VALIDATION OF A FOOD FREQUENCY QUESTIONNAIRE
AND A SPOT URINE SAMPLE FOR THE ASSESSMENT OF
DIETARY SODIUM INTAKE IN LEBANESE ADULTS

by

SALMA ROMANOS CHOUCCAIR

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Signature: ___________________________ Date: 5/5/2016
I would like to take this opportunity to thank everyone who made this thesis possible and made my graduate experience an unforgettable one.

To mom, thank you for fighting against the odds and fulfilling my dream in attending AUB. Thank you for believing in me and relentlessly pushing me to strive for success. I owe every accomplishment to you.

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Title: Validation of a Food Frequency Questionnaire and a Spot Urine Sample for the Assessment of Dietary Sodium Intake in Lebanese Adults

Non-communicable diseases (NCDs) are globally on the rise. In 2010, cardiovascular diseases (CVD) were reported as the primary cause of disability and death in the Arab world. Diet and lifestyle play an important role in determining the risk of CVDs and evidence suggests a link between salt intake and CVDs which sheds the light on the importance of assessing the levels of population salt intake. In Lebanon, the scarcity of data on dietary sodium intake highlights the need for rigorous investigations aiming at assessing the population’s intake of salt and sodium. The gold-standard for the assessment of sodium intake is 24 hour urinary sodium excretion. Even though alternative methods have been proposed for the dietary assessment of Na, the validity, accuracy and/or applicability of these methods have been often criticized in the literature.

Objectives: The objective of the present study is to validate 2 different methods for the assessment of Na intake in adults, namely the sport urinary sodium excretion and the food-frequency questionnaire. Validation will be undertaken through the assessment of sodium urinary excretion, which is recognized as the gold standard in the assessment of sodium intakes.

Methods: A convenience sample of Lebanese adults aged 19 to 55 years were recruited. Subjects provided a 24 hour urine collection and a spot urine sample (particularly the second morning voiding). Subjects were also asked to complete an FFQ on the day of submission of urine samples. Anthropometric measurements (weight, and height) were obtained. Descriptive statistics, Spearman correlations, mean differences, and Bland-Altman plots, Intraclass Correlation (ICC), weighted Kappa, and percent agreement were used to assess the validity of the FFQ and two spot urine formulas: Kawasaki and Tanaka against the gold standard in assessing sodium intake, 24 hour urine. SPSS was used in the analysis and a p<0.05 indicated significance.

Results: Out of the 72 subjects, 60 completed the study. Spearman correlation coefficients between Na intake estimates derived from the FFQ, spot urine (Kawasaki and Tanaka) vs. 24 hour urinary Na excretion were all statistically significant (0.258 for FFQ, 0.504 for spot urine Tanaka, and 0.547 for spot urine Kawasaki). The ICC was significant for 24 hour urine Na excretion vs. spot urine Kawasaki (0.72) and Tanaka (0.610). The ICC for FFQ vs. 24 hour urine Na excretion was not significant (0.327). The mean differences in Na intake between 24 hour urinary Na and the FFQ and spot
urine Kawasaki were significantly different from zero, unlike the mean difference between spot urine Tanaka which was lowest and not significantly different from zero. The slope of the FFQ vs. 24 hour urine was positive and significantly different from zero, while that of the spot urine (Kawasaki and Tanaka) was negative, and significantly different from zero only for the spot urine Tanaka. Both, the adjacent agreement and percent agreement with 24 hour urinary sodium ranked highest for spot Tanaka and spot Kawasaki, and least for FFQ vs. 24 hour urinary sodium. Both spot urine formulas had a fair agreement with 24 hour urine (weighted Kappa = 0.36) with 24 hour urine, while the FFQ had a slight agreement (Weighted Kappa = 0.17).

Conclusion: This study represents the first attempt to validate a short FFQ and spot urine against the gold standard in assessing Na intake; 24 hour urine in Lebanon. It showed that spot urine estimates of sodium intake has an acceptable validity, with significant correlation with 24 hour urine. However, the FFQ did not behave as well as spot urine. Nonetheless, the methods under investigation can be more convenient tools in assessing Na intake.
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Graph 3: Bland Altman Plot for 24 hour Na Urinary excretion Vs. FFQ Na
ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$</td>
<td>US dollars</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>€</td>
<td>Euros</td>
</tr>
<tr>
<td>&lt;</td>
<td>Less Than</td>
</tr>
<tr>
<td>&gt;</td>
<td>Greater Than</td>
</tr>
<tr>
<td>≥</td>
<td>Greater Than or Equal</td>
</tr>
<tr>
<td>24-h</td>
<td>24 hour</td>
</tr>
<tr>
<td>24HUNaV</td>
<td>24-hour urinary sodium value</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>AUBMC</td>
<td>American University of Beirut Medical Center</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Diseases</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>EFOVAL</td>
<td>European Food Consumption Validation</td>
</tr>
<tr>
<td>EPIC-norfalk</td>
<td>European Prospective Investigation of Cancer and Nutrition</td>
</tr>
<tr>
<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
</tr>
<tr>
<td>FHS</td>
<td>Faculty of Health Sciences</td>
</tr>
<tr>
<td>g/day</td>
<td>Grams Per Day</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
</tr>
<tr>
<td>H</td>
<td>Height</td>
</tr>
<tr>
<td>HTN</td>
<td>Hypertension</td>
</tr>
</tbody>
</table>
ICC Intraclass Correlation
IL-17 Interleukin 17
INTERMAP International Study of Macronutrients and Blood Pressure
INTERSALT International Study of Salt and Blood Pressure
ISE Ion-Selective Electrodes
Kg Kilograms
LoA Limits of Agreement
MENA Middle East and North Africa
mEq Milliequivalent
Mg Milligrams
mg/day Milligram per Day
ml Milliliters
mm Hg Millimeters of Mercury
mmol/day Millimol per Day
MPR Multiple Pass Food Recall
Na Sodium
NCD Non-Communicable Diseases
NFSC Nutrition and Food Science
NHANES III Third National Health and Nutrition Examination Survey
PABA Para-Aminobenzoic Acid
SBP Systolic Blood Pressure
SPSS Statistical Analysis Package for Social Sciences
SUcr Spot Urinary Creatinine Concentrations
SUNa Spot Urinary Sodium Concentrations
UcrV24h 24 Hour Urine Creatinine Excretion
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Intake Level</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>W</td>
<td>Weight</td>
</tr>
<tr>
<td>WASH</td>
<td>World Action on Salt Health</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHO MONICA</td>
<td>World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease</td>
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</table>
CHAPTER I
INTRODUCTION

The global burden of cardiovascular diseases (CVDs) has reached epidemic proportions, affecting both developed as well as developing countries. In the recently released Global Status Report 2010 from the WHO, non-communicable diseases (NCDs), which include CVDs, were found to account for approximately two thirds of world deaths (WHO Global status report on non-communicable diseases 2010), with 80% of these deaths reported to occur in low and middle income countries (Mathers CD, 2006). In Lebanon, CVDs account for around 60% of all-cause mortality in persons aged 50 years and older and a recent national study showed that, among adults aged 25-64 years, 13.8% were diagnosed with HTN (Sibai et al, unpublished data). More alarming is the observed increasing trend in the prevalence of hypertension over time, particularly among the older population in the country (Sibai et al. 2010). Being a major risk factor for coronary heart disease and stroke, the World Health Organization (WHO) has estimated that high blood pressure is the leading preventable risk factor for death in the world (Ezzati et al 2002) (Dietary reference intakes for water, potassium, sodium, chloride and sulfate. The national academies press 2004).

In addition to other lifestyle modifications and eating habits that have an impact on blood pressure risk, evidence shows a direct relationship between sodium intake and hypertension; blood pressure rises with increased sodium intake in the general population (Ritz, 2010, Savica et al., 2010; Sodium Reduction Strategy for Canada), and salt intake has been shown to correlate directly with blood pressure in different population groups (Appel et al., 2003; Brook et al.,
The International Study of Salt and Blood Pressure (INTERSALT) indicated that in around 10,000 adults from 32 countries, 24-hour urine sodium excretion was significantly and positively associated with the prevalence of elevated blood pressure (Intersalt, 1988; Stamler, 1997). Importantly, there is good evidence that a reduction in dietary sodium intake will reduce mean population blood pressure as well as the prevalence of hypertension (Dietary reference intakes for water, potassium, sodium, chloride and sulfate. The national academies press 2004) (Cohen and Alderman, 2007; WHO, 2007). Even in non-hypertensive individuals, a reduced salt intake can decrease the risk of developing hypertension (typically defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg) (Dietary reference intakes for water, potassium, sodium, chloride and sulfate. The national academies press 2004). Two meta-analyses of randomized controlled trials that examined the long-term effects of salt reduction in people with and without hypertension have shown that moderate reductions in salt intake (of 2–4·6 g per day) is associated with significant reductions in blood pressure (He FJ, MacGregor 2004; Hooper L, 2004). More importantly, a correlation between the magnitude of salt reduction (within the daily intake range of 3 to 12 g/day of salt) and the magnitude of blood pressure changes has been documented. A decrease of 3 grams/day of dietary salt (1200 mg of sodium present in ½ teaspoon of salt) (He FJ, MacGregor 2003) would result in a decrease of about 5 mmHg in the average systolic blood pressure of people aged over 50 years; diastolic blood pressure would decrease by about half as much.

Available studies suggest that sodium intakes around the world are well in excess of physiological need (i.e. 10-20 mmol/day). Most adult populations have mean sodium intakes >100 mmol/day, and for many, mean intakes are >200 mmol/day (Ian J Brown et al. 2009). In
Lebanon, the scarcity of data on dietary sodium intake highlights the need for rigorous investigations aiming at assessing the population’s intake of salt and sodium. The gold-standard for the assessment of sodium intake is 24 hour urinary sodium excretion (McLean, 2014; Ji et al., 2012). However, this method is recognized as burdensome for both the study subject and the researcher, leading to a low response rate (Bentley, 2006). Accordingly, this method may not be adapted for large-scale studies aiming at assessing the population’s sodium intake (JH Park et al., 2014). Even though alternative methods have been proposed for the dietary assessment of Na (spot urinary sodium excretion; repeated 24 hour dietary recalls; 3-day or 7-day food records; food frequency questionnaires), the validity, accuracy and/or applicability of several of these methods have been often criticized in the literature (Bentley, 2006).

The lack of adequate tools to evaluate sodium intake is still a problem in clinical practice as well as in research settings (Bentley, 2006), and the accurate assessment of sodium intake amongst free-living persons remains a difficult and labor-intensive process. It is important to keep in mind that the routine assessment of the diet of a large number of individuals from different socioeconomic backgrounds requires a quick and simple method of estimating the intake of specific nutrients. In this context, the food frequency questionnaire (FFQ), which is widely used in nutrition research, has been proposed as a precise measure for the evaluation of the intake of nutrients, and has been extensively used for various research purposes (Bentley, 2006). FFQs allow for the assessment of the usual patterns of food intake over an extended period of time, (Subar, 2004) and are able to capture past dietary intake patterns. They are considerably less expensive in both time and cost in comparison to other measurement tools, which is an important consideration in studies involving large cohorts (Willet, 1998). The use of FFQs for the assessment of dietary sodium intake has been proposed in the literature, but none
has yet been developed and validated for the Lebanese or the Middle-Eastern population (Charlton et al., 2008). Besides the FFQ as a potential, suitable approach for the assessment of dietary sodium intake, increasing attention is focusing on substituting 24 hour urine sodium assessment with spot urine sampling (JH Park et al., 2014), whereby participants are required to provide a single urine sample, either overnight, daytime, evening, timed or random (Ji et al., 2012). Spot urine samples are faster to analyze, less tedious compared to 24 hour urine collection, and do not necessitate extensive staff training (Ji et al., 2012; Whelton et al., 2012). Samples can be stored in small containers, with no possibility of over or under collection, and can be frozen for later use (McLean, 2014). This method requires only one visit, and easy collection and analysis protocols, which render it more practical to be in large scale studies (McLean, 2014). The use of a spot urine method, for assessing sodium excretion has previously been examined and shown to strongly correlate with 24-hour sodium excretion (Mann and Gerber, 2010; Tanaka et al, 2002; Kawasaki et al, 1982). Even though this method seems promising, its validity in certain settings has been questioned, thus highlighting the need for the investigation of its validity in various populations (McLean, 2014).

The objective of the present study is to validate 2 different methods for the assessment of Na intake in adults, namely the sport urinary sodium excretion and the food-frequency questionnaire. Validation will be undertaken through the assessment of sodium urinary excretion, which is recognized as the gold standard in the assessment of sodium intakes (Mann and Gerber, 2010). The assessment of sport urinary sodium excretion would require measurement of spot urine sodium concentrations along with a measure of the state of concentration or dilution of the urine, such as the urine creatinine concentrations (Mann and Gerber, 2010). As for sodium intake assessment based on the FFQ, this will be undertaken using a short FFQ that has been previously
developed for the assessment of sodium intake in Lebanese adults (Helou, 2014), and which has been shown to be a reliable tool in evaluating sodium intake in this population group (r=0.866).

The specific objectives of this study are to:

1- Investigate the validity of a short food frequency questionnaire in assessing Na dietary intake amongst Lebanese adults. Validation will be undertaken using 24-hour sodium urinary excretion as the gold-standard.

2- Investigate the validity of spot urinary sodium excretion in assessing Na dietary intake amongst Lebanese adults. Validation will be undertaken using 24-hour sodium urinary excretion as the gold-standard.
A. Salt intake and its association with HTN

1. Prevalence and incidence of HTN

In 2000, almost 972 million individuals were diagnosed with hypertension; a value representing 26.4% of the adult world population, with 333 million in developed and 639 million in developing countries (Hajjar, JM Kotchen, & TA Kotchen, 2006). In 2015 the prevalence of hypertension worldwide has exceeded 1 billion individuals (NRC Campbell, JA Johnson, & TS Campbell, 2012), and this value is expected to reach 1.56 billion by 2025, which is about a 25% increase (Hajjar, JM Kotchen, & TA Kotchen, 2006). About 7.1 million deaths per year are attributed to hypertension (Tohme, Jurjus, & Estephan, 2005), and according to the Global Burden of Disease Study conducted in 1997, a 25% increase in mortality rate due to hypertension, was predicted to occur by 2015 in the Middle Eastern Mediterranean region, which includes most Arab countries (Rahim et al., 2014).

Several epidemiological studies assessed the prevalence of hypertension in different countries; the largest of which was The World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease (WHO MONICA, 1997), which included 22 countries (Hajjar, JM Kotchen, & TA Kotchen, 2006). Prevalence of hypertension was found to vary between and within the countries, ranging between 20% and almost 50% (Anderson et al., 2010). Data from another study published in 2003 estimated the prevalence of hypertension at 27.8% in the United States, 27.4% in Canada, 37.7% in Italy, 38.4% in Sweden, 41.7% in England, 46.8%
in Spain, 48.7% in Finland and 55.3% in Germany (Hajjar, JM Kotchen, & TA Kotchen, 2006). In 2007, more than 65% of the US population was diagnosed with hypertension or prehypertension (Doyle & Glass, 2010).

Hypertension is increasing at a faster rate in developing countries whereby awareness, treatment, and control are not properly implemented (Ibrahim & Damasceno, 2012). A national study conducted in Lebanon in 2005 showed that the prevalence of hypertension among Lebanese is 23.1%, with no statistical difference between males and females (Tohme, Jurjus, & Estephan, 2005). Sibai et al. found the prevalence of hypertension is Lebanon to be 31.2% in 2010 (Sibai et al., 2010). This value increased to 36% according to Azar et al. in 2015 (Azar et al., 2015). Table 1 summarizes the findings reported by Sibai et al. showing the prevalence of hypertension in the Middle East: (Sibai et al., 2010)

Table 1. Prevalence of hypertension among adults in countries of the MENA region.

<table>
<thead>
<tr>
<th>Country</th>
<th>Age</th>
<th>Hypertension %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algeria</td>
<td>≥25</td>
<td>36.2</td>
</tr>
<tr>
<td>Bahrain</td>
<td>≥20</td>
<td>42.1</td>
</tr>
<tr>
<td>Egypt</td>
<td>≥25</td>
<td>26.3</td>
</tr>
<tr>
<td>Iraq</td>
<td>≥20</td>
<td>19.3</td>
</tr>
<tr>
<td>Jordan</td>
<td>≥18</td>
<td>30.2</td>
</tr>
<tr>
<td>KSA</td>
<td>30-70</td>
<td>26.1</td>
</tr>
<tr>
<td>Lebanon</td>
<td>18-65</td>
<td>31.2</td>
</tr>
<tr>
<td>Morocco</td>
<td>≥20</td>
<td>33.6</td>
</tr>
<tr>
<td>Oman</td>
<td>≥20</td>
<td>21.5</td>
</tr>
<tr>
<td>Qatar</td>
<td>≥20</td>
<td>32.1</td>
</tr>
<tr>
<td>Sudan</td>
<td>25-64</td>
<td>23.6</td>
</tr>
<tr>
<td>Syria</td>
<td>18-65</td>
<td>40.6</td>
</tr>
<tr>
<td>UAE</td>
<td>≥20</td>
<td>20.8</td>
</tr>
<tr>
<td>Yemen</td>
<td>≥35</td>
<td>26</td>
</tr>
</tbody>
</table>
Yearly incidence rates of hypertension vary from 3% to 18% based on different factors such as age, ethnicity, body size, gender, and population size. The incidence over a 30-year interval was found to increase with age for both men and women (Hajjar, JM Kotchen, & TA Kotchen, 2006). Accordingly, there is an international consensus to initiate actions that aim at decreasing and preventing the development of hypertension which will significantly improve the quality of life (Doyle & Glass, 2010).

2. Morbidity and mortality; the link with salt intake

An increased blood pressure increases the risk of developing CVD and strokes by two to three times (Tohme, Jurjus, & Estephan, 2005). Both developing and developed countries are suffering from a high burden of CVD, with more than 4.5 million deaths in the developing world being caused by coronary artery diseases (Tohme, Jurjus, & Estephan, 2005). Several epidemiological studies indicate a constant and steady increase in the risk of stroke, cardiovascular disease, diabetes, and renal disease with increasing levels of systolic and diastolic blood pressure (He, Li, & MacGregor, 2013). Consequently, untreated blood pressure increases all-cause mortality (Doyle & Glass, 2010; Hajjar, JM Kotchen, & TA Kotchen, 2006). Uncontrolled blood pressure is expected to double mortality from stroke in the Middle East and Africa by 2030 (Ibrahim & Damasceno, 2012; Tohme, Jurjus, & Estephan, 2005). Moreover, the death from ischemic heart disease in developing countries is expected to rise to 137% for males and 120% for females by 2020 (Ibrahim & Damasceno, 2012).
Many studies, such as EPIC-norfalk, INTERMAP, and INTERSALT have shed light on the link between increased salt intake and elevated blood pressure at both the population and individual levels (De Keyzer et al. 2015; Doyle & Glass, 2010; He Fj, MacGregor, 2009). On the long term, salt was found to have 2 distinct effects: 1) an acute rise in blood pressure due to high intake over days and weeks, and 2) a gradual and slow increase in blood pressure on the long run. This long-term effect might be irreversible and is positively associated with increasing age (Doyle & Glass, 2010). In addition, increased sodium consumption causes a decrease in the elasticity and an increase in the fibrosis of large arteries, theoretically exacerbating hypertension and cardiovascular risk (Kotchen, Cowley, & Frohlich, 2013).

A significant association exists between salt intake and BP (De Keyzer et al. 2015), whereby an increase of 6g/day of salt over a period of 30 years was reported to increase systolic blood pressure by 9mmHg (He, MacGregor, 2009). A newly published meta-analysis of 13 cohorts including 177,025 subjects followed for 3 to 19 years indicated that an excess intake of 5g of salt per day was linked with a 14% and 23% increase in total CVD and stroke risk respectively (He, Jenner, & MacGregor, 2010).

Another meta-analysis of 19 cohorts from 13 studies highlighted a strong association between salt consumption of more than 15g per day (Kotchen, Cowley, & Frohlich, 2013), and increased stroke and cardiovascular risks (De Keyzer et al. 2015). A Japanese study conducted on healthy individuals reported an odds ratio of 1.25 for stroke mortality (95% CI: 1.23-1.27) in areas of high sodium consumption compared to areas of low intake. A 10 year cohort study reported an odds ratio of 2.59 for stroke mortality (95% CI: 1.27-5.28) and a 17% increase in health risks for every increase of 500mg of sodium per day. Overall, a 1.68 hazard ratio (95% CI:
1.06-2.57) was reported for vascular death, myocardial infarction and stroke with a 4000mg/day sodium diet compared to a 1500mg/day sodium diet (Whelton et al., 2012).

The observed association between salt intake and stroke mortality may be partially explained by the increase in blood pressure, and partially explained by other mechanisms independent of blood pressure. Many clinical studies have found a link between increased salt intake and left ventricular hypertrophy, and indicated that a decrease in salt intake reversed this increase in left ventricular mass (Antonios & MacGregor, 1995). Moreover, a positive linear relation was observed between salt intake and posterior wall thickness as well as relative wall thickness independent of other factors. In addition, increased sodium intakes may impair smooth muscle relaxation in arterial endothelium due to increased stress of blood flow (Doyle & Glass, 2010). In animal studies, diets high in sodium speeded up cerebral arterial disease even with the lack of increased blood pressure (Antonios & MacGregor, 1995).

Even though available evidence suggests an increase in both mortality and morbidity due to increased sodium intake, it is hard to establish specific correlations because the effects of sodium progress over a long period of time and are modulated by many other dietary and lifestyle factors (Doyle & Glass, 2010).

3. Pathophysiology of HTN: the role of salt

Hypertension is divided into two main branches: primary or essential (95%) and secondary (5%). Secondary hypertension is mainly due to renal or adrenal problems. Whereas primary hypertension can be caused by several malfunctions in the physiological mechanisms responsible for the maintenance of blood pressure. These mechanisms involve 1) cardiac output and
peripheral resistance, 2) renin-angiotensin-aldosterone system, 3) sympathetic nervous system, and 4) endothelial dysfunction due to several factors: endothelial derived relaxing factor, atrial natriuretic peptide, ouabain, bradykinin, endothelin (Beevers, Lip, & O'Brien, 2001).

The risk of hypertension is mainly modulated by lifestyle and environmental factors in addition to genetically defined characteristics (Ibrahim & Damasceno, 2012). An unhealthy lifestyle, increased body weight, physical inactivity (He, MacGregor, 2009), and the consumption levels of sodium and potassium (Doyle & Glass, 2010) are primary elements responsible for the development of hypertension. Available evidence highlights the fact that increased salt intake in the form of sodium chloride impacts blood pressure the most and in particular induces and worsens hypertension (Intersalt, 1998; Krauss et al., 2000), and may be responsible for the increasing prevalence of prehypertension, hypertension and consequently CVDs (Anderson et al., 2010).

Sodium is an essential nutrient which is involved in many body functions. It regulates extracellular fluid and plasma volume, nervous message transmission, and the active transport across cell membranes. Several minerals interact with sodium, the most important of which are calcium and potassium. A healthy body is programmed to adjust to different levels of salt intake (Doyle & Glass, 2010). Intestinal absorption of sodium is about 98%, and the kidneys are the main organs responsible for its excretion. The sympathetic nervous system, several hormones, and plasma renin activity (He, MacGregor, 2009) modify sodium excretion in urine and sweat according to the levels of intake (Doyle & Glass, 2010). In healthy individuals, almost all of the consumed sodium is excreted in the urine, and a very small quantity is lost through perspiration (Espeland et al., 2001).
Existing research has repeatedly shed the light on inherent salt sensitivity. Individuals at risk of developing salt sensitivity are black skinned individuals, hypertensives, diabetics, uremic patients, and individuals above 51 years of age (Doyle & Glass, 2010; Whelton et al., 2012; He, Li, & MacGregor, 2013). Salt sensitive individuals are not able to excrete salt efficiently compared to salt-resistant individuals, and are more affected by an increased salt consumption (Doyle & Glass, 2010). The extent of sensitivity to salt and its effect on blood pressure augments with age and the baseline level of blood pressure, and with the existence of a family history of hypertension (Hajjar, JM Kotchen, & TA Kotchen, 2006). Moreover, Blacks are more susceptible to hypertension, due to a genetic mutation, whereby renal handling becomes inefficient, and salt retention occurs (Kotchen, Cowley, & Frohlich, 2013). However, several researchers have contested this hypothesis and found that skin pigmentation does not really play a critical role in salt sensitivity. In fact, not only black skinned individuals living in mixed societies are at risk of developing hypertension (Ibrahim & Damasceno, 2012). Many studies have indicated that migration from geographic areas where salt is consumed in small amounts, to areas where salt is highly consumed is followed with an increase in blood pressure due to the adoption of a modern lifestyle (Ibrahim & Damasceno, 2012; Kotchen, Cowley, & Frohlich, 2013).

Renal handling of electrolytes decreases with increased age and with the presence of chronic diseases such as HTN (Doyle & Glass, 2010). High salt intake eventually causes increased arterial pressure (Kotchen, Cowley, & Frohlich, 2013) and induces a big challenge on the kidneys to filter this excess, and consequently can cause volume overload, increased cardiac output, increased systemic vascular resistance and increase in blood pressure and CVD, as well as renal problems (He, MacGregor, 2009). Subsequently, increased blood pressure increases the
risk of developing both CVDs and renal disease, but the mechanisms of which are not fully understood (Doyle & Glass, 2010).

**B. Other health risks associated with high salt intake**

There is an overall consensus that high intake of sodium is a major factor in the rise of blood pressure and CVD risk (He, MacGregor, 2009). Nonetheless, diets high in sodium may induce deleterious health effects other than hypertension and CVD (De Keyzer et al. 2015). These health problems may include: stomach cancer, breast cancer, kidney disease and proteinuria, kidney stones, osteoporosis and exacerbated severity of asthma (Anderson et al., 2010; Doyle & Glass, 2010; He Fj; MacGregor, 2009). Evidence supporting the link between high salt intake and these conditions is not conclusive; however, excessive intake of salt may modify the initiation or severity of some of these conditions (Doyle & Glass, 2010; He, Jenner, & MacGregor, 2010).

1. **Stomach cancer**

Stomach cancer is the fourth most common cancer worldwide, and is the number one cause of cancer related deaths (JH Park et al., 2014). Many studies have related dietary factors, especially highly salted food, to the development of gastric cancer (NRC Campbell, JA Johnson, & TS Campbell, 2012). It is very common in countries where salt is a main constituent of the diets, such as in Japan (JH Park et al., 2014). Tsugane et al. found a linear relation between salt intake and mortality rate from stomach cancer. Moreover, they found that the odds ratio of stomach cancer was 2.25 (95% CI: 1.028-4.939, p=0.043) for individuals excreting more than
240 mEq of sodium in 24 hour urine (JH Park et al., 2014). Other studies have also reported a significant association between salt intake and stomach cancer (JH Park et al., 2014).

Several mechanisms can explain the pathogenesis of cancer caused by increased salt intake; however no final conclusions have been reached. Hypertonic salt solutions can harm the integrity of the gastric mucosa in the same way they damage taste buds (JH Park et al., 2014). On the long run, this may cause inflammation, and consequently atrophic gastritis, a condition frequently seen in salt consuming societies (Antonios & MacGregor, 1995). The constant inflammatory state induces epithelial cell proliferation which can consequently increase the rate of mutations, and on the long run cause tumor development (JH Park et al., 2014). Moreover, hypertonic solutions in the stomach cause an environment favorable for Helicobacter pylori colonization, whereby evolution, cell morphology; survival and virulence are affected by salt concentration (He, MacGregor, 2009; JH Park et al., 2014). H. Pylori is a major risk factor for both stomach and duodenal cancer, and the severity of its effect is increased with increased salt intake (He, MacGregor, 2009).

2. Breast cancer

Several studies have linked unhealthy dietary habits to increased risk of development of breast cancer (Ge et al., 2015; Michels et al., 2007). Breast cancer prevalence was found to significantly increase proportionately with an increase in salt intake, suggesting a link between salt intake and the development of breast cancer (He, MacGregor, 2009; JH Park et al., 2014). Breast cancer cell proliferation is proposed to be induced through epithelial sodium channels, and in turn, high sodium intake may cause a chronic inflammatory environment mediated by
increased reactive oxygen species (Amara et al., 2015). Increased cellular sodium concentrations induce significant upregulation of angiogenic growth factors, and pro-inflammatory cytokines and chemokines, affecting both breast cancer staging and progression (Amara et al., 2015). In fact, Wu et al. showed that increased salt intake causes increased IL-17 secretion (Amara et al., 2015), which was found to be pro-carcinogenic. Moreover, mammary adenocarcinomas where found to have significantly higher sodium concentrations compared to healthy mammary cells (Amara et al., 2015).

3. **Kidney injury**

Continued high sodium intake may result in target organ damage specifically heart failure and kidney injury. Organ injury may occur due to oxidative stress, excessive urine albumin excretion (He, Jenner, & MacGregor, 2010), interstitial fibrosis, renal arteriolar injury, glomerular hyalinization, fibrosis, and increased glomerular hydrostatic pressure, independent of elevated blood pressure (Whelton et al., 2012). Moreover, the amount of salt consumed is a main factor affecting kidney calcium excretion in the urine (He, Jenner, & MacGregor, 2010), therefore increased sodium intake participates in the formation of calcium oxalate stones, known as kidney stones (Doyle & Glass, 2010).

In hypertensive individuals, increased salt intake augments arterial pressure causing microalbuminuria and cardiac hypertrophy (Kotchen, Cowley, & Frohlich, 2013). Moreover, the rise in blood pressure causes hypertensive glomerulosclerosis, which can independently cause renal injury (He, Jenner, & MacGregor, 2010). Accordingly, renal filtering is defected and the kidneys become unable to excrete sodium efficiently. The kidneys will then have to increase the effort to maintain a constant sodium balance, which causes gradual renal failure (Kotchen,
Cowley, & Frohlich, 2013). Conversely, individuals who already have kidney problems are at risk of developing salt related hypertension which in its turn will deteriorated the kidneys gradually, forming a vicious cycle (He, Jenner, & MacGregor, 2010). Hypertensive persons who are slightly salt sensitive can have their sensitivity gradually worsened due to the gradual harmful effect on the kidneys and other target organs (Whelton et al., 2012).

Several animal studies showed that increased salt loading induces changes in vascular endothelial-cell function and causes organ injury. After 3 weeks of such a diet, renal arteriolar injury, interstitial fibrosis, glomerular hyalinization, increased glomerular hydrostatic pressure and consequently proteinuria and renal failure occurred (Kotchen, Cowley, & Frohlich, 2013). Animals with underlying renal problems experienced a worsening in glomerulosclerosis and proteinuria due to high salt intake (He, Jenner, & MacGregor, 2010). Salt restriction suppressed the compensatory work of the kidneys and retarded glomerular injury (Antonios & MacGregor, 1995).

4. Osteoporosis

High intakes of sodium chloride increase the loss of calcium through the urine (Antonios & MacGregor, 1995). Calciuria occurs due to two main reasons: 1) the volume expansion caused by high salt intake and increased glomerular filtration rate, and 2) the antagonism between calcium and sodium at the level of the proximal renal tubule, causing an increased excretion of both ions (Doyle & Glass, 2010). At low calcium intakes, decreased serum calcium activates bone remodeling through the increased secretion of parathyroid hormone. This causes an increase in the production of 1,25(OH)_{2}D_{3} and consequently leads to bone remodeling and bone
loss, which increases the risk of fractures (Heaney, 2006). In addition, increased sodium intake also increases hydroxyproline excretion which is a biological marker indicating bone resorption (Antonios & MacGregor, 1995).

Several studies have shown a statistically significant positive correlation between both, 24-hour urine calcium and sodium excretion in both sexes across all ages. Calcium loss in the urine increases from 0.5 to 1.5 mmol (20-60 mg) for each 100 mmol (2300 mg) ingested sodium (Antonios & MacGregor, 1995). Accordingly, an increase of 100 mmol/day of sodium intake will induce a rise in urinary calcium loss by 1 mmol/day, which equals 40 mg/day. The role of sodium in the pathogenesis of osteoporosis depends on the amount of consumed calcium, if dietary calcium intake is low, the risk of bone remodeling increases (Heaney, 2006). Intakes below 600 mg/day will render the body unable to compensate for the urinary calcium loss, and thus the body will have a negative calcium balance. Individuals consuming the recommended level of calcium (1200 mg/day) may be protected from calciuria and will be in a positive calcium balance (Doyle & Glass, 2010).

5. Asthma outcome

Many epidemiological data proved the presence of a link between asthma prognosis and both population and per capita sodium consumption (Antonios & MacGregor, 1995), whereby high salt diets were positively associated with the increased prevalence of severe asthma (Barros et al., 2015). In addition, high salt diets were found to be positively correlated with increased asthma mortality whereas decreased salt intake improved symptoms even with decreased use of medication (He & MacGregor, 2010). Increased salt intake significantly increases airway
inspiratory pressure due to its interaction with leukotrienes in the airway effector cells exacerbating hyperpnea (Mickleborough & Gotshall, 2004). Increased airway osmolarity due to high sodium concentrations initiates the production of inflammatory cytokines causing the contraction of smooth muscles, increasing vascular permeability and mucus secretion, exacerbating airway obstruction (Mickleborough & Gotshall, 2004).

C. Benefits of decreasing salt intake

1. Health

The numerous health benefits of decreasing salt intake are the exact antagonists of the health problems caused by its increased intake (Intersalt, 1998). This clearly established conclusion has been accepted worldwide, and its assertiveness authorizes the urgent need to considerably decrease salt intake (NRC Campbell, JA Johnson, & TS Campbell, 2012). Even a slight reduction of salt to reach an intake of 5-6g/day (He, Jenner, & MacGregor, 2010) yields considerable health benefits, particularly when it is accomplished on a population level (Whelton et al., 2012).

Decreased salt intake decreases blood pressure in both normotensives and hypertensives, and various meta-analyses of randomized trials have shown this dose-response improvement (Antonios & MacGregor, 1995; De Keyzer et al. 2015; He Fj, MacGregor, 2009; He, Li, & MacGregor, 2013). The Trials of Hypertension Prevention and the Trials of Non-pharmacologic interventions acknowledged a 20% drop in hypertension, and a decreased dose of anti-hypertensive medication with a decrease of salt intake to 100 mmol/day (Krauss et al., 2000). Studies on hypertensives showed that an intake dropped to 6g/day decreases SBP by 7 mmHg
and DBP by 4 mmHg, which is equivalent to the effect of a single antihypertensive drug (He Fj, MacGregor, 2009; NRC Campbell, JA Johnson, & TS Campbell, 2012). On average, a reduction of 1.8g of sodium is followed with a drop of 4mmHg and 2 mmHg in SBP and DBP respectively (Krauss et al., 2000).

A meta-analysis of 28 clinical trials that persisted over 1 month also proved a significantly reduced blood pressure in both hypertensives and normotensives, with a significant dose response relation between salt reduction and blood pressure reduction (He and MacGregor 2002). A cross-over double blind randomized trial of diets of 9.7g and 6.5g of salt per day reinforced that a decrease in salt intake significantly decreased blood pressure in subjects from different races (Asian, white, black), and caused a decrease in arterial rigidity in various individuals (Doyle & Glass, 2010). The Trial of Hypertension Prevention I and II have also showed that a 25-30% reduction in salt intake from a mean of 10g/day caused a drop of 1.7/0.9 mmHg after 1.5 years and 1.2/0.7 mmHg over 3 years (He, Jenner, & MacGregor, 2010). In addition, Pimenta et al. published a crossover study of low and high salt diets, where hypertensive subjects consumed 50 and 250 mmol/d (1.15g and 5.75g respectively) for 7 days each. The decreased intake resulted in a significant drop in both systolic blood pressure and diastolic blood pressure (22.7 and 9.1 mmHg respectively), whereas both remained constant with high salt intake even with antihypertensive medications (Doyle & Glass, 2010).

It is well agreed that hypertensive individuals have a better responsiveness to diminished salt intake compared to normotensives (Kotchen, Cowley, & Frohlich, 2013), whereby the drop in blood pressure is slightly less in non-hypertensives (He, Li, & MacGregor, 2013; Krauss et al., 2000). Gradual et al. showed that hypertensives of different races (Asian, black, and white) benefited more from salt reduction, compared to non-hypertensives (Krauss et al., 2000) (Mean
reduction in SBP of 5.18, 6.44, and 10.21 mm Hg in whites, blacks, and Asians hypertensives, respectively, and of 1.29, 4.02, and 1.27 mm Hg, respectively in normotensives). The outcomes of this meta-analysis are compliant with previous knowledge that a more significant drop is predicted in salt sensitive individuals with advanced stages of blood pressure (Whelton et al., 2012). Dinkinson et al. found that sodium intake of 1.15g/day (just below the recommendation) enhanced artery dilation, independent of the effect on hypertension in obese and overweight subjects compared to sodium intake of 3.46g (the median intake of the U.S. population) (Doyle & Glass, 2010).

Numerous long-term clinical trials have indicated that a lower salt intake results in an average 20% decline in the occurrence of cardiovascular problems (NRC Campbell, JA Johnson, & TS Campbell, 2012) mainly due to the drop in both systolic (SBP) and diastolic blood pressure (DBP) (Kotchen, Cowley, & Frohlich, 2013). A decrease of salt intake by 6g/day could decrease CHD by 18% and strokes by 24% (He, MacGregor, 2009). Accordingly this can save over 35,000 deaths from such incidences in the UK and over 2.5 million deaths worldwide (He Fj, MacGregor, 2009; He, Jenner, & MacGregor, 2010). In the US, a decrease of 3g of salt from the current average consumption will reduce incidence of stroke by 32,000 to 66,000, coronary heart disease by 60,000 to 120,000, myocardial infarction by 54,000 to 99,000 and overall all-cause death by 44,000 to 92,000 (Kotchen, Cowley, & Frohlich, 2013).

In addition, low salt intake induces an overall protective effect on the kidneys. It helps diminish proteinuria, decrease urine calcium excretion and accordingly prevent the formation of kidney stones, and delay the worsening of renal disease. A recently published study on individuals of different races (Whites, Blacks, and Asians) indicated that a decreased salt from 9.7g to 6.5g per day considerably decreased 24 hour urine albumin excretion (He et al., 2009).
Another randomized trial in 40 black skinned hypertensive individuals revealed a decrease of 19% in 24 hour urine albumin when salt intake dropped from 10 to 5 g/day (Swift et al., 2005).

2. **Cost effectiveness and saving of decreasing salt intake**

Many studies have shown that implementing a project to decrease salt intake does not require much funding and is very cost effective and simple to apply (Appel & Anderson, 2010; Cobiac, Vos, & Veerman, 2010; He, Jenner, & MacGregor, 2010; NRC Campbell, JA Johnson, & TS Campbell, 2012; Rahim et al., 2014). Several factors are considered in cost analysis of salt reduction interventions; the required budget, governmental losses from rewarding companies producing less salted foods, and gains from taxing high salted products, as well as the cost benefit of decreasing health conditions associated with high salt intake (Selmer et al., 2000).

In fact, about 10% of Global healthcare cost is spent on treating NCDs (Cobiac, Vos, & Veerman, 2010). As previously mentioned, decreased salt intake may decrease the dose or even the need for antihypertensive medications, as well as hospital stays, and consequently, increase quality of life, life expectancy and productivity (NRC Campbell, JA Johnson, & TS Campbell, 2012; Selmer et al., 2000). Bibbins-Domingo et al. deduced that even a slight decrease in salt intake provides advantages similar to those gained from weight loss, smoking cessation, and the use of medications to treat NCDs (Appel & Anderson, 2010).

In Norway, $43 millions are paid annually on physician fees and $86 million are spent on antihypertensive drugs (Selmer et al., 2000). According to a Norwegian study, a project aiming to decrease salt to 6g/day over a period of 25 years can contribute to a net saving of $240 million (Selmer et al., 2000). Such a project can be comprised of health education, adjusted industrial
recipes, and clear indication of salt content of packaged foods. Moreover, taxes can be charged on highly salted foods on one hand, and grants rewarded for less salted foods on the other hand. Life expectancy was expected to increase by 1.8 months in men and 1.4 months in women, and cost saving was expected to reach $118 million with a 3-6 mmHg decrease in blood pressure of hypertensives (Selmer et al., 2000). In Europe, the cost of treating CVD is about €169B per year (Peterson et al. 2005) and it can reach up to $403.1B in the US (Desmond, 2006; Thom et al., 2006). In the US, many studies have estimated that a decrease of salt intake to 3g per day can save health care costs by $10 billion to $24 billion per year (Appel & Anderson, 2010); this value can even increase to $32 billion (He, Jenner, & MacGregor, 2010). It was also estimated that such interventions can save almost $430 million yearly in treating hypertension in Canada (NRC Campbell, JA Johnson, & TS Campbell, 2012).

In low-income countries, little monetary means are present to treat CVDs (He, Jenner, & MacGregor, 2010). Several assessments have demonstrated that decreasing salt intake has a higher financial benefit than any other intervention (NRC Campbell, JA Johnson, & TS Campbell, 2012). Campaigns that aim at decreasing salt intake were considered to be even more cost saving and cost effective than any other campaigns targeting the prevention of other risk factors of CVDs. For example, projects aiming at tobacco control cost 0.26$ per person, whereas salt reduction projects cost 0.09$ per person (He, Jenner, & MacGregor, 2010). Moreover, the effectiveness of salt intake on treating CVD is 2.75 times more than the effect of tobacco control on CVD (He, Jenner, & MacGregor, 2010).
D. Recommendations for sodium intake and identification of dietary sources

1. Recommended intake

Based on the documented harmful health effects of Na (He Fj & MacGregor, 2009; Loria, Obarzanek, & Ernst, 2001), all governmental and non-governmental organizations recommend the reduction of Na intake levels (NRC Campbell, JA Johnson, & TS Campbell, 2012). The Institute of Medicine of the National Academy of Sciences has specified adequate intakes (AIs) and upper intake levels for sodium and potassium depending on their effect on blood pressure and cardiovascular health. Individuals predisposed to develop increased blood pressure are advised not to exceed sodium’s daily AI level of 1500mg (Doyle & Glass, 2010). Table 2 summarizes the adequate levels of sodium intake per age group, the tolerable upper intake level, and the median intake among males and females (Doyle & Glass, 2010). The WHO recommends salt intake not to exceed 5g/day (2000 mg of dietary sodium) (De Keyzer et al. 2015; Krauss et al., 2000). According to the American Heart Association (AHA), the US Department of Health and Human services, and the US Department of Agriculture daily intake of individuals predisposed to salt sensitivity should not exceed 1500 mg of sodium (4g per day of salt), which is about 2/3 teaspoon of salt (He FJ, MacGregor, 2003; He, Li, & MacGregor, 2013; NRC Campbell, JA Johnson, & TS Campbell, 2012; Whelton et al., 2012).

In 2005, experts from 80 countries formed an international union; WASH (World Action on Salt Health) to raise global awareness about the consequences of salt on health and to collaborate with political bodies and industries to decrease the use of salt. The World Health Organization has then organized an international summit in 2006 to discuss the relation between salt intake and health (Doyle & Glass, 2010). In 2013, the WHO set an action plan that aims to
decrease the incidence and prevalence of chronic diseases by 2020, with one of its nine targets being to reduce average population salt intake by 30% (Krauss et al., 2000; Rahim et al., 2014; McLean, 2014). This decrease should be gradually adapted so that intake of salt decreases to less than 5g/day (Krauss et al., 2000; Rahim et al., 2014) and so that this value is efficiently reached in most countries (McLean, 2014).

Table 2. Recommended sodium intake.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Sodium Intake</th>
<th>Sodium Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AI: 19-50 years</strong></td>
<td>1.5 g/day (65 mmol)</td>
<td>3.8 g</td>
</tr>
<tr>
<td><strong>AI: 51-70 years</strong></td>
<td>1.3 g/day (55 mmol)</td>
<td>3.3 g</td>
</tr>
<tr>
<td><strong>AI: 70 years+</strong></td>
<td>1.2 g/day (50 mmol)</td>
<td>3 g</td>
</tr>
<tr>
<td><strong>UL: tolerable upper intake level</strong></td>
<td>2.3 g/day (95 mmol)</td>
<td>5.8 g</td>
</tr>
<tr>
<td><strong>WHO</strong></td>
<td>2 g/day (83 mmol)</td>
<td>5 g</td>
</tr>
<tr>
<td><strong>Median intake (males)</strong></td>
<td>4.2 g/day (183 mmol)</td>
<td>10.6 g</td>
</tr>
<tr>
<td><strong>Median intake (females)</strong></td>
<td>3.3 g/day (142 mmol)</td>
<td>8.3 g</td>
</tr>
</tbody>
</table>

Several debates have discussed the extent to which sodium should be restricted. The relationship between sodium intake and cardiovascular mortality and morbidity is J-shaped, whereby severe salt limitation was found to affect several biological markers (Kotchen, Cowley, & Frohlich, 2013) especially in diabetics (Gradual, Hubeck, & Jurgens, 2012), and patients with compensated congestive heart failure on diuretics (Paterna et al., 2008; Kotchen, Cowley, & Frohlich, 2013). Many trials have highlighted a significant rise in insulin resistance, triglycerides (7%), total cholesterol (2.5%), plasma levels of adrenaline and noradrenaline, and activity of renin, aldosterone and catecholamine in severe state of Na restriction (Cohen & Townsend, 2015; Gradual, Hubeck, & Jurgens, 2012). An extreme decrease in salt intake decreases blood
volume (Aburto et al., 2013), causing the activation of the sympathetic and renin-angiotensin-aldosterone systems in order to limit this decrease (Aburto et al., 2013; Gradual, Hubeck, & Jurgens, 2012). The activation of these two systems increases the concentration of plasma angiotensin II, which in its turn induces insulin resistance and consequently alters plasma lipid metabolism (Nakandakare et al., 2008). The reduced blood volume also causes hemoconcentration of blood lipids, and consequently a sharp increase in cholesterol and triglycerides (Aburto et al., 2013; Nakandakare et al., 2008). These effects are in general acute and tend to normalize after four weeks, and the variation in these biomarkers are not accurate in predicting future disease risk (Aburto et al., 2013). Gradual decrease in salt intake on the long run was found to slightly increase adrenaline, noradrenaline, aldosterone and catecholamine, with no modification in plasma lipid values (Kotchen, Cowley, & Frohlich, 2013). Thus, recommendations of salt intake should be tailored based on each individual’s health condition and risk factors (Cohen & Townsend, 2015), and should at the same time aim at maintaining blood pressure (Gradual, Hubeck, & Jurgens, 2012).

2. Levels of sodium intake

Due to the alarming rise in NCDs, and the increased awareness about the link between salt intake and such diseases, many countries have launched programs to assess their population salt intake (He, MacGregor, 2009). In general, most of the published data indicate that the current global intake is much higher than the recommended levels (Anderson et al., 2010; Loria, Obarzanek, & Ernst, 2001; McLean, 2014; Sarmugam, & Worsley, 2014). Global Westernization of diets led to increases in sodium intakes compared to the amounts naturally found in food which provided less than 2g of sodium per day (Doyle & Glass, 2010). According to the latest
NHNES III survey (Third National Health and Nutrition Examination Survey), more than 75% of women and 95% of men are surpassing the UL of sodium (Doyle & Glass, 2010; NRC Campbell, JA Johnson, & TS Campbell, 2012). The consumption of salt is higher among men mainly due to their consumption of larger portion sizes, and due to their different food preferences (Appel & Anderson, 2010).

The INTERSALT study (1985-1987), estimated sodium intake in 32 countries around the world to range from 4600 mg to 5600 mg/day (Doyle & Glass, 2010). The INTERMAP study (1996-1999) found that the global average daily intake ranged from 3,660mg to 3,739mg, (Whelton et al., 2012) with 2300 to 4600 mg/day in Europe and North America (1988) (Anderson et al., 2010; Doyle & Glass, 2010), and 5300mg to 6000mg/day in the Far East and Asia (Heaney, 2006). A recent study published in 2010 assessed sodium intake in 66 countries and concluded that the average intake was estimated at 3.95g/day (McLean, 2014), which is almost double the level recommended by the WHO (2000 mg/day) (Anderson et al., 2010). Several studies indicate that most individuals are consuming more than 5,700 mg of sodium per day reaching a daily consumption level of more than 10,000 mg/day (NRC Campbell, JA Johnson, & TS Campbell, 2012). In Turkey, the average intake of salt is about 18g/day compared to 8.5g/day in South India. (Ibrahim & Damasceno, 2012)

A recently published study about the Eastern-Mediterranean regions indicated that Na intake is following the Western trend, since the population is increasingly relying on processed foods, highly salted fast foods and eating out (Ibrahim & Damasceno, 2012; Musaiger, 2002; Rahim et al., 2014). Salt intake in the region ranges from 2 to 19g/day, with 2-7g/day in Lebanon (Rahim et al., 2014). On average, sodium intake in Lebanon is 3.13 g/day (Nasreddine et al., 2014). In 2011, sodium intake in Jordan was estimated at 7.6 g/day (Alkurd, 2011).
3. Dietary sources

Dietary sources of sodium can be divided into 3 main categories: 1) sodium naturally found in food, which accounts for only 10% of sodium intake (Doyle & Glass, 2010; Eckel et al., 2014), 2) salt added during food processing and pickling, and 3) salt added during cooking or at the table (Doyle & Glass, 2010; NRC Campbell, JA Johnson, & TS Campbell, 2012). The different proportions of the last two categories vary between developed and developing countries, and within the same country (Anderson et al., 2010; Ibrahim & Damasceno, 2012). However, the majority of the salt does come from processed foods, and restaurant foods (Anderson et al., 2010). It is very important to identify country-specific food sources of sodium in order to target these food items in projects aiming to decrease the population’s salt intake (Anderson et al., 2010).

In developed countries, mainly European and North American countries (Ibrahim & Damasceno, 2012), almost 75-80% of consumed salt comes from processed foods (Cobiac, Vos, & Veerman, 2010; NRC Campbell, JA Johnson, & TS Campbell, 2012; Sarmugam, & Worsley, 2014), and around 5-10% comes from adding salt at the table (Doyle & Glass, 2010). The INTERMAP study was the first study to assess the contribution of different food products to sodium intake and to compare the main contributors between four countries; Japan, China, The United Kingdom and the United States (Anderson et al., 2010). In these countries, the main sources of salt were commercially prepared cheeses, cereals, grains, bread, sauces, soups, and cured meats, however the exact contribution from each food differed based on local country-specific food habits and traditions (Anderson et al., 2010; Doyle & Glass, 2010; Whelton et al., 2012). In Japan for example, soy sauce accounted for 20% of salt intake. (Anderson et al., 201). In the UK, processed foods contributed to 34% of salt consumption (Anderson et al., 2010) and
processed meat to more than 20% of salt intake (Doyle & Glass, 2010). In the US, bread, grains, and cereals contributed to 19.5% of salt intake (Anderson et al., 2010).

Moreover, staple foods such as bread, can be strong contributors of salt even though they might not contain a considerable amount of salt. This can be explained by the fact that they are highly consumed on a daily basis. Consequently, sodium coming from bread can account for 35 to 50% of sodium intake based on the country’s level of bread intake (Doyle & Glass, 2010). In Arab countries, bread contributes the most to consumed salt (Rahim et al., 2014). The concentration of salt in bread ranges from 1g to 1.34g per 100g, and 0.32g to 0.52g per 100g in cakes and baked products (Doyle & Glass, 2010). In the Middle East and the region, bread and bread-derived products were found to contribute the most to sodium intake, which is almost 20% of the consumed salt (Rahim et al., 2014). Table 3 shows the major sources of sodium in the Lebanese diet from a recently published study (Almedawar at al., 2015).

Table 3. Major contributors of sodium in Lebanon

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Percent contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread, other bread-like products and breakfast cereals</td>
<td>26%</td>
</tr>
<tr>
<td>Processed meats</td>
<td>12%</td>
</tr>
<tr>
<td>Cheese and Labneh</td>
<td>10%</td>
</tr>
<tr>
<td>Salads</td>
<td>9%</td>
</tr>
<tr>
<td>Vegetables based dishes and moughrabiyyeh</td>
<td>8%</td>
</tr>
<tr>
<td>Potato chips and salty snacks</td>
<td>8%</td>
</tr>
<tr>
<td>Legumes based dishes</td>
<td>5%</td>
</tr>
<tr>
<td>Rice and burgul based dishes</td>
<td>4%</td>
</tr>
<tr>
<td>Manaesh and Lebanese pies</td>
<td>4%</td>
</tr>
<tr>
<td>Meat, poultry, fish and egg based dishes</td>
<td>3%</td>
</tr>
<tr>
<td>Other*</td>
<td>13%</td>
</tr>
</tbody>
</table>

* pizza, pasta based dishes, processed poultry, sweets, kishk, tahini and falafel based dishes, gravies, butter, soups, potato based dishes, and milk and milk based products
Available evidence suggests that in some middle and low income countries, a high proportion of salt is added while preparing food at home due to the limited availability of processed foods (NRC Campbell, JA Johnson, & TS Campbell, 2012; Sarmugam, & Worsley, 2014). For example, in African and Asian countries, sauces and seasonings which are used in food preparations are highly salted and are the chief sources of sodium (Ibrahim & Damasceno, 2012). A survey conducted in China concluded that 72-76% of salt is added during cooking, and about 8% of consumed salt comes from soy sauce, which is a main constituent of the Chinese diet (Doyle & Glass, 2010; Ibrahim & Damasceno, 2012).

E. Assessment of sodium intake

The first step in designing a nutrition intervention project targeting a specific nutrient is assessing and monitoring the population’s intake of the specified nutrient (Ji et al., 2012; McLean, 2014). However, evaluating sodium intake can be challenging due to the diversity of its dietary sources and the varying concentrations of Na in foods belonging to the same category, depending on brand, country of origin, traditions, etc. (Ibrahim & Damasceno, 2012; Loria, Obarzanek, & Ernst, 2001). Nonetheless, several objective and subjective methods exist to quantify dietary sodium intake, and each method has its own advantages and disadvantages (Bentley, 2006). There is no agreement as to which method is best in assessing sodium intake at a population level, and both strength and weakness of each method must be considered (Bentley, 2006). These measurement methods branch into two main categories: urinary excretion and dietary assessments (McLean, 2014).
F. Use of biomarkers/urinary excretion

1. **Gold standard: 24 hour urine collection**

   The gold standard for assessing sodium intake is measuring the quantity of sodium in 24-hour urine collection in populations and individuals (Ji et al., 2012; McLean, 2014). This procedure is the most accurate and objective method in assessing sodium intake (Bentley, 2006; De Keyzer et al. 2015; He, MacGregor, 2009). This approach was found to be reliable (Whelton et al., 2012) and is used as a reference to validate and compare other measurement tools of sodium intake (De Keyzer et al. 2015; McLean, 2014). Moreover, it is the most widely adapted method to assess population salt intake in several countries. Some of the studies that used 24-hour urine collection are the INTERSALT study assessing sodium intake in 52 countries, the UK project monitoring sodium intake of population after the implementation of salt reduction project (McLean, 2014), and the SALTURK study (Ibrahim & Damasceno, 2012). However, this approach has not yet been implemented in the Arab countries (Rahim et al., 2014).

   a. **Mechanisms of salt excretion in urine**

   Urine is the primary route of sodium excretion, and in healthy individuals, about 95% to 98% of consumed sodium is excreted over 24 hours (Bentley, 2006; Ji et al., 2012; McLean, 2014). The collection of urine over this duration is essential in order to capture diurnal variation in sodium excretion (Brown et al., 2009). In moderate climate, insignificant losses take place through the skin (sweat and saliva) and feces (GI secretions), which account for less than 10% (Ji et al., 2012; McLean, 2014). These losses can vary with severe climate changes and increased physical activity (Loria, Obarzanek, & Ernst, 2001; McLean, 2014). Moreover, intra-person
variability can reach 30% (Bentley, 2006). Accordingly, it is preferable to average multiple
collections in order to decrease the impact of urine over-collection or under-collection (Whelton
et al., 2012), and increase result precision (Bentley, 2006; Loria, Obarzanek, & Ernst, 2001).

Subjects must be clearly instructed on the right procedure of collecting 24-hour urine in
order to guarantee accurate results and decrease errors during collection (Loria, Obarzanek, &
Ernst, 2001; Whelton et al., 2012). They should be provided with a convenient gallon to collect
and store the urine. The steps for proper collection involves discarding the first morning void,
and collecting all the following urine up until the first morning void of the second day.

b. Urine completeness

24-hour urine collection faces two challenges; over-collection and under-collection, both of
which can affect the result of sodium excretion (Espeland et al., 2001). Accordingly, there is a
need to develop assessment tools that allow the detection of incomplete 24-hour collections
(McLean, 2014). These methods are discussed below:

i. PABA (p-amino benzoic acid)

One of the methods commonly used to evaluate urine collection completeness is the
administration of three 80 mg tablets of Para-aminobenzoic acid (PABA) on the day of collection
with the three main meals (Bentley, 2006), and the quantification of its excretion in the 24-hour
urine sample (De Keyzer et al. 2015; McLean, 2014). PABA is an objective marker that is
almost completely excreted in urine over 24 hours (93% ±4) (Bentley, 2006), and a sample
containing less than 85% of the ingested dose is considered incomplete (McLean, 2014).
However, the use of PABA faces certain limitations, such as possible interference with other
medications, non-adherence to the required number of pills, and the decreasing rate of excretion with age (Bentley, 2006; McLean, 2014).

ii. 24 hour creatinine excretion, urine volume and self-reporting

The evaluation of 24-hour creatinine excretion in the collected urine is also used to measure completeness of collection (McLean, 2014). It is a more practical (Ji et al., 2012), but less accurate method compared to PABA assessment (De Keyzer et al. 2015) due to a wide range of creatinine excretion (Pollack, 1970). Several models have been identified to estimate 24 hour excretion based on gender, age, and weight (Ji et al., 2012), however, they may not have a high sensitivity in identifying all incomplete collections (McLean, 2014).

Urine volume can be also indicative of completeness of collection, whereby a very low volume (<250-500 ml) (McLean, 2014) indicates under-collection. However, due to inter and intra individual variation of urine volume excretion, this method may not be accurate in pinpointing incomplete samples (McLean, 2014). Finally, participants may be asked to report the period of collection (>20 hours), as well as any spilled (more than a few drops) or missed collections (De Keyzer et al. 2015). Few studies even used a combination of these three methods, such as the INTERSALT study (McLean, 2014).

c. **Validity**

24-hour urine collection is a valid method when properly collected (Bentley, 2006). However, similar to any biochemical measurement, validity is threatened by several factors. Analysis of biochemical markers includes several steps, and an error may occur in any of these steps (Bentley, 2006). Moreover, subjects might forget to collect urine, spill part of the urine
during collection, perspire heavily due to severe climates and/or exercise, or lose sodium in breast milk or diarrhea (Whelton et al., 2012).

d. Advantages and disadvantages

The advantage of 24-hour urine collection in estimating sodium intake is that it is an objective measurement, and accordingly is the most valid and reliable method to be used as a reference for validating other methods to assess sodium intake (Bentley, 2006; McLean, 2014). Moreover, it can be used in different populations with different cultures and dietary patterns, permitting valid comparisons between different countries (McLean, 2014). However, this method also has numerous disadvantages that have led researchers to explore other methods that are able to replace it (Bentley, 2006).

The collection of urine samples over 24-hour is unattractive (Loria, Obarzanek, & Ernst, 2001), burdensome, and costly (Bentley, 2006), on both the researcher and the subjects (Ji et al., 2012; McLean, 2014). Participation rate in such studies can be very low because the collection requires that the subject stays at home to collect urine (Ji et al., 2012). Response rates in such studies range from 43-57%, but may reach as low as 10% (McLean, 2014). Accordingly, participants who agree to take part in the study may not be representative of the population studied which can lead to biased results (McLean, 2014). Participants who agree to join such a study are likely to be more health conscious and more interested than those who do not agree (McLean, 2014). This may lead to an underestimation of salt intake (Whelton et al., 2012). Incomplete samples are challenging, since repeating the collection will consume more time, and will double the cost. Moreover, unidentified incomplete samples can also modify the results and
consequently be a source of error (Bentley, 2006). Finally, this method might slightly underestimate sodium intake since it only measures sodium excreted in the kidneys and does not allow the identification of food contributors of Na (Brown et al., 2009; De Keyzer et al. 2015).

2. **Spot urine**

The average of several 24 hour urine collections provides the best estimate for sodium intake (Ji et al., 2012), however the collection of 24 hour urine samples is troublesome and impractical for large-scale studies (JH Park et al., 2014). Therefore, different substitutes to assess sodium intake were proposed to increase the practicality of the study (Ji et al., 2012).

Attention is currently focused on substituting 24 hour urine sodium assessment with spot urine sampling (JH Park et al., 2014), which may be used to analyze several nutritional biomarkers (McLean, 2014). Participants are required to provide a single urine sample, either overnight, daytime, evening, timed or random (Ji et al., 2012). These values are plucked into conversion formulas in order to estimate 24 hour sodium urine concentration (McLean, 2014).

a. **Advantages and disadvantages**

Spot urine samples are faster to analyze, less tedious compared to 24 hour urine collection, and do not necessitate a lot of staff training (Ji et al., 2012; Whelton et al., 2012). Samples can be stored in small containers, with no possibility of over or under collection, and can be frozen for later use (McLean, 2014). This method requires only one visit, and easy collection and analysis protocols, which render it more practical for large scale studies (McLean, 2014).
Even though this method seems promising, it has several disadvantages. Spot urine samples reflect sodium intake over a short period of time, which means that a single sample may not be enough to assess daily sodium intake (McLean, 2014). Spot sodium concentrations depend on diurnal variations and accordingly provide a larger intra-individual variability compared to 24 hour urine (McLean, 2014). For example, overnight collections have lower sodium concentrations compared to day time samples (McLean, 2014). In addition to timing of collection, sodium concentration highly depends on hydration status, physical activity, climate (Koo et al., 2014), and bladder capacity (Ji et al., 2012), which renders spot sampling a weak substitutes for assessing individual sodium secretion (Whelton et al., 2012).

Multiple spot urine samples may be needed in order to obtain accurate results (Ji et al., 2012). Spot urine sodium concentration may not be suitable to assess baseline salt intake and accordingly cannot represent population distributions of baseline intake (Ji et al., 2012). However, it is a valid method in monitoring population change compared to a baseline and in estimating group mean intake which is useful for long term studies and population monitoring (Joint Health Service Unit, 2007) in low income countries, where 24 hour urine method can be implausible (McLean, 2014).

b. **Formulas used to estimate 24 hour sodium excretion**

Several different formulae have been proposed to convert spot urine sodium concentration into an estimate of 24 hour sodium excretion, using spot urine sodium: creatinine ratio as a means to control for urinary concentration (McLean, 2014). Results are recorded in concentration of sodium per liter and then converted to 24 hour urine excretion (Ji et al., 2012).
These formulas were derived from different data including INTERSALT, Western INTERSALT, and NHANES (McLean, 2014), all of which indicated that ethnicity is a paramount factor in estimating 24 hour urine sodium from spot urine samples (JH Park et al., 2014). Accordingly, there is an urgent need to derive country-specific formulas and validate them in each respective country (JH Park et al., 2014) so that spot urine becomes a useful tool in assessing/monitoring population salt intake (McLean, 2014). Table 4 shows the four essential formulas proposed by Pan American Health Organization (PAHO) the regional office of the WHO (the PAHO formula), NHANES, Tanaka, Kawazaki, and Intersalt in North America and Europe (McLean, Williams, & Mann, 2014)
Table 4. Formulas used to assess 24 hour urine sodium excretion from spot urine.

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Estimate 24 h creatinine excretion (24 h Ucreatinine)</th>
<th>Estimate 24 h sodium excretion (24 h UNa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAHO</td>
<td>Measured creatinine from 24 h urine collection</td>
<td>24 hUNa (mg)=spotNa/UCr × measured 24 h urine creatinine (mg)</td>
</tr>
<tr>
<td>PAHO (Milton data)</td>
<td>24 h Ucreatinine excretion from 24 h urines in Milton, New Zealand (mg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>age (years) Men Women</td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>1661 1333</td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>1966 1345</td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>1887 1322</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>1864 1322</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>1751 1265</td>
<td></td>
</tr>
<tr>
<td>60–69</td>
<td>1684 1141</td>
<td></td>
</tr>
<tr>
<td>INTERSALT</td>
<td>For men: 24 hUNa (mg)=23×{25.46+0.46×spot UNa(mmol l⁻¹)–2.75×spot UCr (mmol l⁻¹)}+0.13×spot UK (mmol l⁻¹)+[4.10×BMI (kgm⁻²)]+[0.26×age(years)]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For women: 24 h UNa (mg)=23×{5.07+0.34×spot UNa (mmol l⁻¹)–2.16×spot UCr (mmol l⁻¹)}–0.09×spot UK (mmol l⁻¹)+[2.39×BMI] (kgm⁻²)+[0.35×age (years)]–[0.03×age² (years)]</td>
<td></td>
</tr>
<tr>
<td>Tanaka</td>
<td>24 h UCr (mg)= -2.04×age (years) +14.89×weight (kg)+16.14×height (cm)–2244.45</td>
<td>24 hUNa (mmol )=21.98 (spot UNa/UCr×24 h UCr (mmol ))⁰.₃⁹²</td>
</tr>
<tr>
<td>Kawasaki</td>
<td>For men: 24 h UCr (mg)= -12.63×age (years)+15.12×weight (kg)+7.39×height (cm)–79.90</td>
<td>24 hUNa (mmol )=16.3 (spot UNa/UCr×24 h UCr (mmol ))⁰.⁵</td>
</tr>
<tr>
<td></td>
<td>For women: 24 h UCr (mg)= -4.72×age (years)+8.58×weight (kg)+5.09×height (cm)–74.50</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: 24 h UCr, estimated 24 h urinary creatinine excretion; 24 hUNa, estimated 24 sodium excretion; spot Uc=spot urine potassium; UNa/UCr=spot urine sodium/creatinine ratio. Conversion factors: 1mM Na=23 mg; 1mM Creatinine=113mg.

i. **Tanaka equation**

Tanaka et al. developed a population specific formula that converts spot urine sodium to a 24 hour estimate based on age, weight and creatinine (Ji et al., 2012). The formula was based on a casual urine sample collected at any time (Tanaka et al., 2002). It was derived from the Japanese data on 591 participants (296 females and 295 males) from the INTERSALT study (JH Park et al., 2014; McLean, 2014), and was validated on another external data set (JH Park et al.,
2014). However, it was found to underestimate high sodium consumption and vice versa. It was also evaluated to have a very low specificity (Ji et al., 2012).

ii. Kawazaki equation

Another formula that estimated 24 hour urine sodium by the use of second morning void was formulated by Kawazaki et al. They recruited 159 healthy Japanese subjects (81 females and 78 males) (McLean, 2014), who were required to discard first urine voided at 8:00 a.m., and then collect all following urine voids until the second day at 8:00 a.m. in order to provide 24 hour urine samples. They were then asked to collect the second morning void after 8:00 a.m. in a separate cup. The subjects repeated this procedure for a period of 3-5 days. 24 hour urinary creatinine excretion was calculated through forward stepwise regression by the use of age, height and weight of participants (Kawasaki et al., 1993). Samples that had 24 hour urine creatinine less than 85% or more than 115% of the predicted creatinine value were discarded (Kawasaki et al., 1993). Then, by using second morning void, Kawazaki et al formulated an equation to estimate 24 hour urine sodium (McLean, 2014) by using Na/Cr or K/Cr ratios of the spot urine samples (Kawasaki et al., 1993). The correlation between estimated sodium excretion from spot sample and 24 hour urine sodium was highly significant $r = 0.728$ ($P<0.001$) (Kawasaki et al., 1993).

c. Validity

Several studies validated the accuracy of a single spot urine sample in estimating 24 hour urine excretion (McLean, 2014) through comparing spot urine sodium estimation with 24 hour urine excretion on the same day (Ji et al., 2012). The conclusions reached are controversial, with correlation coefficients ranging from 0.17 to 0.94 (McLean, 2014). In addition to correlation, mean difference ratio of Bland Altman is used to assess the agreement between spot urine and 24
hour urine sodium. This method indicated that spot urine is a poor reflection of 24 hour urine sodium, with formulas behaving differently according to gender (McLean, 2014). However, a validation study in New Zealand using the INTERSALT formula showed that spot urine provided estimates that were 0.46 to 2.56 times the measured 24 hour urine sodium, which indicated that spot urine can give reasonably accurate results compared to 24 hour urine collection (McLean, Williams, & Mann, 2014).

Kawasaki et al. showed that in 242 subjects, a single 24 hour urine collection is not representative of mean individual intake of daily sodium. An average of 3 collections (117 subjects) gave a higher correlation coefficient (0.624). Moreover, they found the correlation coefficient of spot and 24 hour urine to be 0.467, with intra-individual spot urine variation coefficient of 0.725 and 20% SD for creatinine (Ji et al., 2012; Kawasaki et al., 1993). Second morning voids performed on 3 days were more accurate and reliable to estimate 24 hour sodium excretion compared to one day 24 hour urine sample (significant correlation of r=0.774). The correlation was not as high with night samples (Ji et al., 2012; Kawasaki et al., 1993).

According to Wolf et al. spot samples overestimated Na/creatinine ratio and sodium excretion rate compared to 24 hour urine, and early morning spot urine sample preceded by an overnight fast was more associated with 24-hour urine sample (Ji et al., 2012). Tanaka et al. estimated the correlation between 24 hour urine and spot urine correlation at 0.65 (Tanaka et al., 2002), and concluded that spot urine is suitable and precise to assess and monitor mean population Na intake while 24 hour urine sodium is more suitable to assess and monitor individual intake (Tanaka et al., 2002). Another validation study estimated 24 hour urine and spot sample ratio to be 2.0, with a correlation of 0.45, and concluded that spot sampling is not always enough but can be a valid substitute to 24 hour urine collections (Llich et al, 2009). Mann
and Gerber validated AM, PM, and random spot samples against 24 hour urine. After adjusting Na/creatinine ratios for 24 hour creatinine excretion, random samples gave an insignificant correlation (0.17) whereas AM and PM samples gave significant correlations (0.31 and 0.86 respectively). They concluded that spot urine sampling is an inexpensive and practical method in assessing Na excretion in both epidemiological and clinical studies (Mann & Gerber, 2010). A review of 19 studies (6803 participants) established that spot urine is a prospective method in assessing population level sodium intake (Huang et al., 2016). In this review, the formulas developed by Tanaka and Kawasaki were less accurate compared to the INTERSALT formula in different population samples.

G. Dietary assessment

Dietary assessment tools are also being considered as alternatives to 24 hour urine excretion in estimating sodium intake (Bentley, 2006). These include weighted food records, 24 hour recalls and food frequency questionnaires, all of which are subjective measurements of intake (Satoh et al., 2014). Participants are required to list and describe portions of food eaten over a certain period of time by using food models, pictures, household measures or package sizes (Bentley, 2006). This information is then entered in computerized food composition tables and databases which estimate sodium intake from the given dietary information (De Keyzer et al. 2015). The number of required days of collection differ depending on the objective of the study and target population (Bentley, 2006). Diet histories are almost never used to assess sodium intake since they are open-ended, long, and unstandardized, and accordingly impractical for large studies (Brown, 2006).
Dietary assessments might face low reliability (Espeland et al., 2001) and variable validity (McLean, 2014). This is mainly due to the large intra-individual and day-to-day inconsistency in sodium intake (Bentley, 2006), and due to the fluctuating content of sodium in processed foods (Brown et al., 2009). In order to adjust for such errors, it is preferred to account for different dietary patterns and major sodium sources of different populations (McLean, 2014), and to average sodium intake from 7 to 10 days (Whelton et al., 2012). Dietary assessment methods are susceptible to sampling bias (Loria, Obarzanek, & Ernst, 2001). Accordingly, the selected sample should be representative of the population, and sample size should be well calculated in order to compensate for inter-individual variability (McLean, 2014).

In addition, several factors can cause systematic relative biases. These include: inaccurate portion size estimation (Bentley, 2006), incomplete or imprecise food databases, reporting errors, and missing data (Espeland et al., 2001). Subjects can underreport their intake and might be uninterested and less compliant, or unmotivated (Espeland et al., 2001). Moreover, they might be unable to accurately quantify discretionary sodium, i.e. added during cooking, at the table, and in restaurants (Loria, Obarzanek, & Ernst, 2001; McLean, 2014). Subjects’ behavior might also be affected during data collection, which might render the results inaccurate and not representative of their intake (reporting error) (Loria, Obarzanek, & Ernst, 2001).

Another basic problem faced by these methods lies in the adequacy and preciseness of the adopted food composition tables (Loria, Obarzanek, & Ernst, 2001). Data built in such software might rely on few recipes which might not be illustrative of typically applied recipes, and might not include all food types and brands consumed (De Keyzer et al. 2015). Moreover, nutrient content of specific brand names can be inaccurate in case of false labeling, and can vary due to product development, which can affects results if not frequently updated (Brown, 2006). Finally,
the data in food composition tables need to be validated in order to decide on the final sodium contents of foods, along with the quantity of sodium added during cooking and at the table (Loria, Obarzanek, & Ernst, 2001), and sodium found in drinking water or in supplements (Coding error) (Loria, Obarzanek, & Ernst, 2001; Whelton et al., 2012). It is essential to acknowledge the strengths and weaknesses, and reliability and validity of the various dietary assessment methods in order to decide on which is best to answer the needed research question (Jardack, 2006).

1. **Weighted food records**

   The weighted diet record requires subjects to accurately weigh and duplicate what they ate for 24 hours either at home or at a research kitchen. The food sodium content is then measured by chemical analysis (Loria, Obarzanek, & Ernst, 2001). It is a prospective method which permits an easy, on-the-spot observation of dietary intake, with the need of only a small amount of information (Bentley, 2006). In such studies, since the consumed food is closely monitored, the measured sodium accounts for more than 90% of sodium excreted in urine over 24 hours (Loria, Obarzanek, & Ernst, 2001). Several studies have shown that food record analyses provided estimations 15-30% lower than 24 hour urine sodium (Caggiula et al, 1985; Schachter et al, 1980), which makes this method a valid alternative to 24 hour urine with correlation coefficients ranging from 0.5 to 0.6 (Bentley, 2006; Loria, Obarzanek, & Ernst, 2001).

   It has been shown that a minimum of 3 to 6 day food records are needed in order to account for intra-subject variability, and provide an average sodium intake close to 24 hour urinary excretion. Correlations between 24 hour urine and 3 to 6 food record averages were found to range between 0.81 and 0.93 (Espeland et al., 2001). Moreover, absolute differences were found
to be largest with 1 day food records, decreased by almost 50% with an increase of 3 days, and further decreased with 6 day food records (Bentley, 2006). In a second study, sodium estimated from 4 day food records was only 7% lower than the value measured in 24 hour urine (Espeland et al., 2001).

2. 24 hour recall

Another approach to assess sodium intake is 24 hour dietary recall, which is considered the most viable in estimating nutrient intake (Espeland et al., 2001), without requiring any biological sample (Satoh et al., 2014). It consists of an oral, written, or on-the-phone interview in which subjects list what they ate on a previous day, along with detailed descriptions of portion sizes, weights, and preparation methods of these foods (Bentley, 2006). It was found to be very useful in assessing differences between intakes of groups (Bentley, 2006). Two non-consecutive 24 hour recalls are preferred in estimating population or group sodium intake according to a European consensus (De Keyzer et al. 2015). However, there has not been any confirmation on the validity of this method (Satoh et al., 2014).

Both weighted food records and 24 hour dietary recalls are time consuming, and can be hectic for the researchers and study subjects (McLean, 2014). Participants’ intake and behavior might be altered during dietary data collection, especially in prospective methods such as weighted food records, which can cause underreporting of specific foods and nutrients (Gemming et al, 2014). Moreover, subjects might tend to manipulate 24 hour recall data in order to please the researcher (Loria, Obarzanek, & Ernst, 2001). In order for these methods to have good repeatability or reliability, subjects must be able to give precise information (Bentley, 2006).
In 24 hour recalls, consumed sodium is self-reported and not closely monitored, which increases the chance of error occurrence (Bentley, 2006), and renders the agreement between estimated sodium intake and 24 hour urinary excretion very low compared to more controlled conditions (Loria, Obarzanek, & Ernst, 2001). Subjects might not remember all consumed food, and might not accurately report portion sizes especially when considering complex food recipes, or food consumed at restaurants with no information on their salt content (Loria, Obarzanek, & Ernst, 2001). Obese and overweight individuals tend to underreport their energy consumption (McLean, 2014), resulting in false low estimation of sodium intake (Loria, Obarzanek, & Ernst, 2001; Whelton et al., 2012). Accordingly, data collection requires the use of meticulous standardized probes for specific food items (Loria, Obarzanek, & Ernst, 2001). The interviewer should be well trained in order to avoid biased results caused by inadequate probing and leading questions, all of which can be time consuming (Loria, Obarzanek, & Ernst, 2001). In addition, he/she should have a good knowledge about the dietary habits of the subject, and should also be familiar with the used language (Whelton et al., 2012).

Sodium intake estimated from 24 hour recalls is characterized by low repeatability especially for long time intervals due to intra-subject variability (Espeland et al., 2001). Consequently, several recalls per subject are required to reach an accurate result, which makes this method time consuming and costly (Bentley, 2006). In addition, estimates might not be reliable due to the daily the wide diversity of sodium sources, and its fluctuating concentrations in commercial foods (De Keyzer et al. 2015; Whelton et al., 2012). Thus, food records and 24 hour recalls might not be illustrative of a person’s habitual intake, but can be reliable in estimating group intakes (Loria, Obarzanek, & Ernst, 2001).
Several studies used 24 hour recalls as an alternative to assess sodium intake and compared them with 24 hour urine collection, such as the NHANES III (1994-1998) and INTERMAP study (Loria, Obarzanek, & Ernst, 2001). In fact, 24 hour recalls provide sodium estimates which are 72 to 83% the values of 24 hour urine excretion, with fitted ratios ranging from 0.72 to 0.83 for estimated average intake from dietary recall compared to 24 hour collection, which is an average of 22% less than the gold standard (Bentley, 2006). The European Food Consumption Validation (EFOVAL) study validated the use of repeated 24 hour dietary recalls in assessing sodium intake in 5 European countries (October 2007-April 2008). According to this study, two recalls were found to significantly underestimate sodium intake (De Keyzer et al. 2015). Another study found that 24 hour recalls underestimate sodium intake by 22% compared to 24 hour urine sodium (Espeland et al., 2001). Bias in 24 hour recalls was higher among uneducated individuals (Espeland et al., 2001). The INTERMAP study however, indicated that the average of four 24-h recalls gave estimates similar to the average of two 24-h urine collections for participants in the US, UK and Japan, with dietary recall estimates lower than urinary (Brown et al., 2009).

In order to decrease the limitations of 24 hour recalls, the United States Department of Agriculture (USDA) developed a standardized method for the interview called Multiple Pass Food Recall (MPR) (Moshfegh et al., 2008; Raper et al., 2004). In consists of five steps:

- The subjects are asked to give a “quick list” of the foods and drinks they ate in the past 24 hours without being interrupted by the interviewer.
- The interviewer then uses probes in order to remind the subject of foods often omitted in 24 hour recalls.
- The subject is then asked to state the time and meal of each reported food item.
- The interviewer investigates about the foods and drinks consumed: the way of preparation, portion sizes, brand names, and possible supplemental intake.
- Finally, both the subject and the interviewer perform a final evaluation of the whole 24 hour recall.

The validation of this method against 24 hour urine in 30-69 year old subjects gave a ratio of 0.93 (0.89, 0.97) for males and 0.90 (0.87, 0.94) for females, which signifies that it is a valid method in assessing sodium intake (McLean, 2014).

3. **Food frequency questionnaire**

Properly designed and validated food frequency questionnaires (FFQs) can also be used in assessing individual and group sodium intake in both epidemiological (Jardack, 2006) and clinical studies (McLean, 2014). They are self-administered, retrospective questionnaires aiming at assessing a person’s habitual intake of certain foods of interest over the previous week, months, or year depending on study objective (Jardack, 2006). Accordingly, FFQs evaluate intake over a long period of time, and thus compensate for day-to-day variability of a subject’s intake (McLean, 2014).

Several approaches exist in order to well-organize an FFQ. The first step in creating an FFQ is developing a population-specific list of foods that are the highest contributors of the nutrient of study, based on previous validated data (Subar, 2004). The collection, coding and analysis of nutrient of interest should be standardized, and results of the FFQ in use should be verified. Subjects filling an FFQ should have a high level of motivation, and should be familiar with the portions they consume (Bentley, 2006). In addition, the interviewer should be well-
versed in nutrition counseling and the use of food models and images, standard probes and food terminologies in order to help the subject provide accurate responses (Jardack, 2006). Finally, the used food composition tables should be up-to-date and should contain accurate information on the ethnic foods of interest (Jardack, 2006).

Compared to food records and 24 hour recalls, FFQs are easier to use (Brown, 2006; Loria, Obarzanek, & Ernst, 2001), and accordingly more convenient for large population studies (Jardack, 2006). Little training is required, and usually, subjects do not need a lot of instructions in order to complete such a questionnaire (Jardack, 2006). However a successful FFQ necessitates meticulous attention to details and a large commitment. Moreover, FFQs allow the documentation and the quantification of intake over a long period of time for a large sample size (Brown, 2006). They are flexible depending on study objective, whereby they allow the assessment of specific foods or nutrients of interest, and can assess a total diet (Subar, 2004). Finally, FFQs are relatively cheaper compared to other dietary assessment tools (Jardack, 2006; Subar, 2004). According to the Women’s Health Initiative, FFQs cost $1.2 million compared to $25 million for three 24 hour recalls and $23.2 million for 3-day food records (Brown, 2006).

FFQs however have several limitations in assessing nutrient intake. Subjects can find it difficult to estimate the usual frequency of food intake especially for mixed food (Brown, 2006). Portion sizes might seem very broad and vague (Brown, 2006), making FFQs less specific and subject to a greater measurement error (Subar, 2004). In addition, such questionnaires do not consider important details regarding brand names of foods, and salt added in preparation (Loria, Obarzanek, & Ernst, 2001). Univariate analysis of FFQs may vary depending on education level, region and ethnicity, whereas repeatability and relative measurements slightly vary among clinical and demographic subgroups (Espeland et al., 2001). Finally, they do not allow an
accurate estimation of daily intake and are consequently not reliable in monitoring population intake (McLean, 2014).

It is very hard to assess the validity of an FFQ. Usually, a valid FFQ should have a correlation coefficient ranging from 0.4 to 0.7 (Subar, 2004). According to several validation studies, FFQS can give results with are 50% accurate (Brown, 2006), but they tend to overestimate sodium intake when compared to 24 hour urine collection (Jardack, 2006).
CHAPTER III

METHODS

A. Subjects and Study Design:

A convenience sample of healthy adults was recruited through an advertisement that was posted in various recruitment locations (mainly NFSC department, FHS, and AUBMC) at the American University of Beirut. In order to be eligible to participate, individuals must be aged between 19 and 55 years old. Individuals with reported kidney disease, diabetes or who had been prescribed diuretics were excluded from the study. Illiterate and visually impaired subjects were also excluded from the study.

Ethical considerations

A written consent was obtained from participants, prior to their enrollment in the study. All participants were interviewed at the Department of Nutrition and Food Science.

1. Sample Size

A sample of the order of 100 subjects is sufficient to characterize the group mean with 95% confidence intervals (CI) of +/- 12 mmol/d (+/- 276 mg/d) (i.e. +/-2 SE), assuming a standard deviation of urinary sodium excretion of about 60 mmol/d (1.38 g/d) (Elliott & Brown, 2006). Accordingly, our study aimed at recruiting 100 subjects in order to assess the validity of the FFQ, and spot urine Kawasaki and Tanaka in estimating Na intake compared to 24 hour urine.
2. **Study Protocol**

Subjects who agreed to participate in the study were invited to visit the department of Nutrition and Food Sciences. During this visit, subjects were given information about the study methodology and procedures. A trained researcher provided the subjects with a large 2 liter collection container and a plastic bag. The researcher also provided the subjects with detailed instructions for the 24 hour urine collection to the study participants (Appendix 1). An appointment for a second visit in which the 24 hour urine sample should be submitted was booked. During the second visit, each subject was asked to complete a food frequency questionnaire to assess his/her dietary intake of specific foods (Appendix 2). This was done in an interview setting that took 20 minutes on average. Anthropometric measurements were also obtained. In addition, the participant was asked to provide a spot urine sample, consisting of the second morning void, for the assessment of urinary sodium and creatinine levels.

B. **Data collection**

1. **24 hour urine collection:**

Subjects were instructed to collect a 24 hour urine sample by discarding the first voided urine upon arising in the morning then collecting all voided urine up to and including the first void the following morning. They were instructed to pass urine into a 100 mL plastic beaker, and then pour the sample into a large 2 liter collection container which contained the preservative, boric acid. Plastic bags were provided to carry the container if respondents were not at home for some of the collection period. Respondents were asked to record the start and finish times of
their collection, any missed urine passes, and any medication taken during the collection. The full instructions and equipment given to the participants are outlined in Appendix 1.

During the period of collection, 24 hour urine samples were stored at room temperature. The following day, samples were transported to the Department of Pathology at the American University of Beirut Medical Center to be stored. Urine samples were also frozen without preservative at –20°C. 24 hour urine samples were not used for tests other than sodium or creatinine.

2. Spot urine collection:

All study participants were asked to submit a spot urine sample being a second morning void for the assessment of urinary sodium and creatinine levels al (Kawasaki et al., 1993). A 20 ml urine specimen was collected in a plain container for the measurement of sodium and creatinine. Urine samples were stored at -20°C in the Nutrition and Food Science Research laboratory, and were kept for a period of 3 months. Spot urine samples were not used for tests other than sodium and creatinine.

3. Food Frequency questionnaire:

The FFQ questionnaire that was used in this study is a 46 item semi-quantitative questionnaire that was previously developed for the assessment of sodium intake in Lebanese adults (Helou, 2014). The development of this FFQ was based on dietary data that was collected between 2008 and 2009 as part of the national dietary and anthropometric survey (n= 2625 adults), where one 24-hour dietary recall was obtained from the study participants. The data
obtained by means of the 24-hour dietary recall was used in order to identify the food items that are the main contributors to sodium intake among Lebanese adults (Helou, 2014). As such, all individual food items consumed by > 5% of the national survey’s study sample and which provide at least 50 mg Na/serving were selected for inclusion in the FFQ (Charlton et al, 2007). For simplicity purposes, these food items were combined, at a later stage, into food groups that include food items with inherent Na, such as milk, as well as food products with a high amount of added salt, such as processed meat (Charlton et al, 2007; Helou, 2014). A reference portion, expressed in household measures or grams, was specified for each food item in the FFQ (Nasreddine et al, 2006). The FFQ was pilot-tested and previously tested for its reliability (r=0.886) (Helou, 2014). It is shown in Appendix 2.

The FFQ was administered to study participants in an interview setting. Each individual was asked to estimate the number of times per day or week he/she consumes specific food products. The amount usually consumed per food item was estimated by making comparisons with the specified reference portion. Common household measures, measuring cups, spoons and a ruler was used to assist the individual in the estimation process.

4. *Anthropometric measurements:*

Height was measured using SECA height meter and weight was measured SECA digital scale at the Faculty of Agriculture and Food Science at the American University of Beirut.
5. **Analysis of urine samples:**

The 24 hour and spot urinary sodium was measured by ion-selective electrodes (ISE) on the Cobas 6000 instrument at AUBMC. Urinary creatinine was measured at AUBMC by the enzymatic method using Cobas 6000 instrument and Roche CREP-2 products.

C. **Calculation of daily sodium intake**

1. **24 hour urine collection:**

Urine is the primary route of sodium excretion, and in healthy individuals, about 95% to 98% of consumed sodium is excreted over 24 hours (Bentley, 2006; Ji et al., 2012; McLean, 2014). Accordingly, the concentration of sodium in 24 hour urine in mmol per 24 hours was converted to mg per day and considered as the quantity of daily intake.

The method of Knuiman et al, which is based on based on 24 hour urine creatinine, was adopted to assess for the completion of the 24 hour urine samples (Murakami et al., 2008). As such, samples that were found to have a value of less than 0.7 for the Knuiman formula (urinary creatinine [mmol/d] * 113)/(21 * body weight [kg]) were not included in subsequent analyses.

2. **Food Frequency questionnaire:**

Absolute amounts of Na per serving size used for each of the food items on the FFQ were calculated from “Food Composition Tables for Use in the Middle East” (Pellet and Shadarevian, 1970) and the Nutritionist Pro software, version 1.2. The sodium amount (in mg) was multiplied by the frequency factor reported by each individual in order to determine the total daily Na intake per subject.
3. **Spot urine samples:**

For the calculation of 24-hour urinary sodium excretion from the spot urine analysis, the method developed by Kawasaki et al. (Kawasaki 1991, Kawasaki 1993) as well as that developed by Tanaka et al. were used (Tanaka et al., 2002). These methods require the measurement of the spot urine sodium concentration along with a measure of the state of concentration or dilution of the urine by measuring urine creatinine concentration (Mann and Gerber 2010), which is considered to be fairly constant (Arroyave & Wilson 1961; Pollack 1970). The calculation of the 24-hour urinary sodium was based on the application of 2 consecutive steps using 2 consecutive equations for both methods, Kawasaki and Tanaka, respectively:

For the Kawasaki estimation:

**Step 1:**

Adult 24 hour urine creatinine excretion (UcrV24h) was estimated using the following sex-specific equation based on age, bodyweight and height (Kawasaki 1991).

**Equation 1 (males):**

Estimated UcrV24h = -12.63 x Age + 15.12 x W + 7.39 x H - 79.90 (male: mg/day)

**Equation 1 (females):**

Estimated UcrV24h = -4.72 x Age + 8.58 x W + 5.09 x H - 74.50 (female: mg/day)
**Step 2:**

Obtaining the estimated value for $U_{crV24h}$ allowed the calculation of the estimated value for the 24-hour urinary sodium value ($24HUNaV$), as follows:

**Equation 2:**

Estimated value of $24HUNaV$ (mEq/day) = $16.3 \sqrt{X_{Na}}$

Where $X_{Na} = \frac{SUNa}{SUcr} \times$ predicted $24HUcrV$.

$SUNa$ = spot urinary sodium concentrations

$SUcr$ = spot urinary creatinine concentrations

For the Tanaka estimation:

**Step 1:**

Adult 24 hour urine creatinine excretion ($U_{crV24h}$) was estimated using the following sex-specific equation based on age, bodyweight and height. (Tanaka et al., 2002)

**Equation 1 for males and females:**

Estimated $U_{crV24h} = -2.04 \times \text{Age} + 14.89 \times W + 16.14 \times H - 2244.45$ (mg/day)

**Step 2:**

Obtaining the estimated value for $U_{crV24h}$ allowed the calculation of the estimated value for the 24-hour urinary sodium value ($24HUNaV$), as follows:
**Equation 2:**

\[
24\text{HUNa}V (\text{mmol}) = 21.98 \left( \frac{\text{SUNa}}{\text{SUcr}} \times \text{UcrV}_{24h} (\text{mmol}) \right)^{0.392}
\]

\(\text{SUNa} =\) spot urinary sodium concentrations

\(\text{SUcr} =\) spot urinary creatinine concentrations

It is important to note that 24-hour urinary sodium excretion was assumed to be equal to 24-hour dietary sodium intake given that 24-hour urine sodium excretion can account for 95-98% of dietary sodium intake (Bentley 2006).

**D. Statistical analyses**

Statistical analysis was performed using the Statistical Analysis Package for Social Sciences (SPSS, version 19.0) and with the level of significance set at \(p<0.05\). Frequencies and descriptive statistics were performed for the different variables under study. Means and standard deviations were computed for anthropometric and dietary variables. Spearman correlation coefficients were calculated for the relationship between sodium intake as assessed by the FFQ and spot urine estimations (Kawasaki and Tanaka) compared to 24 hour urinary Na.

**E. Validity Statistics**

1. **Mean Difference**

Mean of the 24 hour urine sodium, the FFQ, the urine Kawasaki and urine Tanaka were calculated. In addition, the mean difference of the sodium assessed by the gold standard: 24 hour
urine, with each of the sodium assessment methods under study (FFQ, urine Kawasaki, and urine Tanaka) was computed.

2. **Bland-Altman Method**

   In order to measure the level of agreement between the methods under study and the gold standard, the Bland-Altman statistical method was performed (Bland & Altman, 1986). It allows the evaluation of a new indirect measurement technique against a previously established one, through assessing their agreement across the different ranges of intake (Bland & Altman, 2010; Bland & Altman, 1986), and is adopted in most of the agreement studies (Zaki et al., 2012). The Bland-Altman statistical technique is very useful in the evaluation of the quality of dietary intake validation studies, since it is not affected by inter-person variation while analyzing the standard deviation of the difference between two measurement methods (Bland & Altman, 1999). It considers the differences between the results of the measurement methods on the same subject, and graphs them against the average of both methods per subject. The mean of the differences between the methods are useful in pinpointing the presence of bias of one method with respect to the other. In addition, the standard deviation of the differences evaluates the variability of these differences. The closer the differences are to zero, the more the agreement between the methods. This is represented graphically by a narrow band around the zero (Bland & Altman, 1986). Limits of agreement (LoA), which are two numerical values where 95% of the differences should lie, are also used in order to evaluate to what level the methods disagree/agree. Wide limits indicate the existence of an overall bias (Bountziouka & Panagiotakos, 2010).

   The formulas adopted to calculate LoAs are: \( \text{mean difference} - 1.96 \times \text{standard deviation} \) and \( \text{mean difference} + 1.96 \times \text{standard deviation} \). Bland-Altman
also provided a method of assessing whether the difference between the methods is the same across the range of intakes, and whether the extent of agreement differs for low intakes compared with high intakes (Cade et al. 2004). P < than 0.05 were considered statistically significant.

3. **Spearman Correlation Coefficient**

Spearman correlation was calculated to compare the results provided by the different measures of sodium intake, and measure the association between the estimated levels of intake provided by these methods (Willet 1998):

- 24 hour urine collection and spot Kawasaki
- 24 hour urine collection and spot Tanaka
- 24 hour urine collection and FFQ

This coefficient allows us to assess the strength of the association between the corresponding methods (Bland & Altman, 1986). An acceptable correlation for validity should be between 0.5 and 0.7, however values equal to, or greater than 0.4 are also acceptable. Whereas, a correlation coefficients less than 0.4 drastically decreases validity (Willett 1998). A one-sample t test was performed to assess the significance of the Spearman correlation coefficient.

4. **Intraclass Correlation Coefficient**

In addition to the Spearman Correlation Coefficient, the Intraclass Correlation Coefficient (ICC) was computed along with its P value. ICC is a measure of agreement describing the strength in resemblance of units belonging to the same class (Bland & Altman, 1990). It analyzes data formulated as groups, unlike other correlation coefficients which analyze data in pairs. Unlike weighted Kappa, ICC corrects the agreement expected by chance (Bland & Altman,
and assesses within subject differences instead of observer differences (Bountziouka & Panagiotakos, 2010). However, the ICC treats the two methods of interest as random tools, and does not take into consideration variance orders, all of which are considered in the weighted Kappa statistics.

5. Agreement

Agreement between the gold standard and the methods of interest; FFQ, spot urine Kawasaki and spot urine Tanaka was calculated through weighted Kappa agreement, % agreement and adjacent agreement in quartiles as a means to validate the spot urine estimations (Kawasaki and Tanaka) as well as the FFQ against 24 hour urinary Na excretion.

a. Weighted Kappa agreement

The Kappa was used to study to which extent a subject who belongs to a category in the gold standard (24 hour urinary Na excretion) can belong to the same related category in a second assessment method (FFQ, spot urine Kawasaki and spot urine Tanaka respectively). It assesses the agreement between two or more methods in distributing the data into different categories. In this study, the Weighted Kappa ($\kappa_w$) was used for the three measures of sodium intake: FFQs, Urine Kawazaki and Urine Tanaka, in order to assess how different or how related their ratings were with respect to the 24 hour urinary excretion.

Kappa values were categorized for strength of agreement as suggested by Landis& Koch (1977), and the interpretation of Kappa values is explained in table 5 (Viera & Garrett, 2005):
Table 5. Kappa classes in evaluating agreement of two methods

<table>
<thead>
<tr>
<th>Kappa</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0</td>
<td>Less than chance agreement</td>
</tr>
<tr>
<td>0.01-0.20</td>
<td>Slight agreement</td>
</tr>
<tr>
<td>0.21-0.40</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>0.41-0.60</td>
<td>Moderate agreement</td>
</tr>
<tr>
<td>0.61-0.80</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>0.81-0.99</td>
<td>Almost perfect agreement</td>
</tr>
</tbody>
</table>

b. **Percent agreement**

Percent agreement was calculated as quartiles according to the following formula:

\[
\frac{\text{Number of subjects in quartile 1 + number of subjects in quartile 2 + number of subjects in quartile 3 + number of subjects in quartile 4}}{n=60}
\]

c. **Adjacent agreement**

Adjacent agreement was also calculated in order to measure the ability of these methods in classifying sodium intake with adjacent quartiles compared to 24 hour urine. It was performed according to the following formula:

\[
\frac{\text{Number of subjects in quartile 1 and 2 + number of subjects in quartile 1, 2 and 3 + number of subjects in quartile 2, 3 and 4 + Number of subjects in quartile 3 and 4}}{n=60}
\]
CHAPTER IV

RESULTS

A. Descriptive Characteristics of Study Participants

Seventy-two subjects were recruited, however only sixty subjects (31 males, 29 females) provided a complete 24-hour urine collection based on Knuiman et al. test. Table 6 summarizes the descriptive characteristics of the study population. According to gender, mean BMI, height, weight, 24 hour urinary creatinine and sodium were significantly higher in males compared to females. The mean age was 24.82±6.63 years with no significant difference between genders.

Table 7 shows sodium intake as calculated by the FFQ, spot urine (Kawazaki and Tanaka), and 24 hour urinary excretion. Mean sodium intakes were significantly higher in males compared to females using all the considered methods, except using the Tanaka spot urine. Table 8 shows the percentage of individuals exceeding the WHO limit (2000 mg/d) of sodium intake (De Keyzer et al. 2015; Krauss et al., 2000). Most of the study population was found to exceed the upper limit for Na intake according to the WHO guidelines.
Table 6. Characteristics of the study population by gender:

<table>
<thead>
<tr>
<th></th>
<th>Total (n=60)</th>
<th>Male (n=31)</th>
<th>Female (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.82±6.63</td>
<td>24.97±7.08</td>
<td>24.66±6.24</td>
<td>0.856</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.648±5.253</td>
<td>26.026±3.768</td>
<td>23.174±6.211</td>
<td>0.038</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.693±0.098</td>
<td>1.769±0.0648</td>
<td>1.611±0.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.785±15.992</td>
<td>81.136±10.125</td>
<td>59.721±13.552</td>
<td>0.000</td>
</tr>
<tr>
<td>24 hour urinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>creatinine (g/d)</td>
<td>1.509±0.494</td>
<td>1.874±0.385</td>
<td>1.12±0.228</td>
<td>0.000</td>
</tr>
<tr>
<td>24 hour urinary</td>
<td>3,555.417±1,654.134</td>
<td>4,092.887±1,735.917</td>
<td>2,980.879±1,369.351</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Table 7. Sodium intake (mg/d) as estimated by the FFQ, Spot Urine (Kawasaki, Tanaka), and 24 hour urine sodium excretion in a sample of Lebanese adults (n=60):

<table>
<thead>
<tr>
<th>Methods</th>
<th>Total (n=60)</th>
<th>Male (n=31)</th>
<th>Female (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFQ* (mg/d)</td>
<td>4,968.783±2,374.078</td>
<td>5,990.178±2,003.322</td>
<td>3,876.947±2,277.435</td>
<td>0.000</td>
</tr>
<tr>
<td>Urine Kawasaki b (mg/d)</td>
<td>4,437.199±1,511.448</td>
<td>4,927.26±1,682.316</td>
<td>3,913.34±1,109.618</td>
<td>0.008</td>
</tr>
<tr>
<td>Urine Tanaka c (mg/d)</td>
<td>3,356.675±860.281</td>
<td>3,494.487±951.226</td>
<td>3,209.36±739.254</td>
<td>0.199</td>
</tr>
<tr>
<td>24 hour urine d (mg/d)</td>
<td>3,555.417±1,654.134</td>
<td>4,092.887±1,735.917</td>
<td>2,980.879±1,369.351</td>
<td>0.008</td>
</tr>
</tbody>
</table>

a: Food Frequency Questionnaire
b: Na Urinary Excretion using the Kawasaki Equation (Kawasaki et al., 1991)
c: Na Urinary Excretion using the Tanaka Equation (Tanaka et al., 2002)
d: Na 24 hour Urinary excretion
Table 8. Percentage of individuals exceeding the WHO limit for sodium intake (2000mg/d) based on the FFQ, Spot Urine (Kawasaki, Tanaka), and 24 hour urine sodium excretion in a sample of Lebanese adults (n=60):

<table>
<thead>
<tr>
<th></th>
<th>Total (n=60)</th>
<th>Male (n=31)</th>
<th>Female (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFQa (mg/d)</td>
<td>95</td>
<td>100</td>
<td>89.66</td>
</tr>
<tr>
<td>Urine Kawasakib (mg/d)</td>
<td>96.67</td>
<td>96.77</td>
<td>96.55</td>
</tr>
<tr>
<td>Urine Tanakac (mg/d)</td>
<td>95</td>
<td>93.55</td>
<td>96.55</td>
</tr>
<tr>
<td>24 hour urine (mg/d)</td>
<td>78.33</td>
<td>90.323</td>
<td>65.52</td>
</tr>
</tbody>
</table>

a: Food Frequency Questionnaire  
b: Na Urinary Excretion using the Kawasaki Equation (Kawasaki et al., 1991)  
c: Na Urinary Excretion using the Tanaka Equation (Tanaka et al., 2002)  
d: Na 24 hour Urinary excretion

B. Validity of the FFQ and spot urine

Spearman correlation coefficients between Na intake estimates derived from the FFQ, spot urine (Kawasaki and Tanaka), and 24 hour urinary Na excretion were calculated (Table 9). Spearman coefficients were all statistically significant. The Spearman correlation coefficient ranged between 0.258 for FFQ & 24 hour urinary excretion, 0.504 for spot urine Tanaka & 24 hour urinary excretion, and 0.547 for spot urine Kawasaki & 24 hour urinary excretion. Intraclass correlation coefficients were computed between Na intake estimates derived from the FFQ, spot urine Kawasaki and Tanaka, and 24 hour urine Na excretion. The ICC was significant for spot urine Kawasaki and Tanaka ranging from 0.610 for spot Tanaka to 0.72 for spot Kawasaki vs. 24 hour urine Na excretion. However, the ICC for FFQ vs. 24 hour urine Na excretion was not significant, with a value of 0.327.
Table 9. Association between Na intake estimates derived by FFQ and Spot Urine (Kawasaki, Tanaka), and urinary Na excretion as examined by Spearman and ICC:

<table>
<thead>
<tr>
<th></th>
<th>Spearman</th>
<th>p-value</th>
<th>ICC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Kawasaki</td>
<td>0.547**</td>
<td>0.000</td>
<td>0.720</td>
<td>0.000</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Tanaka</td>
<td>0.504**</td>
<td>0.000</td>
<td>0.610</td>
<td>0.000</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. FFQ Na</td>
<td>0.258*</td>
<td>0.047</td>
<td>0.327</td>
<td>0.066</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

The Bland and Altman approach was used to compare the FFQ and spot urine (Kawasaki and Tanaka) against the gold standard, 24 hour urinary Na excretion. Based on this technique, the difference between 2 methods in measuring Na intake can be assessed by plotting this difference against the average value of the 2 methods. Accordingly, the difference in dietary Na obtained by the FFQ and 24 hour urinary Na excretion and by the spot urine (Kawasaki and Tanaka) and 24 hour urinary Na excretion was compared with the average value of Na intake estimates provided by each of the methods examined. This difference was plotted and analyzed using simple linear regression. The hypothesis that the slope was equal to zero was tested in each case in order to check whether the 2 methods have the same error variance. Mean difference, and limits of agreement between the different methods were determined. The broader the limit of agreements with respect to the mean value are, the lower the level of agreement between the two highlighted methods. A one-sample Student’s t test was performed to examine whether mean difference between each of the methods was significantly different from zero; the closest the mean difference to zero, the higher the agreement.

Based on the Bland and Altman approach, mean differences in Na intake between the FFQ, and spot urine Kawasaki versus 24 hour urinary Na excretion were significantly different.
from zero. Mean difference between spot urine Tanaka and 24 hour Na urinary excretion was not significantly different than zero. The slope of the FFQ vs. 24 hour urine was positive and significantly different from zero, demonstrating that the FFQ tends to overestimate Na intake principally at high intake levels of sodium. In addition, it indicates that the higher the levels of sodium intake, the weaker the agreement between the FFQ and 24 hour urinary Na excretion. However, the slope of the spot urine (Kawasaki and Tanaka) was negative, and significantly different from zero only for the spot urine Tanaka, indicating that they over-estimate Na intake at low levels of intake, and underestimate Na intake at high intake levels.

Bland Altman graphs were plotted and the mean difference, 95% limits of agreement (LoA), intercept, slope and confidence interval for all methods are presented in Table 10. The method having the highest agreement with 24 hour urinary sodium is the spot urine Tanaka; providing the lowest mean difference being 198.741 ± 1396.945 mg sodium with limits of agreement -2,9992.631 mg and 2,595.148 mg. The next agreeable method with 24 hour urinary sodium is the spot urine Kawasaki with mean difference 881.782 ± 1,482.928 and limits of agreement -2,084.075mg and 3,847.639. The widest limits of agreement, and largest mean difference and standard deviation were observed for FFQ versus 24 hour urinary Na excretion suggesting a lower correlation/agreement between the two methods.
Table 10. Differences between each of the methods for estimating Na intake based on the Bland Altman approach:

<table>
<thead>
<tr>
<th>Difference</th>
<th>Mean Difference</th>
<th>Standard Deviation</th>
<th>Limits of Agreementa</th>
<th>Slopeb</th>
<th>Sign</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Kawasaki</td>
<td>881.782*</td>
<td>1482.928</td>
<td>-2,084.075; 3,847.639</td>
<td>-0.115</td>
<td>0.408</td>
<td>171.438; 2,512.821</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Tanaka</td>
<td>-198.741</td>
<td>1396.945</td>
<td>-2,992.631; 2,595.148</td>
<td>-0.798c</td>
<td>0.000</td>
<td>1,643.275; 3,476.929</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. FFQ Na</td>
<td>1413.366*</td>
<td>2595.531</td>
<td>-3,777.713; 6,604.428</td>
<td>0.580c</td>
<td>.006</td>
<td>-2,889.239; 775.757</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. FFQ Na calibrated</td>
<td>312.541</td>
<td>1624.745</td>
<td>-2,936.949; 3,562.031</td>
<td>-1.501c</td>
<td>0.000</td>
<td>4918.488; 6851.273</td>
</tr>
</tbody>
</table>

*: the mean difference significantly different from zero, as examined by t-test  
a: LoA determined as mean difference ± 2×standard deviation of the differences  
b: Slope of the average of methods regressed on difference between methods  
c: slope values significantly different from zero

The Bland-Altman plots shown in Figure 1 graphically represent the difference and the LoA between the 24 hour Na urinary excretion and the other 2 methods under investigation (FFQ and spot urine Kawasaki and Tanaka). The FFQ only tended to overestimate sodium intake compared to the 24 hour Na urinary excretion since its regression line was positive. All of the three methods had quite large limits of agreement.
Figure 1. Bland Altman plots for Na intake as estimated by the FFQ, Spot Urine (Kawasaki, Tanaka), and 24 hour urine sodium excretion:

Graph 1: Bland Altman Plot for 24 hour Na Urinary excretion Vs. Spot urine Na Kawasaki

Graph 2: Bland Altman Plot for 24 hour Na Urinary excretion Vs. Spot urine Na Tanaka

Graph 3: Bland Altman Plot for 24 hour Na Urinary excretion Vs. FFQ Na
C. Agreement with 24 hour urine.

Percent agreement, adjacent agreement, and weighted linear Kappa agreement between the different methods (Table 11) were calculated to compare categorization of Na consumption data into quartiles. Moreover, percent agreement of these methods with 24 hour urine collection in classifying Na intake above and below the WHO guideline of 2000mg/day was computed. (Table 12)

Both, the adjacent agreement and percent agreement ranked highest for the spot urine formulas; Tanaka (81.66%) and Kawasaki (80%) vs. 24 hour urinary sodium. Kappa values ranged from 0.17 for the FFQ Na vs. 24 hour urine Na, and 0.36 for both the spot urine Na Tanaka and spot urine Na Kawasaki. Both spot urine formulas has a fair agreement with 24 hour urine, while the FFQ had a slight agreement. The three methods had a good agreement with 24 hour urine in classifying sodium intake above and below the WHO guideline of 2000 mg/day. Both the Tanaka equation and the FFQ admitted an 80% agreement and the Kawasaki equation admitted an 81.66% agreement.

Table 11. Agreement in classification of Na intake in quartiles:

<table>
<thead>
<tr>
<th>Method</th>
<th>% adjacent agreement</th>
<th>% Agreement</th>
<th>Kappa</th>
<th>95% CI</th>
<th>Kappa Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Kawasaki</td>
<td>80</td>
<td>41.67</td>
<td>0.36</td>
<td>0.1861; 0.5339</td>
<td>0.21-0.40 Fair agreement</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Tanaka</td>
<td>81.66</td>
<td>41.67</td>
<td>0.36</td>
<td>0.1843; 0.5357</td>
<td>0.21-0.40 Fair agreement</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. FFQ Na</td>
<td>71.66</td>
<td>30</td>
<td>0.17</td>
<td>-0.0097; 0.3563</td>
<td>0.01-0.20 Slight agreement</td>
</tr>
</tbody>
</table>
Table 12. Agreement in classification of Na intake above and below the WHO guideline (2000mg/day):

<table>
<thead>
<tr>
<th>Method</th>
<th>% agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Kawasaki</td>
<td>81.66</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. Spot urine Na Tanaka</td>
<td>80</td>
</tr>
<tr>
<td>24 hour Na Urinary excretion Vs. FFQ Na</td>
<td>80</td>
</tr>
</tbody>
</table>

D. Calibration of the FFQ

Calibration of the FFQ was performed by linear regression model: FFQ calibrated = constant (α) + slope (γ) * FFQ; with 24 hour urine sodium concentration being the dependent factor, and estimated sodium intake by the FFQ being the independent factor estimating a linear ‘calibration factor’ as the slope of this regression line. Accordingly, calibrated FFQ was calculated using the following formula:

FFQ calibrated = 2,834.451 + 0.208* 4,968.783

<table>
<thead>
<tr>
<th>Collection 24 hour</th>
<th>Original FFQ</th>
<th>Calibrated FFQ</th>
<th>α</th>
<th>Λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,555.417</td>
<td>4,968.783</td>
<td>3,867.957</td>
<td>2,834.451</td>
<td>0.208</td>
</tr>
</tbody>
</table>

The calibrated FFQ can be further assessed for validity against 24 hour urine in future research.
CHAPTER V
DISCUSSION

The strong association between increased salt intake, increased stroke and cardiovascular risks (De Keyzer et al. 2015), elevated blood pressure at both the individual and population levels (De Keyzer et al. 2015; Doyle & Glass, 2010; He Fj, MacGregor, 2009), in addition to other deleterious health effects (Anderson et al., 2010), sheds the light on the urgency to decrease salt intake (Doyle & Glass, 2010; He Fj, MacGregor, 2009; Loria, Obarzanek, & Ernst, 2001). The first step in initiating a salt reduction intervention necessitates a detailed assessment of salt intake and of its sources (Ji et al., 2012: McLean, 2014). The gold standard in assessing sodium intake is the 24 hour urine collection (Ji et al., 2012: McLean, 2014). Even though this method is known to provide the most accurate estimate for sodium intake (Bentley, 2006; De Keyzer et al. 2015; He Fj, MacGregor, 2009; JH Park et al., 2014), it is burdensome, expensive (Bentley, 2006), challenging and unattractive (Loria, Obarzanek, & Ernst, 2001), especially in large-scale studies (JH Park et al., 2014). There is no agreement as to which substitute to the gold standard is the most valid, which poses a problem in clinical and research practices (Bentley, 2006). Spot urinary sodium excretion is one of the suggested alternative methods for assessing dietary intake of sodium (JH Park et al., 2014), even though its validity has been questioned in some studies (Ji et al., 2012). Dietary assessment tools have also been suggested as possible alternative methods for the assessment of the population’s sodium intake, while other studies have contested the validity of such tools (Kelly et al., 2015). This study aimed at investigating the validity of spot urinary sodium excretion in estimating sodium intake in healthy Lebanese adults. For this purpose, 2 spot urine methods were selected: the Tanaka method (Tanaka et al., 2002), and the
Kawasaki method (Kawasaki et al., 1993). In addition, this study aimed at examining the validity of a short food frequency questionnaire in assessing sodium intake amongst adults. The validation of both the sport urinary sodium excretion and the FFQ was performed against 24-hour urinary excretion, the gold-standard method in the evaluation of sodium intake (Charlton et al., 2008).

A. Major Findings of the Study

The study showed that the FFQ, which was specifically developed for the assessment of sodium intake in Lebanon (Helou, 2014), may not have an acceptable validity in estimating dietary sodium intake at the individual level, whereas spot urine had acceptable validity in assessing sodium intake. Both spot urine formulas behaved better than the FFQ with higher correlation coefficients and agreement with 24 hour urine. However, the three methods under investigation had very good agreement in classifying intake of individuals above or below the WHO limit (2000mg/day). Accordingly, the FFQ can be a useful tool in comparing intake with respect to a certain baseline at the population level.

B. Findings on Relative Validity of the FFQ and spot urine

1. FFQ vs. 24 hour urine Na

In this study, the Spearman correlation coefficient between dietary Na intakes assessed by the FFQ and 24 hour urine Na excretion was of 0.258 (P<0.05) which indicates a poor but significant correlation between the two methods. This coefficient is slightly higher than the Spearman correlation reported by Charlton et al. (2008) which was of 0.224. It is important to note that correlation coefficients of the FFQ when validated against the gold standard should be
at least 0.3-0.4 in order to detect associations between diet and disease (Cade et al., 2002). Despite the significant correlation between the FFQ and 24 hour urinary Na, the mean difference of these two methods was high and significantly different from zero, suggesting poor agreement (Bland & Altman, 1999). The Bland Altman plot showed a positive slope that was significantly different than zero indicating that the FFQ tends to over-estimate Na intake at increased levels of Na dietary intake. Moreover, the FFQ had the widest limits of agreement, suggesting that it may not accurately predict 24 hour Na intake. This was similar to the results of Kelly et al. who showed that the Bland Altman plot of FFQ Na vs. 24 hour urinary sodium had a large mean difference and very wide limits of agreement (Kelly et al., 2015).

The ICC obtained for the Na estimated from the FFQ vs. 24 hour urinary sodium was low and non-significant with a value of 0.327 (P>0.05). The ICC is sensitive to inter-subject variability and ranges from 0 to +1, with values closer to 0 indicating that there is a discrepancy due to the subject’s replies (Graham, Milanowski, & Miller, 2012). An ICC less than 0.5 indicate that inconsistency due to error represents at least 50% of the total inconsistency (Cantin et al., 2015). A reliable tool should receive an ICC of +0.7 to +1 in order to decrease this variance due to error (Bountziouka & Panagiotakos, 2010; Graham, Milanowski, & Miller, 2012). With respect to our FFQ, ICC suggests that it may not be a reliable tool in estimating daily sodium intake.

Percent agreement between the FFQ and 24 hour urine collection in classifying subjects into quartiles based on sodium intake was low (30%). Adjacent percent agreement of the FFQ was higher than the percent agreement but also lowest compared to the other methods under investigation (i.e. the spot urine samples) with a value of 71.6% indicating that it behaved better in classifying sodium intake in adjacent quartiles. A value of 75% to 90% is usually needed to
achieve a good agreement (Graham, Milanowski, & Miller, 2012), and thus the FFQ also did not prove that it has a good agreement with the gold standard. Kappa value was also low, 0.17 (-0.0097; 0.3563), indicating a slight agreement. The FFQ had a good agreement with 24 hour urine in classifying individuals with respect to a standard, in this case, the WHO guideline of Na intake, 2000mg/day. Accordingly, the FFQ can be beneficial in classifying levels of intake at the population level and comparing the intake to the standard. This can very beneficial in replacing 24 hour urine in assessing the percentage of the population exceeding the requirement and helps highlight the need to implement salt reduction programs, especially since FFQs are relatively cheaper compared to 24 hour urine collection (Jardack, 2006; Subar, 2004).

Our results are very similar to the results in the literature regarding the validity of the FFQ in assessing sodium intake. A semi-quantitative FFQ administered in Brazil indicated that there was no correlation between the FFQ and 24 hour urinary sodium, but was found to have a better validity when considering discretionary sodium (Ferreira-Sae et al., 2009). Another validation study in Ireland also showed that FFQ is not a valid tool in assessing sodium intake, but van be useful in classifying population intake with respect to a certain standard (Kelly et al., 2015). These findings may be explained by the fact that the FFQ is based on retrospective recall, and is highly dependent on the subject’s memory of both, the frequency of the food item eaten and its exact portion size. In addition, since FFQs assess intake over a long period of time (McLean, 2014), it tends to be less specific and more prone to measurement error (Subar, 2004). The salt content of foods may vary between brands, restaurants, home cooked dishes, various sources making it difficult to accurately estimate sodium consumed from each meal (Loria, Obarzanek, & Ernst, 2001). The food composition data are old (1970s) and we relied on data coming from the USDA database which may not be accurate for the Lebanese context. Since Lebanese dishes
tend to be mixed, subjects might also find it difficult to estimate the usual frequency and portions eaten (Brown, 2006). Finally, the FFQ is a tool that is known to overestimate sodium intake (Jardack, 2006), and several studies in the literature have suggested the use of linear regression in calibrating an FFQ.

2. **Spot urine vs. 24 hour urine Na**

The Spearman correlation coefficient between sodium intakes estimated by the spot urine Tanaka and 24 hour urine Na excretion was significant with a value of 0.504 (P=0.000) which was higher than that of the FFQ. Accordingly, a moderate and significant correlation exists between these two methods. This coefficient is higher than that reported by Seok Koo et al. (2014) which was of 0.490 (P<0.001), and that reported by Toft et al. (2014) which was of a value of 0.39 in a Caucasian (Danish) sample of 473 adults (25-65 years) (Toft et al., 2013). A Spearman correlation of 0.50 was found between spot urine Na Tanaka and 24 hour urine Na by Cogswell et al. (2013) in Washington DC which is very close to our findings (Cogswell et al., 2013) and a slightly higher correlation was reported by McLean et al. (2014) in New Zealand with a significant value of 0.58 (P<0.001) (McLean, Williams, & Mann, 2014).

The mean difference of the spot urine Na Tanaka and the 24 hour urine Na was the lowest (when compared to the FFQ and to the Kawasaki method), and was not significantly different from zero, suggesting a good correlation. Kelly et al. also reported that the mean difference of spot urine Tanaka and 24 hour urinary Na is low amongst Irish employees with a value of 251.79mg, which was slightly higher than our result (198.74mg) (Kelly et al., 2015). This was similar to the results of a meta-analysis of 14 validation studies which concluded that the mean difference between Tanaka estimation and the gold standard was the least with a P value not
significantly different from zero (Huang et al., 2016).

The Bland Altman plot of these two methods showed a negative slope, significantly different than zero indicating that the spot urine Tanaka tends to over-estimate Na intake at low levels of Na dietary intake, and under-estimate Na intake at high levels of Na dietary intake. This result differs from that of Kelly et al. whereby spot urine Tanaka was found to underestimate Na intake at low levels of dietary intake, and overestimate intake at high levels of Na intake (Kelly et al., 2015). However, the results of our study were similar to those reported by Toft et al. (2013) (Toft et al., 2013) and Tanaka et al. (Van Huysduynen et al., 2014), and a meta-analysis of 14 studies assessing the validity of spot urine Tanaka (Huang et al., 2016). Accordingly, spot urine Tanaka might not be useful in estimating sodium intake among individuals who consume high levels of sodium. It can be suggested that estimating Na from several spot urine samples for the same subject can provide a more accurate estimation, especially among individuals who either consume a very low amount of Na, or a very high amount of sodium.

Moreover, the Tanaka spot urine showed the narrowest limits of agreement, indicating that it might accurately predict 24 hour Na intake at moderate intake levels. Both Kelly et al. (Kelly et al., 2015), McLean et al. (2014) (McLean, Williams, & Mann, 2014), and Rhee et al. (2014) (Rhee et al., 2014), in addition to the previously mentioned meta-analysis (Huang et al., 2016) showed that the Bland Altman plot of spot urine Na Tanaka vs. 24 hour urinary sodium had narrow limits of agreement (Kelly et al., 2015).

The ICC between Na estimated from spot urine Tanaka vs. 24 hour urinary sodium was higher than that of the FFQ, with a significant value of 0.610 (P=0.000). This value is closer to +1 but still less than 0.7. This indicates that it can be a reliable tool in estimating 24 hour urinary sodium, and that the variation due to error represents at least 39% of the total variation.
The percent agreement in quartiles for the spot urine Tanaka was higher than that of the FFQ with a value of 41.6%, indicating that it had a better agreement with the 24 hour urine in classifying sodium intake compared to the FFQ. Adjacent percent agreement of the spot urine Tanaka was the highest amongst the methods under investigation, with a value of 81.6%, which is greater than that reported by McLean et al. (2014) (McLean, Williams, & Mann, 2014) (68%), demonstrating that it behaved best in classifying sodium intake in adjacent quartiles. Since this value is in the range of 75% to 90%, the spot urine Tanaka can be considered to have a good agreement with the gold standard. Kappa value was 0.36 (0.1843; 0.5357), which is close to the Kappa value reported McLean et al. (2014) in New Zealand (0.33) suggesting fair agreement (McLean, Williams, & Mann, 2014).

When looking at the Kawasaki method for estimating sodium intake based on spot urine samples, the Spearman correlation coefficient between spot urine Kawasaki and 24 hour urine Na excretion was the highest compared to the other methods under investigation, with a significant value of 0.547 (P=0.000). This coefficient is comparable to that reported by McLean et al. (2014) amongst New Zealanders (0.56) (McLean, Williams, & Mann, 2014), while higher than that reported by Kawasaki et al. (1993) amongst Japanese (0.53). Similarly, Rhee et al. demonstrated that Kawasaki spot urine showed the highest Spearman correlation with 24 hour urine collection when compared to the Tanaka equation (Rhee et al., 2014). Accordingly, a moderate and significant correlation exists between these two methods.

However, the mean difference of the spot urine Kawasaki Na and the 24 hour urine Na was higher than that of the spot urine Tanaka and significantly different from zero. This result was similar to that reported by Kelly et al. Irish employees whereby Kawasaki spot urine Na showed the largest mean difference, which was almost double that of spot urine Tanaka with a value of
395.01mg compared to 251.79mg (Kelly et al., 2015). Accordingly, Kawasaki spot urine may over-estimate Na intake, a conclusion also highlighted by Kelly et al. (Kelly et al., 2015), Rhee et al. (Rhee et al., 2014), and by a meta-analysis of 9 validation studies (Huang et al., 2016).

The Bland Altman plot of Kawasaki spot urine Na vs. 24 hour urine Na also showed a negative slope however, which was not significantly different than zero indicating that the spot urine Kawasaki was not modulated by levels of dietary Na intake. However, the Kawasaki spot urine showed limits of agreement which are wider than those of the spot Tanaka, making it less accurate in assessing sodium intake. Kelly et al. (Kelly et al., 2015) stated that the Kawasaki spot urine had the largest bias among the different formulas that estimate 24 hour Na intake from spot urine. A similar result was also reached by McLean et al. (McLean, Williams, & Mann, 2014), Rhee et al. (Rhee et al., 2014), and by a meta-analysis of 9 studies validating the Kawasaki formula against 24 hour urine (Huang et al., 2016).

The ICC between Na estimated from spot urine Kawasaki vs. 24 hour urinary sodium was highest among the three methods under investigation, with a significant value of 0.72 (P=0.000). This value is more than 0.7, suggesting that the Kawasaki formula can be an acceptable tool in estimating 24 hour urinary sodium, and that the variation due to error represents at least 28% of the total variation, which is less than the spot urine Tanaka.

The percent agreement in quartiles for the spot urine Kawasaki was similar to that of the spot urine Tanaka with a value of 41.6% indicating that compared to the Tanaka equation, it had a similar agreement with 24 hour urine in classifying sodium intake, and a better agreement compared to the FFQ. Adjacent percent agreement of the spot urine Kawasaki was second best, with a value of 80%, which is slightly less than of the Tanaka equation. Since this value is in the range of 75% to 90%, the spot urine Kawasaki can also be considered to have a good agreement
with the gold standard. Kappa value was 0.36 (0.1861; 0.5339) indicating a fair agreement, which is also similar to that of Tanaka. This result is higher than the Kappa value reported McLean et al. (2014) which was 0.24 for spot urine Kawasaki vs. 24 hour urine Na (McLean, Williams, & Mann, 2014).

Compared to the FFQ, both spot urine Tanaka and Kawasaki behaved better in assessing Na sodium intake in the study sample of Lebanese healthy adults. The correlations were higher compared to those of the FFQ, with spot urine Kawasaki showing the highest correlations. However, Kawasaki spot urine tended to overestimate sodium intake over different intake levels, while the spot urine Tanaka showed smaller limits of agreement in the Bland Altman plots, indicating a more accurate assessment. Our results indicate that even though spot urine estimations can be valid, they also are subjected to several biases which affect their level of accuracy. According to several studies, spot urine always tends to over or under estimate sodium intake (Huang et al., 2016; Rhee et al., 2014) since spot urine signifies Na excretion at a specific point in time. This excretion is highly affected by daily variations of sodium intake, climate, and physical activity (Koo et al., 2014), rendering the excretions not constant during the same day and between days (McLean, 2014; Van Huysduynen et al., 2014). However, our results indicate that spot urine estimates can be more valid than the FFQ in assessing sodium intake, and can be used as a means of estimation of population intake in order to highlight the need of the initiation of salt reduction programs in Lebanon. It is also important to note that formulas that estimate sodium intake by spot urine Na concentrations are population specific, so they might not behave as well in populations of different countries (Huang et al., 2016). This also raises the need to develop specific formulas that allow a valid estimation of sodium intake for the Lebanese population.
C. Comparison of Intake with Upper Limit

According to our study, mean sodium intake (mg/d) in Lebanese adults exceed the WHO upper limit of 2000mg/day, ranging from 3,555.42 mg/day according to the 24 hour urine, 3,356.68 mg/day according to the urine Tanaka, 4,437.2 mg/day according to the urine Kawasaki, and 4,968.78 mg/day according to the FFQ. These results are close to the value obtained by Powels et al. (2010) which was of 3130 mg per day.

The four sodium assessment methods under investigation indicated that our study sample surpassed the WHO recommendations of Na for adults aged 19 to 55 years. According to the 24 hour urine, 78% of the study population exceeded the limit set by the WHO, followed by 95% according to the spot urine Tanaka and FFQ, and 96.67% according to the spot urine Kawasaki. The percentage of individuals exceeding the acceptable level of sodium intake was higher among males. This result is consistent with other studies whereby males tend to consume more sodium than females, a reflection of their higher food intake and higher portion size (Appel & Anderson, 2010; Doyle & Glass, 2010).

Moreover, the FFQ and spot urine formulas (Kawasaki and Tanaka) had a good agreement with 24 hour urine collection in classifying individuals as exceeding the WHO limit of Na intake. Both the Tanaka equation and the FFQ admitted an 80% agreement and the Kawasaki equation admitted an 81.66% agreement. Accordingly, the three methods can be good substitutes of 24 hour urine collection in assessing whether a certain population is over-consuming sodium, and subsequently highlighting the need for sodium reduction programs.

D. Strengths, Limitations and Potential Biases

Several studies have attempted to validate alternative methods (FFQ and spot urine) in
assessing sodium intake against 24 hour urinary sodium excretion, however, no such study have been done in the Middle East and in Lebanon. Our study is the first to attempt to validate an FFQ and spot urine formulas (Kawasaki and Tanaka) against the gold standard; 24 hour urine collection in assessing dietary sodium intake. The strengths of this study included the fact that the used FFQ is adapted specifically to the Lebanese population and includes food items that belong to the local culture and that are mostly consumed. The food items included in the FFQ were based on data collected for a national dietary survey in 2008/2009, which allowed the selection of the food items that are the paramount contributors of Na in Lebanon. Both the frequency section and the portion section in the FFQ were designed to minimize any burden on the participants. Subjects were asked to assess portion sizes based on a 2-D visual poster (Nutrition Consulting Enterprises, Framingham, MA), standard portions, and commonly used household portions, allowing the decrease in errors due to inaccurate reporting of portion intakes. Moreover, data collection was performed by a research dietitian, and data collection during periods when subjects altered their regular dietary practices, such as Lent, Christmas, Ramadan and official holidays was avoided.

Subjects were given clearly instructions on the procedure of collecting 24 hour urine samples. Biochemical analysis was also performed in a certified lab by trained technicians at AUBMC. Another strength of this study is the fact that validation was against the gold standard; 24 hour urine. A final strength of our study is that it admitted a low drop-out rate (17%).

This study has several limitations to consider, the first of which is the small sample size of 72 subjects, 83.3% of whom provided a complete 24 hour urine collection. Accordingly, the sample size is not large enough to reach solid conclusions on the validity of the investigated methods in assessing sodium intake, when compared to 24 hour urine collection. Another
limitation is the method used in assessing completion of 24 hour urine samples. We adopted the Knuiman ratio of creatinine to weight, and considered all values of less than 0.7 as indicators of incomplete collection. Even though this formula is widely used in the literature (Rhee et al., 2014), it may not be as accurate as the use of PABA assessment (Toft et al., 2013; De Keyzer et al. 2015). However the use of PABA in validation studies may be associated other medications, non-adherence to the required number of pills, and decreased rate of excretion with age (Bentley, 2006; McLean, 2014), which can also render it inefficient in assessing the completion on 24 hour urine.

An additional limitation to highlight is the use of a single spot urine sample and a single 24 hour urine sample in assessing the validity of the Kawasaki and Tanaka formulas (Rhee et al., 2014). Due to varied sodium intake, it was suggested that in order to reach a better estimate of Na intake, three to eight 24 hour urinary samples are required, which is impractical and highly burdensome to study participants (Liu et al., 1979; Liu & Stamler., 1984; Langford & Watson., 1973). In addition, the collection of 24 hour urine is burdensome on study participants (Ji et al., 2012; Loria, Obarzanek, & Ernst, 2001). Accordingly, not many individuals agree to provide more than one sample had they agreed to participate. Individuals who agree to collect urine for several days might lose their motivation and preform the collection wrongly (Mann & Gerber., 2010). Hence, the assessment of sodium intake from spot urine samples, which is associated with a considerably higher response rate, using Na/Cr ratio can be accurate (El Bokl et al., 2009) and more desirable due to its ease of application and practicality (Stine et al., 2004). Even though studies have found that several spot urine samples are needed to attain a better correlation for validity (Kawasaki et al., 1993), Kawasaki et al. reported that it is enough to use a single urine collection and a second morning void sample in order to achieve a reliable approximation of 24
hour urinary excretion (Kawasaki et al., 1990; Kawasaki et al., 1992).

The limitations of use of FFQ in nutrient intake analysis, and in Na assessment should also be accounted for. Since FFQs measure intake over a long period of time and basically rely on memory (Jardack, 2006), subjects can face a difficulty in accurately recalling the frequency and portion of the food consumed (Subar, 2004). In addition, the sodium content of the food items considered in the FFQ was based on the Food Composition Tables for Use in the Middle East (Pellett & Shadarevian, 1970) for Lebanese foods and their nutritive value, a reference last updated in the 1970’s, and on the USDA’s food and nutrient database for foods of international nature. Accordingly, sodium content of the actually consumed food can differ from the databases we have used considering the fact that sodium content of food highly differs from one brand to another (Webster, Dunford, & Neal, 2010). Nonetheless, after extensive review, the USDA nutrient database was the most frequently updated and was inclusive to almost all food items of interest (Merchant & Dehghan, 2006).

Moreover, individuals who agreed to participate in the study accepted the burden of collecting urine for 24 hours. 72 subjects were recruited, 12 of which did not complete the 24 hour urine collection. The 60 subjects who provided complete samples demonstrated a high level of interest and motivation and accordingly can be interested in the topic and more health conscious that individuals who did not agree to do so. Accordingly, the study subjects might not be representative of the study population, leading to a potential selection bias. However, since study participants voluntarily agreed to participate, this bias could not be avoided. Another bias to consider is the recall bias which cannot be avoided since we were using an FFQ to assess intake, which measures intake over a long period of time. In order to avoid this bias, accurate portion estimation tools were used.
E. Conclusion and Recommendations

This study represents the first attempt to validate a short FFQ and spot urine against the gold standard in assessing Na intake; 24 hour urine in Lebanon. It showed that spot urine estimates of sodium intake has an acceptable validity, with significant correlation with 24 hour urine. The FFQ did not behave as well as spot urine against the 24 hour urine collection, however, it had good agreement with the gold standard in classifying individuals exceeding the WHO limit of 2000mg of Na per day. The FFQ may not be applicable in assessing Na intake on the individual level, but it may be beneficial in assessing sodium intake on the population level.

Nonetheless, the methods under investigation; the FFQ and spot urine can be useful tools in assessing Na intake since they cause less burden on the study subjects and are more convenient for large-scale studies. FFQs allow the evaluation of intake over a long period of time. (McLean, 2014). They are easier to use (Brown, 2006; Loria, Obarzanek, & Ernst, 2001), flexible (Subar, 2004), and relatively cheap (Jardack, 2006; Subar, 2004), and accordingly more convenient for large population studies (Jardack, 2006). Spot urine samples are easy to collect, (McLean, 2014), faster to analyze, less tedious compared to 24 hour urine collection, and do not necessitate a lot of staff training (Ji et al., 2012; Whelton et al., 2012). Samples can be stored in small containers, with no possibility of over or under collection, and can be frozen for later use (McLean, 2014). Accordingly, further research is needed in updating the food composition database or in elaborating the FFQ in a way to more accurately assess sensitive nutrients, such as sodium. In addition, we suggest that such a study should be performed on a larger sample size in order to reach more conclusive data on the validity of these methods.
APPENDICES

APPENDIX 1: 24 HOUR URINE COLLECTION PROCEDURE

Why 24 hours?

The content of some nutrients in urine fluctuates according to various factors such as what we last ate or how much fluid we drink. Collecting urine over 24 hours gives information about the typical intakes of these nutrients in a person’s diet.

Equipment:

- One 2 L screw-capped plastic collection bottles to store the collected urine
- A safety pin (to attach to your underclothes or nightwear simply as an aide memoire)
- One plastic bags for carrying the equipment outside the home
- A sheet to record the essential information about the collection (Collection Sheet).

N.B: The 2 L plastic bottle contains a boric acid preservative for keeping the urine at room temperature. This could cause skin or eye-irritations by contact or could cause stomach upset if swallowed. Please be sure to keep it out of the reach of young children.

Instructions:

Before the procedure

- Generally, no prior preparation, such as fasting, is required.
- The sample should be collected during the agreed 24-hour period.
- If possible, choose a 24-hour period when you will be mostly at home so you do not have to transport your urine.
- If you are female, you should not make your collection during your period.
- Notify your health care provider of all medications (prescription and over-the-counter) and herbal supplements that you are taking.

Collection your urine for the 24 hour sample

Generally, 24-hour urine collection follows this process:

1. On the day that you start your collection, you will pass urine – discard this urine, do not put it into the container. Record the date and time on the Collection Sheet. This will be the start time of the 24-hour collection.
2. From then onwards until the next day, ALL urine you pass, after the first (flushed) specimen, in the next 24 hours, both during the day and night, must be collected
   - Pass all urine directly into the collection container
   - If you need to open your bowels, always remember to pass urine first before you pass a stool
   - Each time you add a new urine specimen to the large container, screw the lid tight and swirl the urine around a few times, to mix it with the preservative.
3. Try to urinate again at the same time, 24 hours after the start time, to finish the collection process, but if you cannot urinate at this time, it is not a problem. This completes the 24h hour collection. Record the day and time on the Collection Sheet.

N.B: Do not worry if you have not collected for ‘exactly’ 24 hours, as long as you record exact time of start and finish.

If you miss a sample

If during the 24hour collection period a sample is missed for any reason, such as because of a bowel movement, record this on the Collection Sheet.

Once you have completed your collection

Once the urine collection has been completed, store the urine containers in a cool, dark place before taking it to the lab as agreed with the researcher.

If you have any other questions

We hope this document answers the questions you may have. If you have any other questions, contact the health professional. You are free to withdraw from this study at any point.

Collection Sheet

24 hour urine collection:
Date started: Day________; Month________; Year__________
Time started: ___________________

Date finished: Day________; Month________; Year__________
Time finished: ___________________
APPENDIX 2: FFQ

Assessment of Sodium Dietary Intake in Lebanese Adults

Assigned ID number .............................................

Date: .............................................................

Age: ..............................................................

Sex: ...............................................................

Education level:

a. Primary school or less
b. Intermediate school
c. High school
d. Technical diploma
e. University degree
f. Refused to answer

Weight (kg): .........................................................

Height (m): ...........................................................

BMI (kg/m²): ........................................................

Spot urinary sodium concentrations:

| Spot | urinary | creatinine | concentrations |
|------|---------|------------|----------------|----------------|

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# Food-Frequency Questionnaire

<table>
<thead>
<tr>
<th>Code #</th>
<th>Food item</th>
<th>Proposed serving size</th>
<th>Portion usually consumed by individual</th>
<th>Frequency of consumption</th>
<th>Rarely or Never consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Per day</td>
<td>Per week</td>
<td>Per month</td>
</tr>
<tr>
<td></td>
<td>Bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(White/Whole bread)</td>
<td>-1 med pita (70g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 large pita (120g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-French baguette 16 cm (68g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaak</td>
<td>-1 finger kaak (15g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3 round kaak (15g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Markouk</td>
<td>½ loaf (53 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Croissant</td>
<td>-1 med (57g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 large (120g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 mini (15g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other Pastries</td>
<td>-1/2 crepe (57g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(crepe, brioche, muffin)</td>
<td>-1 brioche (57g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 English muffin (57g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manaecsh (zaatar, cheese and Kishk)</td>
<td>1 piece= 159g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pasta based dishes &amp; moughrabieh</td>
<td>1 cup= 186g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice based dishes</td>
<td>(Riz bi lahmeh, kabseh, mdardara)</td>
<td>1 cup= 185 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Description</td>
<td>Example Unit and Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Burgol based dishes</strong></td>
<td>(burgol bi banadoura burgol bi dfin )</td>
<td>1 cup = 19 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Potato and potato-based dishes</strong></td>
<td>(French fries, potato baked, grilled, yakhnet potato, potato kebbe)</td>
<td>1 medium potato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>= 1 medium plate of French fries</td>
<td>= 20 items = 100 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1 item = 5g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Traditional pies</strong></td>
<td>(lahm bi 3ajin cheese rolls fried, sambousik meat, fatayer silk)</td>
<td>1 piece = 23g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pizza</strong></td>
<td></td>
<td>1 slice = 103g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Milk and Dairy products</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cheese</strong></td>
<td>(white, yellow processed)</td>
<td>1 slice = 22 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Labneh</strong></td>
<td></td>
<td>1 Tbsp = 27 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Yogurt and yogurt based dishes</strong></td>
<td>(Laban, frozen yogurt)</td>
<td>1 cup = 208g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td>1 cup = 244 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kishk based dishes</strong></td>
<td></td>
<td>1 cup = 253 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Meats, Poultry, Fish and Eggs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cured meats</strong></td>
<td>(hotdogs, ham, mortadelle, sausages,</td>
<td>- 1 frankfurter or sujuk (50g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 2 hot dogs (50g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Description</td>
<td>Serving Size</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>----------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ready prepared meat</td>
<td>(kibbe, kafta, shawarma, hamburger patty)</td>
<td>90 g (3oz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ready prepared poultry</td>
<td>(taouk, chicken burger patty, chicken breaded, Chicken flour coated, Chicken batter fried)</td>
<td>90g (3 oz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat based dishes</td>
<td>(Steak, Lamb cooked, Pork cooked, raw meat)</td>
<td>90g (3 oz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry based dishes</td>
<td>(Stewed, grilled &amp; roasted chicken)</td>
<td>90g (3 oz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>(canned, fish fingers)</td>
<td>90g (3 oz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vegetables and Vegetables based dishes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salads</td>
<td>(fattouch, Tabouleh, coleslaw, green salad)</td>
<td>1 cup=182g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables ragouts</td>
<td>(mouloukhieh, green peas ragout, spinach ragout, okra ragout, loubieh bi zeit)</td>
<td>1 cup =243g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stuffed vegetables</td>
<td>(stuffed zucchini, stuffed grape leaves, stuffed cabbage)</td>
<td>-1 piece of stuffed cabbage (35g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1 piece of stuffed grape</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

89
<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>(16 g)</td>
</tr>
<tr>
<td>-1 piece of stuffed zucchini med</td>
<td>(69 g)</td>
</tr>
<tr>
<td>Canned vegetables</td>
<td></td>
</tr>
<tr>
<td>(peas, corn, asparagus)</td>
<td>0.5 cup</td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
</tr>
<tr>
<td>Legumes based dishes</td>
<td></td>
</tr>
<tr>
<td>(foul moudamaas, mjaddara, adas bi zeit, balila, msabaha, chickpeas cooked)</td>
<td>1 cup=265g</td>
</tr>
<tr>
<td>Falafel</td>
<td>90g (3 pieces)</td>
</tr>
<tr>
<td>Salty Snacks</td>
<td></td>
</tr>
<tr>
<td>Potato chips</td>
<td></td>
</tr>
<tr>
<td>-1 bag 500l.l = 40g</td>
<td></td>
</tr>
<tr>
<td>-1 bag of 250l.l = 25g</td>
<td></td>
</tr>
<tr>
<td>-1 bag of 1500l.l = Pringles (big) = 150 g</td>
<td></td>
</tr>
<tr>
<td>Pop corn</td>
<td></td>
</tr>
<tr>
<td>-1 cup = 11g</td>
<td></td>
</tr>
<tr>
<td>-Microwave bag = 4 cups</td>
<td></td>
</tr>
<tr>
<td>-Movies large container = 20 cups</td>
<td></td>
</tr>
<tr>
<td>-Movies medium container = 15 cups</td>
<td></td>
</tr>
<tr>
<td>-Movies small container = 10 cups</td>
<td></td>
</tr>
<tr>
<td>Roasted nuts, seeds and lupin (salted)</td>
<td>1 cup=160g</td>
</tr>
<tr>
<td>Sweets</td>
<td></td>
</tr>
<tr>
<td>Kneffehe (without kaak)</td>
<td>1 piece (111 g)</td>
</tr>
<tr>
<td><strong>Biscuits and cookies</strong></td>
<td>1 biscuit (40 g)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Chocolate bars</strong></td>
<td>1 bar chocolate (60g)</td>
</tr>
<tr>
<td><strong>Sfouf, akras tamer</strong></td>
<td>1 piece (45 g)</td>
</tr>
<tr>
<td><strong>Cakes</strong></td>
<td>1 piece (100g)</td>
</tr>
</tbody>
</table>

**Soups, Condiments and Sauces**

<table>
<thead>
<tr>
<th><strong>Soups</strong></th>
<th>1 cup=274g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Olives, pickles, makdouss</strong></td>
<td>-1 olive ( 3 g)</td>
</tr>
<tr>
<td></td>
<td>-1 pickle-cucumber (30g)</td>
</tr>
<tr>
<td></td>
<td>-1 makdouss (59g)</td>
</tr>
<tr>
<td><strong>Dry thyme</strong></td>
<td>1 Tbsp=16g</td>
</tr>
<tr>
<td></td>
<td>(thym and oil)</td>
</tr>
<tr>
<td><strong>Condiments and tomato sauces</strong></td>
<td>1 Tbsp=15g</td>
</tr>
<tr>
<td>(ketchup, mustard, tomato paste canned, tomato sauce canned)</td>
<td></td>
</tr>
<tr>
<td><strong>Soya sauce</strong></td>
<td>1 Tbsp=18g</td>
</tr>
<tr>
<td><strong>Cream, gravies, and mayonnaise</strong></td>
<td>1 Tbsp=14g</td>
</tr>
<tr>
<td><strong>Tahini-based dishes</strong></td>
<td>1 Tbsp or 1 cup</td>
</tr>
<tr>
<td>(tarator, tahini, hummos bi thineh, baba ghanouj)</td>
<td></td>
</tr>
<tr>
<td><strong>Table salt</strong></td>
<td>1 pinch = 0.25 g</td>
</tr>
<tr>
<td></td>
<td>1 tsp = 6g</td>
</tr>
<tr>
<td></td>
<td>1 sachet = 1 g</td>
</tr>
</tbody>
</table>
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laboratory centre for disease control at health canada, heart and stroke foundation of canada.

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