…… 0 ……... 1 Yes ……...…… ….	**0 → 2.13**
2.12	How often did this happen in the past [4 weeks/30 days]? Rare-less than 5 days Sometimes- up to 7 days Often –More than 14 days	Rarely……. 1 ……... 2 Sometimes 3 ...…….	

		Often ……	
2.13	In the past [4 weeks/30 days] did you or any household member go to sleep at night hungry because there was not enough food?	No…… 0 …….. 1 Yes ……..…… ….	**0 → 2.15**
2.14	How often did this happen in the past [4 weeks/30 days]? Rare-less than 5 days Sometimes- up to 7 days Often –More than 14 days	Rarely……. 1 …….. 2 Sometimes 3 ...…….. Often ……	
2.15	In the past [4 weeks/30 days] did you or any household member go a whole day and night without eating anything at all because there was not enough food?	No…… 0 …….. 1 Yes ……..…… ….	**0→ 3.1**
2.16	How often did this happen in the past [4 weeks/30 days]? Rare-less <5days Sometimes- 6-14 days Often –>15 days	Rarely……. 1 …….. 2 Sometimes 3 ...…….. Often ……	
3.0	**BEHAVIORAL ASSESSMENT**		
<u>3.1</u>	Do you currently smoke regularly or have you ever smoked cigarettes?	No…… 0 …….. 1 Yes ……..…… ….	**0 →3.7**
<u>3.2</u>	If you are a current or ex-smoker, how many cigarettes do you or did you previously smoke on average per day	<5…… 1 ……. 2 6- 3 10…… 4 …… 5 11- 20…… …... 21- 30…… …... >30…… …….	
3.3	If you are an ex-smoker, how many years ago did you stop? years **(If less than one year, please insert a zero**	<5…… 1 ……. 2 6- 3 10…… 4 …… 5 11- 20…… …...	

				21-30…… …... >30…… …….	
3.4	Do you currently smoke regularly or have you ever smoked a pipe/cigar?			No…… ……... Yes ……...…… …. 0 1	
3.5	If you are a current or ex-pipe/cigar smoker, how many do you now, or did you previously, smoke on Average per day?			<5…… ……. 6-10…… …… 11-20…… …... 21-30…… …... >30…… ……. 1 2 3 4 5	
3.6	If you are an ex-pipe/cigar smoker, how many years ago did you stop? years (If less than one year, please insert a zero)			< Than 1 year…… …… 2-5 years …… ……. 5-10 years…… ……... >10years…… ……..…… 1 2 3 4	
3.7	Do you drink alcohol?			No…… ……... Yes ……...…… …. 0 1	**0→4.1**
		3.8.What type of alcohol do you drink	3.8a. On Average, how many units of alcohol do you drink per day Unit=500/300ml beers; I glass wine; I glass(250ml) local brew Spirit/whisky-25ml tot	3.8b.On Average, how many units of alcohol do you drink per week Unit=500/300ml beers; I glass wine; I glass(250ml) local brew	
	3.81	Beer			
	3.82	Wine			
	3.83	Changaa			

	3.84	Busaa			
	3.85	Whisky			
	3.86	Muratina			
	3.87	Other			

4.0 MEDICAL HISTORY

4.1 Is there family History of any of the following medical conditions?

4.2 If yes, who in the family

4.3 Have you ever been diagnosed as suffering from any of the following conditions or illnesses(please indicate this in column A

4.4. If yes, are you currently receiving treatment or medication on or a special diet please indicate this in Column B

Disease/ Condition	**4.1. Family History No=0 Yes=1**	**4.2.If yes whom**	**4.3.Column A Ever Diagnosed No = 0 Yes =1**		**4.4. ColumnB Current Tx No=0 Yes=1**
Heart Attack	4.1a	4.2a	4.3a	4.4a	
Angina(chest Pain or exertion)	4.1b	4.2b	4.3b	4.4b	
High Blood Pressure	4.1c	4.2c	4.3c	4.4c	
Diabetes	4.1d	4.2d	4.3d	4.4d	
Arthritis/joint pain	4.1e	4.2e	4.3e	4.4e	
Chronic Back pain	4.1f	4.2f	4.3f	4.4f	
Other(specify)	4.1g	4..2g	4.3g	4.4g	

HEALTH RISK ANALYSIS

4.4	Family history of Heart Disease occurring before 50 years old; Indicate the number of members of your direct family who have died or been diagnosed with Heart Disease before the age of 50.	None…… ……. 1 person …… >1 person…… …… 99 Don't Know……	1 2 3 4

4.5	Family history of Heart Disease occurring after 50 years old. Indicate the number of members of your direct family who have died or been diagnosed with after the age of 50.	None…… 1 ….. 2 1 person 3 …….……..… 4 >1 person…… … 99 Don't Know……	
4.6	Personal history of heart disease. Have you ever been diagnosed with any form of heart disease?	No…… 0 … 1 Yes…… 2 …… Don't Know……	
4.7	Have you had any major illness within the past five years?	No…… 0 …….. 1 Yes…… 2 …… Don't Know……..	**0 → 4.8**
4.7a	If yes Specify		
4.8	Do you have any disability	No…… 0 …… 1 Yes…… 2 …… Don't Know……	**0 → 4.9**
4.8a	If yes, please specify	☐☐	
4.9	In the past year how many times have you visited your doctor?	☐☐	
4.10	In the past year how many days have you been in hospital? (Days)	☐☐	
5.0	**SLEEP** **The next question is about getting enough rest or sleep.**		
5.1	What time do you go to sleep?	☐☐ pm	
5.2	How long does it take you to fall asleep?	☐☐☐	
5.3	What time do you get up in the morning?	☐☐	
5.4	Do you have disrupted sleep?	No…… 0 …… 1 Yes…… 2 …… Don't Know……	0 → 5.5
5.4a	If yes when		

5.4 b	If yes why		
5.5	During the past 30 days, for about how many days have you felt you did not get enough rest or sleep?	Rarely……. 1 …….. 2 Sometimes 3 ...……. Often ……	
5.6	If yes above, does it affect you when you wake up the following day?	No…… 0 …… 1 Yes…… 2 …… Don't Know……	
5.7	If it affects your feelings in the morning, please explain how(summarize e.g. Tired, weak, cannot concentrate etc)		

DIETARY ASSESSMENT

1.0 FRUITS CONSUMPTION IF NONE INDICATE 00

7.1	Do you consume fruits	NO…… 0 YES…….. 1	
7.2	How often do you consume any type of fruits **(if answer is none indicate 00)**	per day ☐☐ Per week …… ☐☐	
7.3	What are your **FOUR (4)** favorite fruits starting with the most favorite to the least 1_____ 2_____ 3_____ 4_____	Apple…….. 1 Mango……. 2 Pawpaw…… 3 Pineapple……. 4 Watermelon…… 5 Oranges…….. 6 Ripe Banana…… 7 Passion fruit…… 8 Quava…….. 9 Loquards……. 1 Quava……. 0 Sweet melon……. 1 Tomato fruit…… 1 Other_____ 1 Non specific …….. 2	
	VEGETABLE CONSUMPTION		

7.4	How often do you consume any type of vegetables **(if answer is none indicate 00)**	per day ☐☐ Per week …… ☐☐	
	DIETARY FAT CONSUMPTION		
7.3	What type of oil or fat do you use for frying food	Vegetable fat …….. 1 Vegetable oil …….. 2 Ghee……. 3 Both…….. 4 Do not Usefat/oil……. 5 Do not know……. 6	
7.4	Please give reasons as to why you use the type of fat you have indicated above	1 2 3 4	
7.5	At what point do you add salt to your food?	At the beginning……. 1 Half way through Cooking…… 2 When Food is ready before serving…… 3 .. 4 At the Table only…… 5 Both (table /cooking)…… 6 I do not Use salt ……..	
7.7	What is your habit in regard to adding salt to food at the table	Never……. 1 Sometimes…… 2 Always…….. 3 Rarely…… 4 . 5 Add before tasting food…….	
	BEVERAGES		
7.8	Do you drink coffee?	No…… 0 Yes…….. 1	**0 → 7.9**
7.8 a	If yes how many cups of coffee do you drink per day /week	Per day ☐☐ Per week ☐☐	
7.9	Do you have any food intolerances that you know off?	No…… 0 Yes…….. 1	
7.9 a	If yes specify	1 2 3	

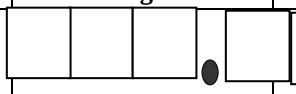
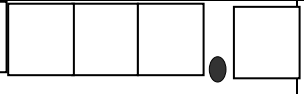
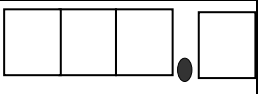
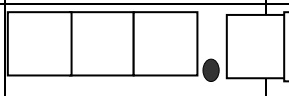
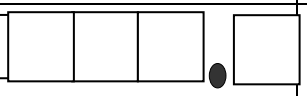
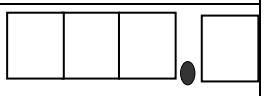
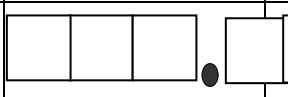
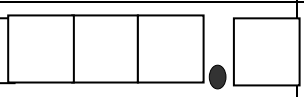
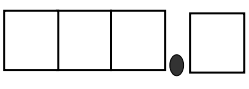
	MEATS AND MEAT PRODUCTS		
7.10	Do you consume red meat?	No…… 0 Yes…….. 1	**0 ➔ 7.14**
7.11	How often do you consume red meat in a day?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…… 5 .. None ……. Other…… …	
7.12	If you do not eat meat daily, how often per week?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…… .. Other …….	
7.13	If you do not eat pee week, how often per month?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…… .. Other …….	
7.14	Do you consume white meat (chicken /fish)?	No…… 0 Yes…….. 1	**0 ➔ 7.18**
7.15	How often do you consume white meat in a day?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…… 5 .. None ……. Other…… …	
7.16	If you do not eat white meat daily, how often per week?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…… .. Other …….	

7.17	If you do not eat per week, how often per month?	Once…… 1 …. 2 Twice…… 3 … 4 Thrice…….. Other …….	
	CONSUMPTION OF WATER		
7.18	Do you drink Water?	No…… 0 Yes…….. 1	**0 →7.20**
7.19	How much water do you consume per day ?	No of glasses/Cups ☐☐ Volume of Glass/cup ☐☐	
7.20	How much salt do you think you consume? (READ LIST)	Far too much..... 1 Too much..... 2 right amount..... 3 Too little..... 4 Far too little..... 5 Don't know..... 6 7	
7.21	Do you think that a high salt diet could cause a serious health problem?	No…… 0 Yes…….. 1	**0 → 8.1**
7.22	If Yes in 4 above, what sort of problem?	High Blood Pressure/hypertension…… 1 …. 2 Stomach…… 3 … 4 Asthma ……. 5 Osteoporosis…….. Other (specify)……	
8.0	**CLINICAL ASSESSMENT** The next few questions are about health-related problems or symptoms**.**		
8.1	During the past 30 days, for about how many days did pain make it hard for you to do your usual	Please indicate actual No. of days	

	activities, such as self-care, work, or recreation?		
8.2	During the past 30 days, for about how many days have you felt very healthy and full of energy?	Please indicate actual No. of days	

8.3	Do you suffer from any of the following? **Signs and Symptoms**	**0=No 1=Yes**	**8.4 If yes When**	**1Week=1 2week=2 3week=3 4week=4**	**8.5. For how long**	
8.31	Severe thirst		8.41a		**8.41b**	
8.32	Frequent passing of urine		8.42a		8.42b	
8.33	Numbness of feet		8.43a		8.43b	
8.34	Numbness of fingers		8.44a		8.44b	
8.35	Pins and needless		8.45a		8.45b	
8.36	Severe hunger		8.46a		8.46b	
8.37	Loss of appetite		8.47a		8.47a	
8.38	Wounds on your legs		8.48a		8.48b	
8.39	Chest pains		8.49a		8.49b	
8.311	Severe anxiety		8.50a		8.50b	
8.312	Shortness of breath		8.51a		8.51b	
8.313	Swollen feet		8.52a		8.52b	
8.314	Swollen ankles		8.53a		8.53b	
8.315	Nausea		8.54a		8.54b	
8.316	Severe headaches		8.55a		8.55b	
8.318	Nose bleeding		8.56a		8.56b	

Anthropometric Assessment

		1st Reading	**2nd Reading**	**3rd Reading**
11.1	**Weight (kg)**			
11.2	**Height (cm)**			
11.3	**Waist C (cm**			

11.4	**Hip C(cm)**			

Blood Pressure Measurements

		1st Reading	**2nd Reading**	**3rd Reading**
11.5	**SYSTOLIC**			
11.6	**DIASTOLIC**			
11.7	**PULSE**			

SCHOOL AGE CHILDREN (8-12Yrs)

Effects of Iodine Exposure on Blood Pressure Among Female School Children and Women in Makueni , Kenya

Randomized Crossover Trial

BASELINE SURVEY

IDENTIFICATION		
HH01. CLUSTER (EA) NAME……	HH02. CLUSTER NUMBER: ____ ____ ____ ____	
HH03. HOUSEHOLD NUMBER: ____ ____ ____	HH04. SUB-LOCATION . …… ____ 1=THAVU 2: ITUKA	
HH05.CLUSTERS: 1. KIUUMONI 2. KATHAMBANGI 3. MATHEMBA 4.MWAANI 5.YEEMULWA 6.KIVANI 7. 8. KOSYA 9. KIVANI 10. KITHAYONI 11. KILAANI/NGUTHUNI 12.KIUSINI 13. ITUKA 14. MASAKU NDOGO		
HH07. INTERVIEWER _____ : ____ ____ NAME CODE	HH08. TEAM LEADER _____ : ____ ____ NAME CODE	
_____ /_____ /_____ DD MM YY	_____ /_____ /_____ DD MM YY	

IDENTIFICATION		
HH01. CLUSTER (EA) NAME……	HH02. CLUSTER NUMBER: ____ ____ ____ ____	
HH03. HOUSEHOLD NUMBER: ____ ____ ____	HH04. SUB-LOCATION . …… ____ 1=THAVU 2: ITUKA	
HH06. IDENTIFICATION NO…… _____ _____ _____		
CLUSTERS: 1. KIUUMONI 2. KATHAMBANGI 3. MATHEMBA 4.MWAANI 5.YEEMULWA 6.KIVANI 7. 8. KOSYA 9. KIVANI 10. KITHAYONI 11. KILAANI/NGUTHUNI 12.KIUSINI 13. ITUKA 14. MASAKU NDOGO		
HH07. INTERVIEWER _____ : ____ ____ NAME CODE	HH08. TEAM LEADER _____ : ____ ____ NAME CODE	
_____ /_____ /_____ DD MM YY	_____ /_____ /_____ DD MM YY	

INTERVIEWER VISITS		
HH10. VISIT 1	**HH11. Follow up visit**	
DATE _____ /_____ /_____ DD MM YY TIME: START: ____:_____ STOP: ____:_____ **RESULT ..... ____	DATE _____ /_____ /_____ DD MM YY TIME: START: ____:_____ STOP: ____:_____ **RESULT ..... ____	HH12. Result Of Individual Interview: 2. COMPLETED 3. NOT AT HOME 4. POSTPONED 5. REFUSED 6. PARTLY COMPLETED 7. INCAPACITATED 8. OTHER

2. SOCIAL DEMOGRAPHIC DATA

Now I would like to ask you some questions about yourself.

No.	QUESTION	CODING CATEGORIES	SKIP
2.1	Girl's Name (three names)		
2.2	Girl's Household Number (ID)	☐☐☐☐	

2.3	Identification No. of Girl selected in the household	
2.4	Relationship to the WRA in the study	aughter..... 1 ister..... 2 iece (sister to father)..... 3 iece (Sister to Mother)..... 4 ousin..... 5 randdaughter..... 6 ther 7
2.5	Age (Years)	
2.6	Date of Birth	_____ /_____ /_____ DD MM YY
2.7	Gender of Girl	1= Female
2.8	Name of respondent for the girl	
2.10	Age of the respondent for the girl(yrs) maybe retain	
2.11	Date of birth	_____ /_____ /_____ DD MM YY
2.12	Gender of the respondent for the girl	1=FEMALE 2=MALE

2.13	What class are you in? 152	None……..…… 1 ……..…... 2 Primary (1-4) …… 3 Primary (5-8)……..	
3.0	BEHAVIORAL ASSESSMENT We know you are still young but we would like to ask you some questions regarding behavior. Do not feel embarrassed to answer them, we will not punish are report you to any one		
3.1	Do you currently smoke regularly or have you ever smoked cigarettes?	No…… 0 ……... 1 Yes ……...…… ……	
3.3	Do you drink alcohol	No…… 0 ……... 1 Yes ……...…… ……	
4.0	**MEDICAL HISTORY**		
4.1	**Is WRA family relation**	NO…… 0 …… 1 YES…… ……...	**1→4.6**
4.2	Family history of Heart Disease occurring before 50 years old; Indicate the number of members of your direct family who have died or been diagnosed with Heart Disease before the age of 50. (**Link to the WRA)**	None…… 1 …… 2 1 person 3 …… 4 >1 person…… …… 99 Don't Know……	
4.3	Family history of Heart Disease occurring after 50 years old. Indicate the number of members of your direct family who have died or been diagnosed with after the age of 50. (**Link to the WRA**)	None……. 1 …. 2 1 person 3 ……... 4 >1 person…… 99 Don't Know	
4.4 **4.5** **4.6**	Is there family History of any of the following medical conditions? If Yes whom Have you (SAC)ever been diagnosed as suffering from any of the following conditions or illnesses(please indicate this in column A If yes, are you (SAC) currently receiving treatment or medication on or a special diet please indicate this in Column B		

Disease/ Condition	**4.1. Family History No=0**	**4.2.If yes whom**	**4.6.Column A Ever Diagnosed No = 0 Yes =1**		**4.7. ColumnB Current Tx No=0 Yes= 1**

	Yes= 1					
Heart Attack	4.1a	4.2a	4.6a	4.7 a		
Angina(chest Pain or exertion)	4.1b	4.2b	4.6b	4.7 b		
High Blood Pressure	4.1c	4.2c	4.6c	4.7 c		
Diabetes	4.1d	4.2d	4.6d	4.7 d		
Arthritis/joint pain	4.1e	4.2e	4.6e	4.7 e		
Chronic Back pain	4.1f	4.2f	4.6f	4.7 f		
Other(specify)	4.1g	4.2g	4.6g	4.7 g		

	HEALTH RISK ANALYSIS		
4.8	Personal history of heart disease. Have you ever been diagnosed with any form of heart disease?	No…… 0 …….. 1 Yes…… 2 … Don't Know……	
4.9	Have you had any major chronic disease (CVD, heart disease, diabetes) illness within the past five years?	No…… 0 …… 1 Yes…… 2 …… Don't Know……	**0 → 4.10**
4.10a	If yes Specify		
4.10	Do you have any disability	No…… 0 …….. 1 Yes…… 2 …… Don't Know……	**0 → 4.10**
4.10a	If yes, please specify	☐☐	
4.11	In the past year how many times have you visited your doctor?	☐☐	
4.12	In the past year how many days have you been in hospital? (Days)	☐☐	
5.0	**SLEEP** **The next question is about getting enough rest or sleep.**		
5.1	What time do you go to sleep?	☐☐ pm	

5.2	How long does it take you to fall asleep?	☐☐	
5.3	What time do you get up in the morning?	☐☐ a m	
5.4	Do you have disrupted sleep?	No…… 0 …… 1 Yes…… 2 …… Don't Know……	0 →5.5
5.4 a	If yes when	1 2	
5.4 b	If yes why	1 2	
5.5	During the past 30 days, for about how many days have you felt you did not get enough rest or sleep?	☐☐	

6.0	**PHYSICAL ACTIVITY**		
	Part 1: SCHOOL RELATED PHYSICAL ACTIVITY		
	The first section is about your school physical activity.		
6.1	Do you participate in any school physical activities?	NO…… 0 …… 1 YES…… ……..	
6.2	If yes Which ones	1 2 3	
6.2	What work do you perform away from school for example at home	1 2 3	

DIETARY ASSESSMENT

7.0 FRUITS CONSUMPTION IF NONE INDICATE 00

7.1	Do you eat fruits?	NO…… 0 ……. 1 Yes…… ……	0-7.4
7.2	How often do you consume any type of fruits **(if answer is none indicate 00)**	per day ☐☐ Per week …… ☐☐	
7.3	What are your **FOUR (4)** favorite fruits starting with the most favorite to the least 1_____ _	Apple…….. 1 Mango……. 2 Pawpaw…… 3 Pineapple……. 4 Watermelon…… 5	

	2_____ _ 3_____ _ 4_____ _	Oranges…….. 6 Ripe Banana…… 7 Passion fruit…… 8 Quava…….. 9 Loquards……. 1 Quava……. 0 Sweet melon…… 1 Tomato fruit…… 1 Other_____ 1 Non specific ……... 2 1 3 1 4 1 5	
	VEGETABLE CONSUMPTION		
7.4	How often do you consume any type of vegetables **(if answer is none indicate 00)**	per day ☐☐ Per week …… ☐☐	
	DIETARY FAT CONSUMPTION		
7.5	What type of oil or fat is used for frying food	Vegetable fat …….. 1 Vegetable oil …….. 2 Ghee…… 3 . 4 Both……. 5 . 6 Do not Usefat/oil……. Do not know……	
7.6	Please give reasons as to why you use the type of fat you have indicated above	Price…… 1 … 2 Availability…… 3 …. Other…… ….	
7.7	At what point do you add salt to your food?	At the Table …… 1 Both…… 2 … 3 I do not Use salt …….. 4 At the beginning……. 5 Half way through Cooking…… When Food is ready before 6 serving…… 7 ….. Do not add salt ……..	
7.8	How often do you add salt to your food at the table or in cooking?	Never…… 1 Sometimes…… 2 Always……. 3 Rarely……. 4	
	BEVERAGES		
7.9	Do you drink coffee?	No…… 0 Yes…….. 1	**1 → 7.10**
7.9a	If yes how many cups of coffee do you drink per day	☐☐	
7.10	Do you have any food intolerances that you know off?	No…… 0 Yes…….. 1	

7.10 a	If yes specify		
	MEATS AND MEAT PRODUCTS		
7.11	Do you consume red meat?	No…… 0 Yes…….. 1	**1 → 7.15**
7.12	How often do you consume red meat in a day?	Once…… 1 Twice…… 2 Thrice…… 3 None 4 ……. 5 Other……	
7.13	If you do not eat meat daily, how often per week?	Once…… 1 Twice…… 2 Thrice…… 3 Other 4 …….	
7.14	If you do not eat pee week, how often per month?	Once…… 1 Twice…… 2 Thrice…… 3 Other 4 …….	
7.15	Do you consume white meat (chicken /fish)?	No…… 0 Yes…….. 1	**1 → 7.19**
7.16	How often do you consume white meat in a day?	Once…… 1 Twice…….. 2 Thrice…… 3 None…… 4 Other…… 5	
7.17	If you do not eat white meat daily, how often per week?	Once…… 1 Twice…….. 2 Thrice…….. 3 Other ……. 4	
7.18	If you do not eat per week, how often per month?	Once…… 1 Twice……. 2 Thrice……. 3 Other …… 4	
	CONSUMPTION OF WATER		
7.19	Do you drink Water?	No…… 0 Yes…….. 1	**0 → 7.21**
7.20	How much water do you consume per day ?	No of glasses/Cups ☐☐ Volume of Glass/cup ☐☐	
7.21	How much salt do you think you consume? (READ LIST)	1 Far too much..... 2 Too much..... 3 right amount..... 4 Too little..... 5 Far too little..... 6 Don't know..... 7	
7.22	Do you think that a high salt diet could cause a serious health problem?	No…… 0 Yes…….. 1	**0 → 8.1**
7.23	If Yes in 4 above, what sort of problem?	High Blood Pressure/hypertension…… 1 Stomach…… 2 Asthma …… 3 Osteoporosis…… 4	
8.0	**CLINICAL ASSESSMENT**		

	The next few questions are about health-related problems or symptoms.		
8.1	During the past 30 days, for about how many days did pain make it hard for you to do your usual activities, such as self-care, work, or recreation?	Please indicate actual No. of days	
8.2	During the past 30 days, for about how many days have you felt very healthy and full of energy?	Please indicate actual No. of days	
8.3	Have you started your menstrual cycles?	No…… 0 Yes…….. 1	**0→8.6**
8.9	If Yes, at what age did you start? Or date	☐☐	
8.5	Are your menstrual cycles regular (every month?)	No…… 0 Yes…….. 1	
8.6	Do you suffer from any of the following?		

8.4	Do you suffer from any of the following? **Signs and Symptoms**	**0=No 1=Yes**	**8.4 If yes When**	**1Week=1 2week=2 3week=3 4week=4**	**8.5. For how long**	
8.41	Severe thirst		8.41a		**8.41b**	
8.42	Frequent passing of urine		8.42a		8.42b	
8.43	Numbness of feet		8.43a		8.43b	
8.44	Numbness of fingers		8.44a		8.44b	
8.45	Pins and needless		8.45a		8.45b	
8.46	Severe hunger		8.46a		8.46b	
8.47	Loss of appetite		8.47a		8.47a	
8.48	Wounds on your legs		8.48a		8.48b	
8.49	Chest pains		8.49a		8.49b	
8.50	Severe anxiety		8.50a		8.50b	
8.51	Shortness of breath		8.51a		8.51b	
8.52	Swollen feet		8.52a		8.52b	
8.53	Swollen ankles		8.53a		8.53b	
8.54	Nausea		8.54a		8.54b	
8.55	Severe headaches		8.55a		8.55b	
8.56	Nose bleeding		8.56a		8.56b	

Anthropometric Assessment

		1st Reading	2nd Reading	3rd Reading
11.1	Weight (kg)			
11.2	Height (cm)			
11.3	Waist C (cm			
11.4	Hip C(cm)			

Blood Pressure Measurements

		1st Reading	2nd Reading	3rd Reading
11.5	SYSTOLIC			
11.6	DIASTOLIC			
11.7	PULSE			

INTERVIEWER'S OBSERVATIONS

TO BE FILLED IN AFTER COMPLETING INTERVIEW

COMMENTS ABOUT RESPONDENT:

_____

_____

COMMENTS ON SPECIFIC QUESTIONS:

_____

_____

_____

_____

APPENDIX 3.8. SAMPLE COLLECTION TOOL

BLOOD PROCESSING FORM: PLAIN TUBE

EIE Project

Date: / /2013

H/H ID:

	Target group e.g.(SAC)	**Label ID: 4 digits**	**Blood volume(ml)**	**Prepared CRP/AGP (circle)**	**Prepared TSH/Free T4(circle)**	**Stored sample(serum (circle)**	**comments**
1.				Y N	Y N	Y N	
2.				Y N	Y N	Y N	
3.				Y N	Y N	Y N	
4.				Y N	Y N	Y N	
5.				Y N	Y N	Y N	
6.				Y N	Y N	Y N	
7.				Y N	Y N	Y N	

APPENDIX 3.9: ANTHROPOMETIC ASSESSSMENT PROTOCOL FOR HEIGHT AND WEIGHT

Standing height was measured as stretch stature using portable height meters. Participants stood upright on their feet (without shoes) without headgear, with arms hanging loosely on the sides and the back against the stadiometer. Measurements were recorded to the nearest 0.1 cm. Body weights were obtained using electronic weighing scales for accuracy in reading and recording. The scales were placed on a flat surface and zero checked periodically before every measurement. Participants were weighed without shoes, while wearing minimal clothing. The readings were recorded in duplicate. The scales were also be periodically calibrated using weights.

Body mass index calculation

Body mass index was calculated relating to the WHO references (2000), where any BMI below 18.5 kg/m^2 is underweight while 18.65-24.9 kg/m^2 were considered normal weight and a BMI above 24.9kg /m^2 is overweight. Overweight is also classified into pre-obese 25.00 kg/m^2 to 29.99, kg/m^2 obesity class 1, 30.00 kg/m2 to 34.99 kg/m^2, obesity class 11, 35.00 kg/m2 to 39.99 kg/m2, and obesity class 111 above or equal to 40.00 kg/m^2.

Anthropometric assessment in children

The children were classified into severely thin, thin, normal, overweight or obese based on WHO recommendations (WHO, 2006; WHO, 2007). A child with a BAZ below -3SD was classified as severely thin (severe acute malnutrition), -3SD to -2SD as thin (moderate malnutrition), +1SD to +2SD as overweight and ≥ +2SD indicated that a child is obese.

Body Circumferences

Waist circumference, hip circumference, and subsequently waist hip ratio is an independent risk factor assessment tool, for determination of cardiovascular risks irrespective of the weight. All the results were classified according to WHO standards. The WHO standards indicates that the waist circumference should be equal to or below 80cm and 95cm among women and men respectively.

APPENDIX 3.10. ANALYSIS OF URINARY SODIUM, POTASSIUM, LITHIUM

Preparation of Standard solutions
Standard solutions were prepared from the salts of the elements in required concentrations. 1000 ppm standard solution was dissolved in 1000 mg of element in 1000 ml of double distilled water.

Sodium Standard Solution (Na)
635 mg of "Anal R" grade of NaCl was weighed and dissolved in exactly 250 ml of double distilled water. The solution so obtained had 1000 ppm of Na. The solution was diluted to 1:10 with double distilled water to make a standard of 100 ppm. 40 ml of this solution was dissolved in 60 ml double distilled water to make 100 ml of 40 ppm solution of Na.
Potassium Standard Solution (K)
477 mg of "Anal R" grade of KCl was weighed and dissolve in exactly 250 ml of double distilled water. The solution so obtained had 1000 ppm of K. The solution was diluted to 1:10 with double distilled water to make standard solution of 100ppm. 40 ml of this solution was dissolved in 60 ml double distilled water to make 100 ml of 40 ppm solution of K.
Lithium Standard Solution (Li)
1331 mg of "Anal R" grade $Li2CO^3$ was weighed and dissolve in minimum quantity of 1:1 HCl to make up exactly 250 ml of double distilled water. The solution so obtained had 1000 ppm of Ca. the solution was diluted to 1:10 with double distilled water to make standard solution of 100 ppm. 40 ml of this solution was dissolve in 60 ml double distilled water to make 100 ml of 40 ppm solution of Li.
Analysis Procedure for sodium, potassium and lithium

1. The machine was switched on and allowed average of 30 minutes for warm up
2. Calibration was then done using five standards
3. The standards were then read as samples to verify their concentrations
4. The controls and samples were diluted appropriately
5. Bench controls were aspirated and their readings in Meq/L recorded and later converted to mg/L
6. Samples were aspirated with at least three controls aspirated after every ten samples, and the machine recalibrated after every 30 samples

Bench controls
Minimum of 20 readings was obtained per each control in three different days. The mean standard deviation and coefficient of variation computed after the 20 readings. This was used to get the range of the bench controls to be used in order to check if the samples readings would fall within the accepted range during analysis (SD±2). The controls (in Meq/L without dilution factor) with ranges are summarized as below:

APPENDIX 3.11: LOCAL QUALITY CONTROL CHART FOR URINARY IODINE CONCENTRATION

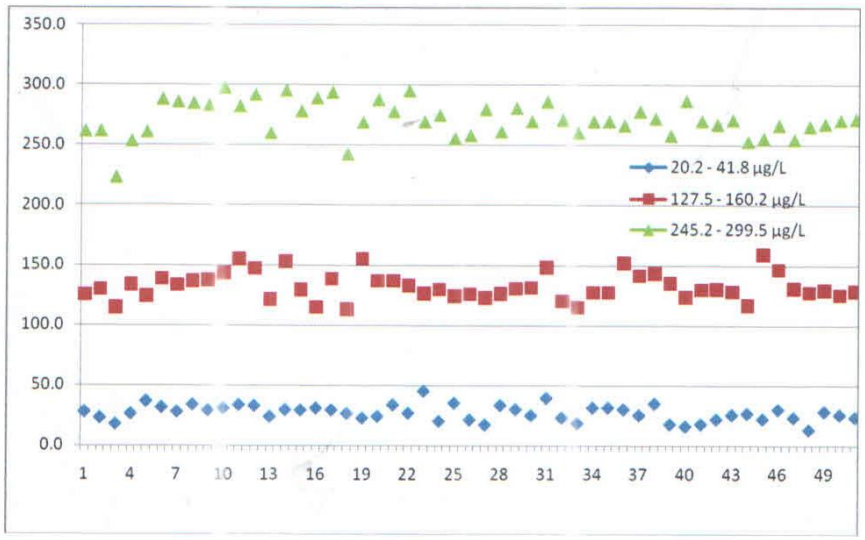
Control	Element	Mean	SD	CV	Range (SD±2)	
A	Na	136.4	2.5	1.8	131.5	141.5
	K	1.8	0.2	1.1	1.8	1.9
B	Na	228.4	10.3	4.1	227.5	269.3
	K	4.2	0.1	2.7	4	4.5
C	Na	170.2	2.9	1.7	164.4	176.1
	K	2.7	0.05	1.8	2.7	2.9
S	Na	158.2	4.9	3.1	148	168
	K	2.4	0.2	4.2	2	2.8
E	Na	124	4.6	3.7	115.3	133.4
	K	3.9	0.1	3.2	3.7	4.2
R	Na	131	5.4	4.1	110.2	142.2
	K	3.4	0.1	3	3.2	3.6
X	Na	27.6	0.64	2.33	26.3	28.9
	K	0.3	_	_	0.3	0.3

Date	Run No	QC-Low		QC-Medium		QC-High		
		20.2 - 41.8 µg/L	Remarks	127.5 - 160.2 µg/L	Remarks	245.2 - 299.5 µg/L	Remarks	Final score of the run
07.11.2014	1	28.1	Pass	125.3	Fail	261.6	Pass	Accepted
07.11.2014	2	23.2	Pass	129.9	Pass	261.7	Pass	Accepted
09.11.2014	3	18.0	Fail	114.8	Fail	223.1	Fail	Rejected
09.11.2014	4	26.5	Pass	133.8	Pass	253.5	Pass	Accepted
09.11.2014	5	36.8	Pass	124.4	Fail	261.1	Pass	Accepted
10.11.2014	6	31.7	Pass	138.6	Pass	288.5	Pass	Accepted
10.11.2014	7	28.1	Pass	133.5	Pass	286.4	Pass	Accepted
10.11.2014	8	34.0	Pass	137.0	Pass	285.1	Pass	Accepted
10.11.2014	9	29.2	Pass	137.4	Pass	283.3	Pass	Accepted
10.11.2014	10	30.9	Pass	143.6	Pass	298.0	Pass	Accepted
11.11.2014	11	33.7	Pass	155.2	Pass	282.5	Pass	Accepted
11.11.2014	12	33.0	Pass	147.4	Pass	292.1	Pass	Accepted
11.11.2014	13	24.0	Pass	121.4	Pass	260.1	Pass	Accepted
11.11.2014	14	29.7	Pass	153.0	Pass	295.6	Pass	Accepted
11.11.2014	15	29.5	Pass	129.5	Pass	278.6	Pass	Accepted
11.11.2014	16	31.2	Pass	115.1	Fail	289.4	Pass	Accepted
12.11.2014	17	29.6	Pass	138.5	Pass	294.0	Pass	Accepted
12.11.2014	18	26.7	Pass	113.1	Fail	242.6	Fail	Rejected
12.11.2014	19	22.8	Pass	155.7	Pass	269.3	Pass	Accepted
12.11.2014	20	24.3	Pass	137.3	Pass	288.2	Pass	Accepted
12.11.2014	21	33.8	Pass	137.1	Pass	278.1	Pass	Accepted
12.11.2014	22	27.0	Pass	133.2	Pass	295.4	Pass	Accepted
12.11.2014	23	45.3	Pass	126.5	Fail	269.7	Pass	Accepted
12.11.2014	24	20.3	Pass	129.7	Pass	275.2	Pass	Accepted
12.11.2014	25	35.4	Pass	124.2	Fail	256.0	Pass	Accepted
13.11.2014	26	21.4	Pass	126.2	Fail	258.2	Pass	Accepted
13.11.2014	27	17.3	Fail	122.9	Fail	280.1	Pass	Rejected
13.11.2014	28	33.5	Pass	126.4	Pass	261.5	Pass	Accepted
13.11.2014	29	30.1	Pass	130.7	Pass	281.2	Pass	Accepted
13.11.2014	30	25.1	Pass	131.2	Pass	270.0	Pass	Accepted
13.11.2014	31	39.5	Pass	148.8	Pass	286.4	Pass	Accepted
13.11.2014	32	23.2	Pass	120.3	Pass	271.4	Pass	Accepted
14.11.2014	33	18.6	Fail	115.0	Fail	260.6	Pass	Rejected
14.11.2014	34	31.6	Pass	127.5	Pass	270.1	Pass	Accepted
14.11.2014	35	31.6	Pass	127.5	Pass	270.1	Pass	Accepted

14.11.2014	36	30.2	Pass	152.3	Pass	266.7	Pass	Accepted
14.11.2014	37	25.3	Pass	141.7	Pass	278.5	Pass	Accepted
15.11.2014	38	34.9	Pass	143.5	Pass	272.5	Pass	Accepted
15.11.2014	39	17.8	Fail	135.5	Pass	258.3	Pass	Accepted
15.11.2014	40	16.0	Fail	123.7	Fail	287.2	Pass	Rejected
15.11.2014	41	17.9	Fail	129.7	Passed	270.5	Pass	Accepted
15.11.2014	42	22.0	Pass	130.3	Pass	267.6	Pass	Accepted
17.11.2014	43	25.7	Pass	128.4	Pass	271.3	Pass	Accepted
17.11.2014	44	26.8	Pass	117.1	Fail	253.2	Pass	Accepted
17.11.2014	45	22.3	Pass	159.8	Pass	256.0	Pass	Accepted
17.11.2014	46	30.1	Pass	146.7	Pass	266.9	Pass	Accepted
17.11.2014	47	23.4	Pass	131.0	Pass	255.1	Pass	Accepted
17.11.2014	48	13.3	Fail	127.5	Pass	265.9	Pass	Accepted
20.11.2014	49	28.4	Pass	129.6	Pass	268.1	Pass	Accepted
20.11.2014	50	25.7	Pass	125.5	Fail	271.3	Pass	Accepted
20.11.2014	51	23.6	Pass	128.8	Pass	272.1	Pass	Accepted

APPENDIX 3.12: URINARY IODINE CONCENTRATION QUALITY CONTROL -TNFC

iii. Quality Control Trend Chart (Between assay results of internal QC materials)

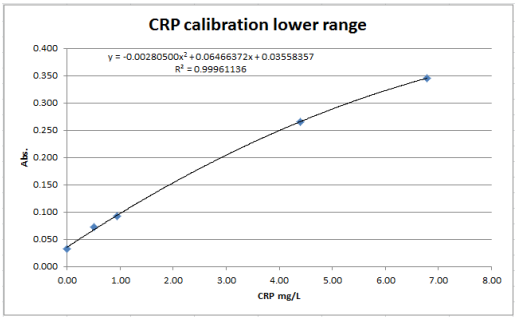


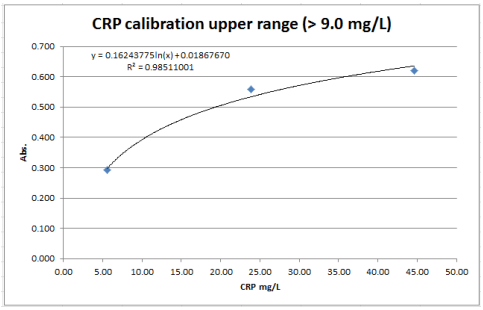
APPENDIX 3.13: QUALITY CONTROL FOR CRP AND AGP

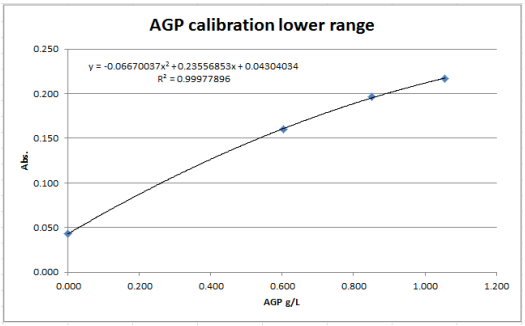
All values represent the mean of an independent double measurement. Measurements with a high CV were repeated and obvious outliers removed. To calculate the results, the calibration curves were adjusted with a control sample which were measured in 10 wells and Biorad Liquickeck controls in 3 different concentrations in 6 wells on each plate. CV of the different indicators on the 384-plates (n = 26) for a pooled plasma sample (expected concentration in brackets)
Quality Control for Ferritin, sTfR, RBP –Juegen Lab, Germany

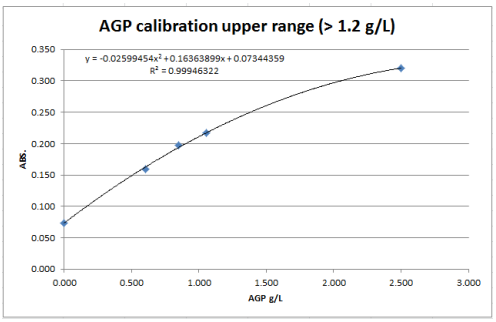
Indicator	Mean	%
CRP (6.86mg/L)	6.79	6.55
AGP (0.975 g/L)	0.97	5.96

Calibration curves of the different indicators (certified QC samples from the CDC/Atlanta and Biorad Liquicheck controls)









APPENDIX 3.14. COMPUTATION OF BLOOD PRESSURE PERCENTILES IN CHILDREN

To compute the systolic blood pressure (SBP) percentile of a girl aged y years and height h inches with SBP = x mmHg:

1. Refer to the most recent CDC growth charts, which are available online, and convert the height of h inches to a height Z-score relative to boys of the same age; this is denoted by Zht.
2. The expected SBP (μ) for girls of age y years and height h inches given by $\mu = \alpha + \Sigma\, \beta j\, (y-10)\, j + \Sigma\, \gamma k\, (Zht)k$ was computed

where α, β 1…, β 4 and γ1…, γ4 are given in the 3rd column of appendix table B–1.

3. The girls' observed SBP was converted to a Z-score (Zbp) given by $Zbp = (x - \mu)/\sigma$

where σ was given in the 3rd column of appendix table B–1.

4. To convert the bp Z-score to a percentile (P), $P = \Phi(Zbp) \times 100\%$ was computed where Φ (Z) = area under a standard normal distribution to the left of Z.
   Thus, if Zbp = 1.28, then Φ (Zbp) = .90 and the bp percentile = .90 x 100% = 90%.
5. To compute percentiles for SBP for girls, diastolic blood pressure DBP (K5) for girls, we used the regression coefficients from the 4th, 5th, and 6th columns of appendix table B–1.
6.

For example, a 12-year-old girl, with height at the 90th percentile for her age-sex group, has a height Z-score = 1.28, and her expected SBP (μ) was
$\mu = 102.19768 + 1.82416\,(2) + 0.12776\,(22) + 0.00249\,(23) - 0.00135\,(24) + 2.73157\,(1.28) - 0.19618\,(1.28)^2 - 0.04659\,(1.28)^3 + 0.00947\,(1.28)^4 = 109.46$ mmHg.
Suppose her actual SBP is 120 mmHg (x); his SBP Z-score is then:
$\text{SBP Z-score} = (x - \mu)/\sigma = (120 - 109.46)/10.7128 = 0.984$
The corresponding SBP percentile = $\Phi(0.984) \times 100\% = 83.7$th percentile.

APPENDIX 3.15A: REGRESSION COEFFICIENTS FROM BLOOD PRESSURE REGRESSION MODELS

TABLE B–1

Regression Coefficients From Blood Pressure Regression Models*

Variable Name	Symbol	Systolic BP		Diastolic BP5	
		Male	Female	Male	Female
Intercept	α	102.19768	102.01027	61.01217	60.50510
Age					
Age–10	β_1	1.82416	1.94397	0.68314	1.01301
(Age–10)^2	β_2	0.12776	0.00598	-0.09835	0.01157
(Age–10)^3	β_3	0.00249	-0.00789	0.01711	0.00424
(Age–10)^4	β_4	-0.00135	-0.00059	0.00045	-0.00137
Normalized height					
Zht	γ^1	2.73157	2.03526	1.46993	1.16641
Zht^2	γ^2	-0.19618	0.02534	-0.07849	0.12795
Zht^3	γ^3	-0.04659	-0.01884	-0.03144	-0.03869
Zht^4	γ^4	0.00947	0.00121	0.00967	-0.00079
Standard deviation	σ	10.7128	10.4855	11.6032	10.9573
ρ†		0.4100	0.3824	0.2436	0.2598
n (persons)		32,161	31,066	24,057	23,443
n (*visits*)		*42,074*	*41,017*	*29,182*	*28,794*

BP, blood pressure; Diastolic BP5, diastolic measurement at Korotkoff 5.

* The coefficients were obtained from mixed-effects linear regression models.

† The value of ρ represents the correlation between BP measurements at different ages for the same child after correcting for age and Zht. This computation was necessary because some studies contributing to the childhood BP database provided BP at more than one age.

Source: NIH, 2005

APPENDIX 3.15B: BLOOD PRESSURE CHARTS FOR GIRLS BY AGE AND PERCENTILES

TABLE 4

Blood Pressure Levels for Girls by Age and Height Percentile*

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88

12 The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean. For research purposes, the standard deviations in appendix table B–1 allow one to compute BP Z-scores and percentiles for girls with height percentiles given in table 4 (i.e., the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles). These height percentiles must be converted to height Z-scores given by (5% = -1.645; 10% = -1.28; 25% = -0.68; 50% = 0; 75% = 0.68; 90% = 1.28; 95% = 1.645) and then computed according to the methodology in steps 2–4 described in appendix B. For children with height percentiles other than these, follow steps 1–4 as described in appendix B.

Source: NIH 2005

APPENDIX 3.16: CLASSIFICATION OF HYPERTENSION IN CHILDREN

TABLE 5

Classification of Hypertension in Children and Adolescents, With Measurement Frequency and Therapy Recommendations

	SBP or DBP Percentile*	Frequency of BP Measurement	Therapeutic Lifestyle Changes	Pharmacologic Therapy
Normal	<90th	Recheck at next scheduled physical examination.	Encourage healthy diet, sleep, and physical activity.	—
Prehypertension	90th to <95th or if BP exceeds 120/80 mmHg even if below 90th percentile up to <95th percentile†	Recheck in 6 months.	Weight-management counseling if overweight, introduce physical activity and diet management.‡	None unless compelling indications such as CKD, diabetes mellitus, heart failure, or LVH exist
Stage 1 hypertension	95th percentile to the 99th percentile plus 5 mmHg	Recheck in 1–2 weeks or sooner if the patient is symptomatic; if persistently elevated on two additional occasions, evaluate or refer to source of care within 1 month.	Weight-management counseling if overweight, introduce physical activity and diet management.‡	Initiate therapy based on indications in Table 6 or if compelling indications as above.
Stage 2 hypertension	>99th percentile plus 5 mmHg	Evaluate or refer to source of care within 1 week or immediately if the patient is symptomatic.	Weight-management counseling if overweight, introduce physical activity and diet management.‡	Initiate therapy.§

BP, blood pressure; CKD, chronic kidney disease; DBP, diastolic blood pressure; LVH, left ventricular hypertrophy; SBP, systolic blood pressure

* For sex, age, and height measured on at least three separate occasions; if systolic and diastolic categories are different, categorize by the higher value.

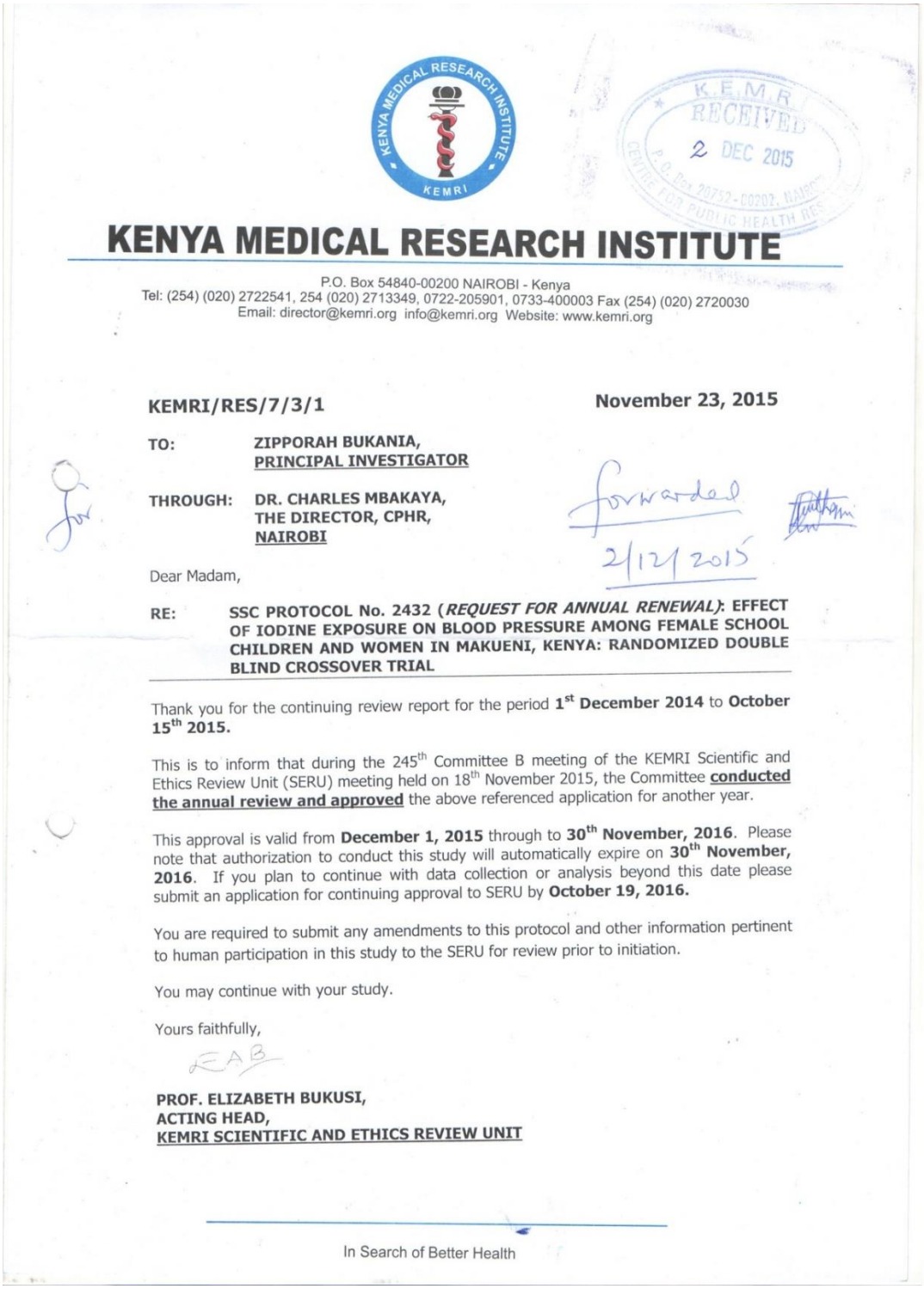
† This occurs typically at 12 years old for SBP and at 16 years old for DBP.

‡ Parents and children trying to modify the eating plan to the Dietary Approaches to Stop Hypertension (DASH) eating plan could benefit from consultation with a registered or licensed nutritionist to get them started.

§ More than one drug may be required.

Source NIH 2005

APPENDIX 3.17: ETHICS CERTIFICATE



KENYA MEDICAL RESEARCH INSTITUTE

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Email: director@kemri.org info@kemri.org Website: www.kemri.org

K.E.M.R.I. RECEIVED 2 DEC 2015 CENTRE FOR PUBLIC HEALTH RES.

KEMRI/RES/7/3/1 **November 23, 2015**

TO: **ZIPPORAH BUKANIA,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. CHARLES MBAKAYA,**
THE DIRECTOR, CPHR,
NAIROBI

forwarded
2/12/2015

Dear Madam,

RE: **SSC PROTOCOL No. 2432 (*REQUEST FOR ANNUAL RENEWAL*): EFFECT OF IODINE EXPOSURE ON BLOOD PRESSURE AMONG FEMALE SCHOOL CHILDREN AND WOMEN IN MAKUENI, KENYA: RANDOMIZED DOUBLE BLIND CROSSOVER TRIAL**

Thank you for the continuing review report for the period **1st December 2014** to **October 15th 2015.**

This is to inform that during the 245th Committee B meeting of the KEMRI Scientific and Ethics Review Unit (SERU) meeting held on 18th November 2015, the Committee **conducted the annual review and approved** the above referenced application for another year.

This approval is valid from **December 1, 2015** through to **30th November, 2016**. Please note that authorization to conduct this study will automatically expire on **30th November, 2016**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to SERU by **October 19, 2016.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SERU for review prior to initiation.

You may continue with your study.

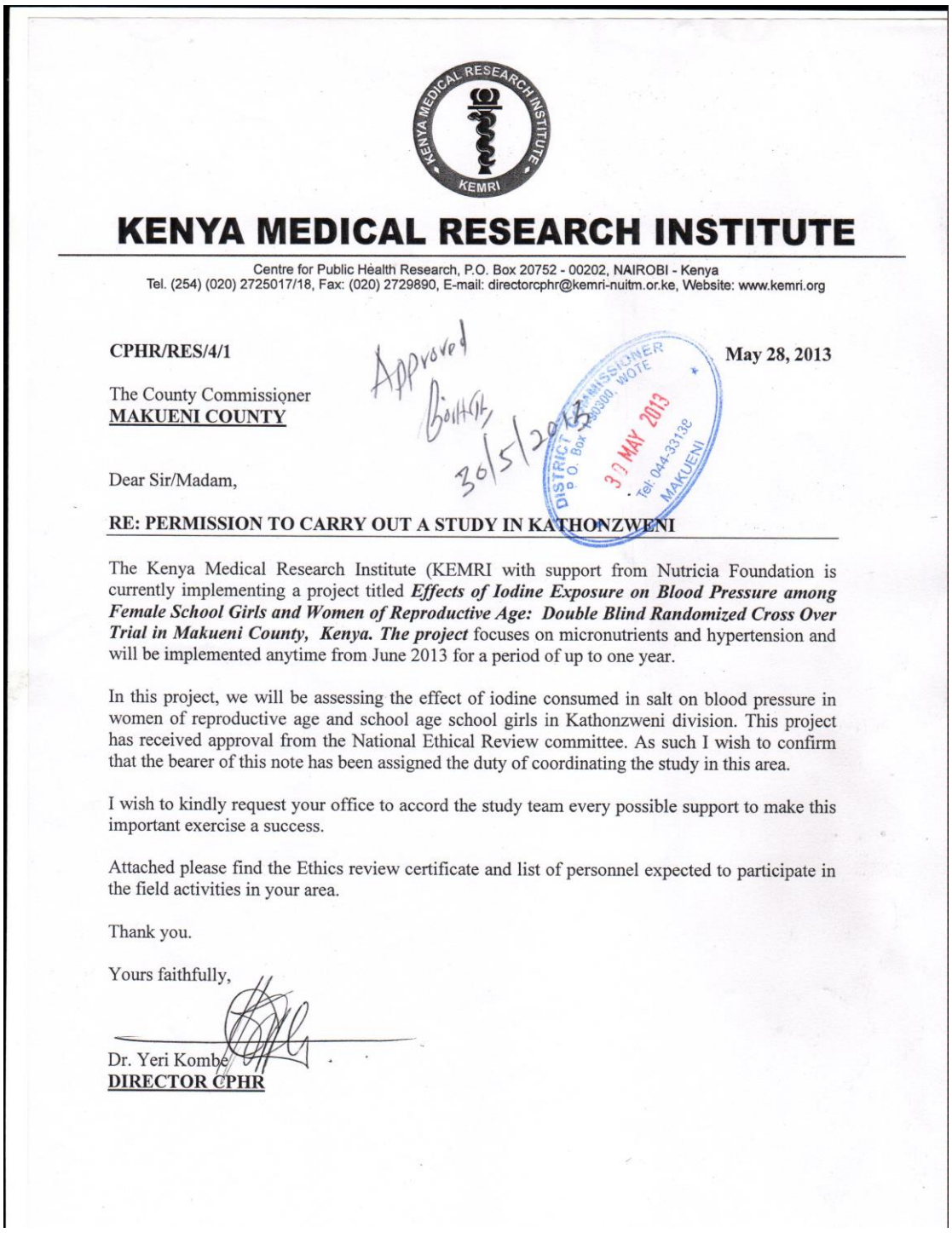
Yours faithfully,

EAB

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

APPENDIX 3.18: MAKUENI COUNTY GOVERNMENT APPROVAL LETTER



KENYA MEDICAL RESEARCH INSTITUTE

Centre for Public Health Research, P.O. Box 20752 - 00202, NAIROBI - Kenya
Tel. (254) (020) 2725017/18, Fax: (020) 2729890, E-mail: directorcphr@kemri-nuitm.or.ke, Website: www.kemri.org

CPHR/RES/4/1 **May 28, 2013**

Approved
30/5/2013

DISTRICT COMMISSIONER P. O. Box 300300, WOTE 31 MAY 2013 Tel: 044-331 38 MAKUENI

The County Commissioner
MAKUENI COUNTY

Dear Sir/Madam,

RE: PERMISSION TO CARRY OUT A STUDY IN KATHONZWENI

The Kenya Medical Research Institute (KEMRI with support from Nutricia Foundation is currently implementing a project titled ***Effects of Iodine Exposure on Blood Pressure among Female School Girls and Women of Reproductive Age: Double Blind Randomized Cross Over Trial in Makueni County, Kenya. The project*** focuses on micronutrients and hypertension and will be implemented anytime from June 2013 for a period of up to one year.

In this project, we will be assessing the effect of iodine consumed in salt on blood pressure in women of reproductive age and school age school girls in Kathonzweni division. This project has received approval from the National Ethical Review committee. As such I wish to confirm that the bearer of this note has been assigned the duty of coordinating the study in this area.

I wish to kindly request your office to accord the study team every possible support to make this important exercise a success.

Attached please find the Ethics review certificate and list of personnel expected to participate in the field activities in your area.

Thank you.

Yours faithfully,

Dr. Yeri Kombe
DIRECTOR CPHR

APPENDIX 3.19. INFORMED CONSENT DOCUMENT

EFFECT OF IODINE EXPOSURE ON BLOOD PRESSURE AMONG FEMALE SCHOOL CHILDREN AND WOMEN IN MAKUENI, KENYA: RANDOMIZED CROSSOVER TRIAL

PART A. INFORMATION SHEET

THE FIRST PART EXPLAINS THE REASONS FOR THE STUDY AND DESCRIBES THE STUDY. IT WILL BE READ ALOUD TO PARTICIPANTS. THE SECOND PART WILL BE READ TO THE PARTICIPANTS INDIVIDUALLY TO OBTAIN THEIR CONSENT.

Introduction: The Kenyan Medical Research Institute (KEMRI) is carrying out a study among free-living individuals in Makueni. The relationship between blood pressure (BP) and risk of cardiovascular disease (CVD) events is continuous, consistent, and independent of other risk factors. The higher the BP, the higher the risk of heart attack heart failure, stroke and kidney disease. There is a posible association between iodine status and blood pressure because thyroid function interacts with other systems involved in cardiovascular regulation. (Pingtone and Iervasi, 2007). Thyroid dysfunction, both hypothyroidism and hyperthyroidism, may increase the risk of hypertension. An understanding of the possible association between iodine and high blood pressure can be made through this study. It is also important to note that too much salt consumption has been associated with health risks. The common and known link between excess salt consumption and health is predisposition to developing high blood pressure, in addition to other risk factors.

Objectives of the Study

The main aim of this study is to compare the blood pressure effects after consuming salt with low and high iodine content and the effects on urinary iodine, sodium and potasium levels and thyroid status in female school children and adults in Makueni County.

Participation in the study

We are asking you to join this research study. Joining the study is voluntary. Through your participation, we shall be able find out what are the nutritional health behaviours we need to address in-order to prevent people from developing hypertension. We wish to assure you that all materials and instruments we shall use are new, sterile and clean. Our staff who will attend to you are all qualified to look after patients. And the relevant government departments have allowed us to conduct this study.We would like you to know that some of the tests will be done here BUT some will be done in our laboratories in KEMRI. There are no perceived risks of taking part in this study. Alternatively, if you choose not to participate in the study, we will not victimise you in any way. By participating in this study you will be followed up for 56 days with assessments at the beginning, mid and at the end.

What your participation will involve:

1. If you decide to join the study we shall enroll you. Then our staff attached to the project will ask you a number of questions regarding yourself, your home, and health.

2. If you decide to join the study, we shall request you to honour your scheduled appointments at the designated places and to provide accurate information. .

Procedures

Information will be collected about your general lifestyle and a clinical and physical examination will be done by the study medical personnel. You will be asked about your usual dietary patterns and habits. You will be assessed for nutritional status by taking a set of measurements which include weight, height, and body composition. A blood sample of 5 ml will be taken to determine in more detail your biochemical status and to help determine your thyroid function. During the period you are in the study, all the costs of these tests will be paid by the project.

Confidentiality

All information you provide us throughout the study will remain confidential. Only the study team will have access to this information and it will not be relayed to any other persons unless you give your permission. You will be made aware of any important issues related to your health should such a need arise.

Benefits for participating clients:

By participating in this study you will benefit from:

1. You will receive free medical check-up. Those found ill will be referred to hospitals for further treatment.

2. Medical and laboratory tests All tests performed as part of the study will be provided free of charge.

3. Additional nutritional assessments will be available to you, such as the composition of your body.

4. You might feel a little discomfort when blood is being drawn, however there are no other expected complications associated with this exercise.. The team's well-trained and experienced staff will guide you through this exercise and will take necessary precaution to ensure minimum discomfort. Precautions will be taken to ensure the blood draws are conducted safely.

5. Participation in the study will require you to spend slightly extra time for the next 2 months. This may take time away from other activities you may wish to do.

Withdrawal from the study:

You may withdraw from participating in this study at any time without giving the reason. It is only necessary that you inform us incase you make such a decision.

PART B: CONSENT FORM

Please read the information sheet (PART A) or have the information read to you carefully before completing and signing this consent form. If there are any questions you have about the study, please feel free to ask them to the investigators prior to

signing your consent form. If you have any questions that you think affect your rights of participation in the study feel free to contact the secretary National/KEMRI Ethical Review Committee (ERC) on Tel: 2722541/2713349

FOR COMPLETION BY ALL PARTICIPANTS

I have read the information sheet concerning this study and I understand what will be required of me if I take part in the study. Any questions I have concerning this study have been answered.

I understand that at any time that I may wish to withdraw from this study I can do so without giving any reason and without affecting my access to normal health care and management.

I agree to take part in this study.

Name_____Signed_____Date_____
__

Principle Investigtor _____signed_____date_____

APPENDIX: 3.20. ENROLLMENT ASSENT DOCUMENT-ENGLISH

EFFECT OF IODINE EXPOSURE ON BLOOD PRESSURE AMONG FEMALE SCHOOL CHILDREN AND WOMEN IN MAKUENI, KENYA: RANDOMIZED CROSSOVER TRIAL

PART A. INFORMATION SHEET

THE FIRST PART EXPLAINS THE REASONS FOR THE STUDY AND DESCRIBES THE STUDY. IT WILL BE READ ALOUD TO PARTICIPANTS. THE SECOND PART WILL BE READ TO THE PARTICIPANTS INDIVIDUALLY TO OBTAIN THEIR CONSENT.

Introduction: The Kenyan Medical Research Institute (KEMRI) is carrying out a study among free-living individuals in Makueni. The relationship between blood pressure (BP) and risk of cardiovascular disease (CVD) events is continuous, consistent, and independent of other risk factors. The higher the BP, the higher the risk of heart attack heart failure, stroke and kidney disease. There is a posible association between iodine status and blood pressure because thyroid function interacts with other systems involved in cardiovascular regulation. (Pingtone and Iervasi, 2007). Thyroid dysfunction, both hypothyroidism and hyperthyroidism, may increase the risk of hypertension. An understanding of the possible association between iodine and high blood pressure can be made through this study.

Objectives of the Study

The main aim of this study is to compare the blood pressure effects after consuming salt with low and high iodine content and the effects on urinary iodine, sodium and potasium levels and thyroid status in female school children and adults in Makueni County.

Being in the Study is Your Choice:

Allowing your child to be in the study is your choice. This consent form gives you information about the study and the risks will be explained to you. Once you understand the study, and if you agree for your child to take part, you will be asked to sign your name or make your mark on this form.

Before you learn about the study, it is important that you know the following:

1. Your child's participation in this study is entirely voluntary
2. You may decide not to answer questions, give any specimens or even withdraw from the study at any time.

Participation in the Study

In this study, we are trying to learn more about find out what are the nutritional health behaviours we need to address in-order to prevent people from developing hypertension. We wish to assure you that all materials and instruments we shall use are new, sterile and clean. We would like you to know that some of the tests will be done here BUT some will be done in our laboratories in KEMRI.

If you agree your child to participate in this study by signing at the end of this form, you will participate in the study. Your child or you the parent/guardians will be asked questions about your child's past medical dietary, social and economic history. A clinical and physical examination will be done by the study medical person for signs

and symptoms of diseases related. For children aged **8-12years**, they will be requested to give 3 mls of blood and urine for further testing. While those 15 -17 years will be requested to give 5 mls of blood for testing. All of this should take about 45 minutes. By participating in this study the child will be followed up for 56 days with assessments at the beginning, mid and at the end.

Study Groups

The study groups will comprise children of 8-12 years and non pregnant women aged 15 to 49 years, in all selected households. All groups of people mentioned here are very important to this study.

Risks and Benefits of the Study

The study has no serious risks to subjects. However we shall require a small amount of blood from the participants, amounting to just over 1 teaspoonful in quantity. This process will involve injecting the participants with a small needle which might make them feel a little discomfort when blood is being drawn. At the sites of the needle prick. However, the team's well trained and experienced staff will guide you through this exercise and will take necessary steps to ensure minimum discomfort. By agreeing your child to participate in this study, your child will receive a free medical check-up and advice where necessary. If we find that the child is sick, we will refer her/him to the nearest hospital for treatment.

Cost to you

There is no cost to you for your child participating in the study.

Confidentiality

All information provided to us throughout the study will remain confidential and will only be used to provide for the objective it is intended to. Only the study team will have access to this information and it will not be relayed to any other persons . Data will be securely managed.

Your Rights as a Study Participant

This research has been reviewed and approved by the Ethical Review Committee of the Kenyan Medical Research Institute (KEMRI), if you have any questions about your child's rights as a research participant you may contact the secretary of the KEMRI ERC (a group of people who review the research to protect your rights) at 020-272-2541, or 020-272-6781.

Withdrawal from the Study

No one will be upset if you do not want your child to participate, or if you change your mind later and want to stop. You can also skip any of the questions you do not want to answer.If there are any questions you have about the study, please feel free to ask them to the investigator prior to signing your assent form. You may contact the secretary National/KEMRI Ethical Review Committee (ERC) on Tel: 2722541/2713349

Your Statement of Consent and Signature

If you have read the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree to join this study, please carefully read the statements below and think about your choice before signing your name or making your mark below. No matter what you decide, it will not affect your rights in anyway:

- I have been given the chance to ask any questions I have and I am content with the answers to all of my questions.

- I know that my child's records will be kept confidential and that my child may leave this study at any time
- The name, phone number and address of whom to contact in case of an emergency has been told to me, and has also been given to me in writing.
- I agree for my child to take part in this study as a volunteer, and will be given a copy of this informed consent form to keep.

Name of Parent/guardian	**Signature and date**
Name of Research staff	**Signature and date**

APPENDIX 3.21. INFORMED ASSENT FORM FOR MINORS –ENGLISH

EFFECT OF IODINE EXPOSURE ON BLOOD PRESSURE AMONG FEMALE SCHOOL CHILDREN AND WOMEN IN MAKUENI, KENYA: RANDOMIZED CROSSOVER TRIAL

My name is _____ (identify yourself to the child by name), and I am from the Centre for Public Health Research in KEMRI. We are asking you to participate in this research study because you are a child aged between 15-17years

Purpose

In this study we are trying to learn if there is a possible association between iodine status and blood pressure because thyroid function interacts with other systems involved in cardiovascular regulation.

Participation

You will do the following today: You will be asked to answer some questions on what you ate yesterday, you will be weighed and your body measurements taken, and the nurse will examine you for any signs or symptoms of illness.

Risks & benefits

You might feel a little discomfort when blood is being drawn, however, the team's well trained and experienced staff will guide you through this exercise and will take necessary steps to ensure minimum discomfort. By agreeing to participate in this study, you will receive a free medical check-up and advice where necessary. If we find that you are sick, we will refer you to the nearest hospital for treatment.

Compensation

We have already asked your parents if it is alright for me to ask you to take part in this study. Even though your parents said I could ask you, you still get to decide if you want to be in this research study. You can also talk with your parents, grandparents, and teachers before deciding whether or not to take part. No one will be upset if you do not want to participate, or if you change your mind later and want to stop. You can also skip any of the questions you do not want to answer.

You can ask questions now or whenever you wish. If you want to, you may call our study leader _____ (provide phone number).

Please sign your name below, if you agree to be part of my study. I will give both you and your parents a copy of this form after you have signed it

<u>Participants' Statement</u>

I, _____understand that my parents (mom and dad)/guardian have/has given permission (said it's okay) for me to take part in a project about done by

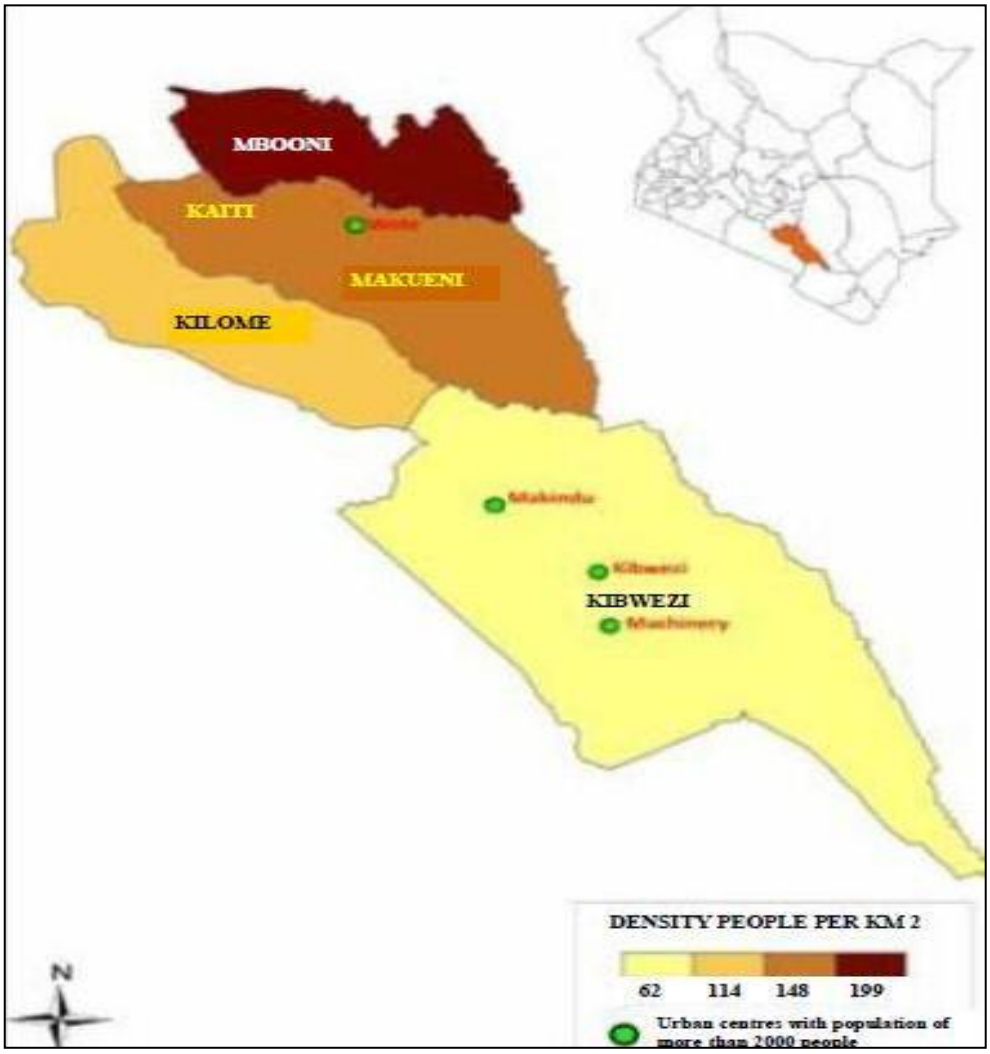
I am taking part because I want to. I have been told that I can stop at any time I want to and nothing will happen to me if I want to stop.

Name of Participant **Signature and date**

_____ _____

Name of Research staff **Signature and date**

APPENDIX 3.22. MAP OF MAKUENI COUNTY



APPENDIX 3.23. PUBLICATIONS

(Attached)

1. **Bukania ZN, Mwangi M, Kaduka LU, Kimiywe J and van der Haar F.(2015).** Differences in Blood Pressure between Arms of Normotensive Female Farmers in Semi-arid Midlands of Kenya. Epidemiology; Vol, 2015. https://www.omicsonline.org/open-access/difference-in-blood-pressure-between-arms-of-normotensive-female-farmers-in-the-semiarid-midlands-of-kenya-2161-1165-S1-002.pdf

2. **Bukania ZN, Kimiywe J, John T, Mwangi M, Kaduka LU, et al. (2017)** Inadequate and Excessive Urinary Iodine Concetration in School Age Girls and Women in Makueni, Eastern Kenya. J Epid Prev Med 3(1): 125 https://www.elynsgroup.com/journal/article/inadequate-and-excessive-urinary-iodine-concentration-in-school-age-girls-and-women-in-makueni-eastern-kenya