AMERICAN UNIVERSITY OF BEIRUT

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

by
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AN ABSTRACT OF THE THESIS OF

Rita Michel Helou for Master of Science
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Title: Development and Validcation of a Food Frequency Questionnaire for the Assessment of Sodium Dietary Intake in Lebanese Adults

The global burden of cardiovascular diseases (CVDs) has reached epidemic proportions, affecting both developed and developing countries. 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. 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. 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; and salt intake has been shown to correlate directly with blood pressure in different population groups. 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. Eventhough several methods have been proposed for the dietary assessment of Na (24 hour urinary sodium excretion; repeated 24 hour dietary recalls; 3-day or 7-day food records), the validity, accuracy and/or applicability of several of these methods have been often criticized in the literature. 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 population. The objective of the present study is to develop and validate a short food-frequency questionnaire for the assessment of dietary sodium intake among Lebanese adults. The questionnaire will be developed in a way as to include culture-specific food items that represent the biggest contributors to sodium intake. The developed questionnaire will be pilot-tested and its validity evaluated against urinary sodium excretion as well as a repeated 24 hour dietary recalls. The reliability of the questionnaire will also be tested. For this purpose, a convenient sample of 100 healthy male and female individuals aged between 18 and 55 years will be recruited. Through the development and validation of a questionnaire that can be practically used for the assessment of dietary sodium intake, this project should be viewed as the first step towards the establishment of a preventive strategy to control the population's salt intake and to combat hypertension and CVD in the country.

Objectives: The objective of the present study is therefore to develop and validate a short food-frequency questionnaire for the assessment of dietary sodium intake among Lebanese adults.

Methods: Lebanese adults aged 19 to 55 years were recruited. Subjects

completed the FFQ (FFQ-1), and one 24-hour recall in the first visit. Subjects were also asked to provide a spot urine sample (particularly the second morning voiding) in a plain container. Anthropometric measurements (weight, and height) were obtained. During the following 4 weeks, 2 24-hour recalls were collected, 2 and 4 days after the initial interview, by phone. At Week 4, the FFQ (FFQ-2) was completed a second time. Descriptive statistics, Spearman correlations, mean differences, and Bland-Altman plots, Intraclass Correlation (ICC), and percent agreement were used to assess the validity and reproducibility of the developed FFQ. SPSS was used in the analysis and a $p<0.05$ indicated significance.

Results: Out of the 100 subjects, 87 completed the study. Spearman correlation coefficients between Na intake estimates derived from the FFQ, 24-hour dietary recall, and urinary Na excretion were computed. Based on the Bland and Altman approach, mean difference between FFQs and urinary excretion was not significantly different than zero, indicating that the two methods are compatible. However, the slopes for the FFQ1, FFQ2, and FFQ average were positive and significantly different from zero. This indicates that the FFQ tend to overestimate Na intake particularly at high levels of intake.Spearman coefficients were all statistically significant. The values ranged between 0.215 for FFQ1 & urinary excretion, and 0.549 for FFQ1 & repeated 24-hr recall. The ICC was significant for all methods, ranging from 0.332 for FFQ1 vs. urinary excretion to 0.512 for FFQ Average vs. repeated 24-hr recall. The ICC statistic used to evaluate reliability between FFQ-1 and FFQ-2 was 0.86. The percent agreement ranked highest for 24-hour recall vs FFQ1. The percent of individuals that were correctly classified into the same tertile, was also highest for the same methods stated above (52.33%). Agreement in classifying Na intake according to WHO limit was also computed. The percent agreement, the values were between 76.19% for urinary excretion vs. repeated 24-hr recall and 92.94% for repeated 24-hr recall vs. FFQ1. As for the percent agreement between FFQ-1 and FFQ-2 in ranking participants into the same tertiles and according to the WHO limit was 73.56 % and 93.02% respectively.

Conclusion: Our findings indicate that the developed FFQ is reliable with acceptable validity in assessing dietary Na intake. The results of this study are similar to previous Na-based FFQ validation studies in adults. This FFQ will serve as a useful dietary assessment tool for researchers, health care professionals, dietitians and policy makers in assessing dietary sodium intake of adults in the MENA region against dietary guidelines, tracking intake over time, and establishing clear and useful nutritional messages and interventions.

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ABBREVIATIONS

24-HR	24-hour dietary recall
AUB	American University of Beirut
AI	Adequate Intake
BMI	Body mass index
CI	Confidence interval
Cm	Centimeter
CVD	Cardiovascular Disease
DR	Dietary Record
FAO	Food and Agriculture Organization
FFQ	Food frequency questionnaire
FFQ-1	The first administration of the Food Frequency Questionnaire
FFQ-2	The second administration of the Food Frequency Questionnaire
HTN	Hypertension
ICC	Intraclass correlation
IRB	Institutional Review Board
κ	Kappa coefficient
κ w	Weighted Kappa
Kg	Kilograms
LoA	Limits of Agreement
MENA	"Middle East and North Africa
MPR	Multiple Pass 24-hour dietary Recall
n	Sample size
NHANES	National Health and Nutrition Examination Survey
NFSC	Nutrition and Food Sciences Department at AUB
RDA	Recommended Dietary Allowance

SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
Tbsp.	Tablespoon
Tsp.	Teaspoon
UL	Tolerable Upper Limit
WHO	World Health Organization

CHAPTER I

INTRODUCTION

A. General Overview

Evaluating populations' dietary intake represents a true challenge for nutrition research (Serra-Majem *et al*., 2009). Dietary assessment methods are used to evaluate nutritional intake and to investigate the association between dietary factors and disease risk (Yaroch *et al.*, 2000). Because it is crucial to properly assess populations' dietary intake and regularly monitor it, dietary assessment tools must be valid, reliable, and inexpensive as well as tailored to the population's social, ethnic and cultural background. They must also be able to collect information that reflects dietary habits over an extended period of time without exposing participants and researchers to a heavy burden (Torheim *et al*., 2001).

Dietary assessment of sodium has recently gained a lot of attention given that sodium intake has been associated with increased risk of cardiovascular diseases (WHO, 2007). Evidence shows a direct relationship between sodium intake and hypertension; blood pressure rises with increased sodium intake in the general population (Sodium Reduction Strategy for Canada) (Ritz, 2010, Savica et al., 2010), and salt intake has been shown to correlate directly with blood pressure in different population groups (CD frost et al.1991, Savica et al., 2010; Brook et al., 2001; Sharma, 2002; Appel et al., 2003). 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 **(**Dietary reference intakes for water, potassium, sodium, chloride and sulfate, 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. 230-460 mg/day) (Brown et al., 2009). In 2004, the IOM set Dietary Reference Intakes (DRIs) for sodium. The Adequate Intake (AI) was set at 1,500 mg per day for those aged 9 to 50 years, with lower values for younger and older individuals. The Tolerable Upper Limit (UL) for sodium was set at 2,300 mg per day for people aged 14 years and older, with lower values for those less than 14 years of age. The WHO recommends a reduction to <2000 mg/day sodium (5 g/day salt) in adults (Sodium Intake for Adults and Children, WHO 2012). As for the AHA, it recommends that the daily value for sodium be lowered to 1,500 mg by 2020 with an intermediate goal of 2,000 mg for 2013 (American Heart Association Dietary Guidelines, 2010). Most adult populations have mean sodium intakes >2300 mg /day, and for many, mean intakes are >4600 mg/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. Even

though several methods have been proposed by the literature for the dietary assessment of Na (24 hour urinary sodium excretion; repeated 24 hour dietary recalls; 3-day or 7-day food records), the validity, accuracy and/or applicability of several of these methods have been often criticized in the literature (Bentley, 2006). The lack of valid 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 a wide range of 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 population groups (Willet, 1998). FFQs rely on recall from 'generic' memory, which may be more easily recalled than 'episodic' memory, but because of the importance of cultural sensitivity, all FFQs require some adjustments and validation when used for a select cultural group (Coultson M, 2008).The use of FFQs for the assessment of dietary sodium has been proposed in the literature, but none has yet been developed and validated for the Lebanese population (Charlton et al., 2008)

B. Objectives

The objective of the present study is therefore to develop and validate a short food-

frequency questionnaire for the assessment of dietary sodium intake among Lebanese adults. The specific objectives of the project are to:

- Develop a FFQ for the assessment of Na dietary intake in Lebanese adults
- Investigate the validity of the developed questionnaire against repeated 24-hour dietary recalls in assessing Na dietary intake
- Investigate the validity of the developed questionnaire against urinary sodium excretion.
- Investigate the reliability of the developed questionnaire for the assessment of Na dietary intake.

CHAPTER II

LITERATURE REVIEW

A. Burden of Cardiovascular Disease

Cardiovascular diseases (CVD) are the leading cause of death and a major cause of disability worldwide. In 2005, according to the World Health Organization (WHO), 35 million people died from chronic diseases worldwide and 30% of these deaths were due to CVDs (Preventing Chronic Diseases, a Vital Investment Report). The National Center for Chronic Disease Prevention and Health Promotion in the United States concluded that CVD accounted for 34.4% of the 2.4 million deaths in 2003 and remain a major cause of health complications and rising health care costs (Mensah & Brown, 2007).Thom et al (2006) stated that an estimated one in three U.S. adults (about 71.3million) suffers from one or more types of CVDs, the prevalence of which increases with advancing age and varies within racial, ethnic, geographic, and socio-demographic groups.In 2006, Centers for Disease Control and Prevention in Atlanta reported that health care spending and lost productivity from CVD exceeded 400 billion dollars (Mensah, & Brown, 2007).Strong et al (2006) described CVD as the largest cause of mortality among people of working age, hence, having detrimental effects on a nation's economic development and creates challenges for public health and clinical care in resource scarce settings. As for the Middle East and North Africa (MENA) countries, Khatib (2004) described CVDs and strokes as rapidly growing problems representing the major underlying causes of morbidity and mortality. In Lebanon for example, CVDs account for around 60% of all-cause mortality in persons aged 50 years and older (Sibai et al, 2009). Mensah et al (2005) projected that the obesity epidemic, underuse of prevention strategies, and suboptimal control of risk factors, coupled with an ever growing aging population, could all worsen the CVD burden worldwide. Therefore, increased adherence to clinical and

community-level guidelines and renewed emphasis on policy, environmental, and lifestyle changes will be central for the successful prevention and management of CVDs.

Mensah & Brown (2007) found that among the 71.3 million adults with one or more forms of CVD, the most prevalent conditions are hypertension or high blood pressure (65 million), coronary heart disease (13.2 million), stroke (5.5 million), heart failure (5 million), and congenital heart defects (1 million). Ezzati et al (2002) ranked high blood pressure as the most important risk factor for CVD. Gaziano et al (2010) reported that 50% of the CVDs worldwide are caused by elevated blood pressure. In fact, 7.1 million (13%) of worldwide deaths were estimated to be attributable to blood pressure levels above the optimum of 115 mmHg (systolic pressure). Approximately one third of DALYs attributable to high blood pressure occur in developed countries, and two thirds in developing countries; thus strategies for blood pressure control should be considered as a public health priority around the globe and not just in developed countries (WHO, 2002). 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 (Global Health Risks: mortality and burden of disease attributable to selected major risks, 2009).

1. Hypertension: definition and prevalence

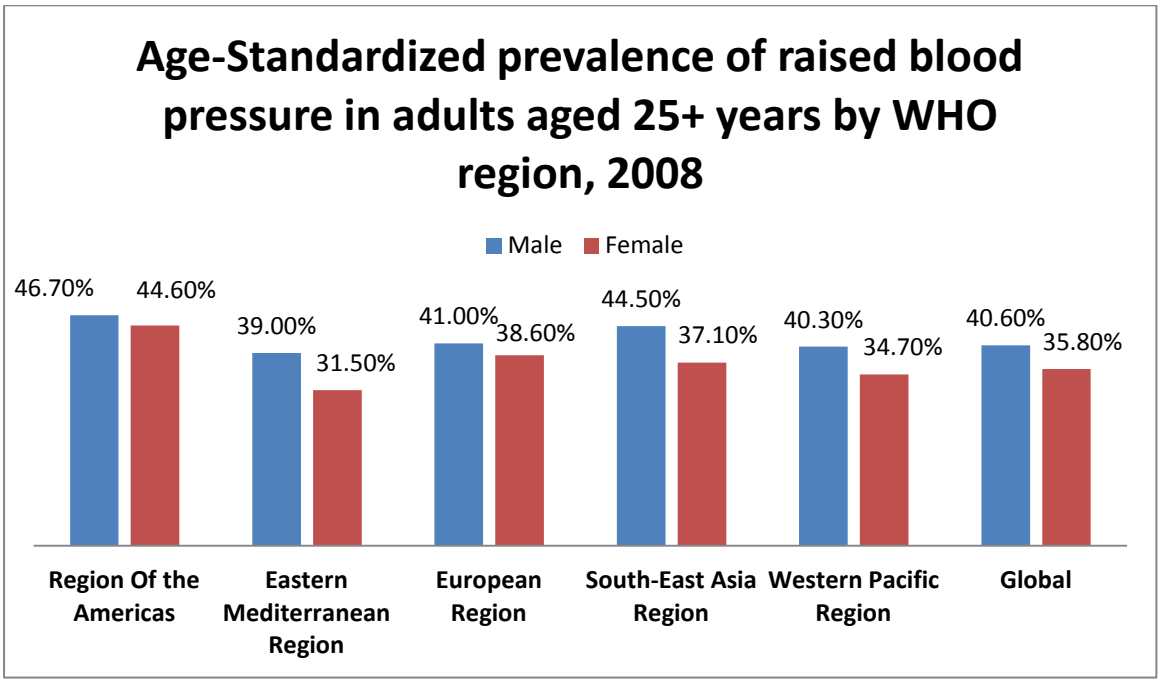
Hypertension is rarely accompanied by any symptoms, and its identification is usually through screening, or when seeking healthcare for an unrelated problem (Fisher & Willliams, 2005).According to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (2003), hypertension is diagnosed when the average of 2 or more diastolic BP measurements on at least 2 subsequent visits is greater than or equal to 90 mm Hg or when the average of multiple systolic BP readings on 2 or more subsequent visits is consistently greater than or equal to 140 mm Hg.

Individuals with high normal BP tend to maintain pressures that are above the average of the general population and are at greater risk for development of definite hypertension and cardiovascular events than the general population (Oscar et al., 2000).

Muntner et al (2002) reported that 25% of the US adult population is diagnosed with hypertension, that is, blood pressure of 140/90 mm Hg or higher and/or current use of anti-hypertensive medication. This proportion was found to vary with race, being higher in blacks (32.4%) compared to whites (23.3%) and Mexican Americans (22.6%) and with age. Systolic BP rises throughout life, whereas diastolic BP rises until the age of 55 to 60 years and thus the greater increase in prevalence of hypertension among the elderly is mainly due to systolic hypertension. Muntner et al (2002) also showed that the prevalence of HTN varies with gender, with hypertension being more prevalent in men (though menopause tends to eliminate this difference); and with socioeconomic status, which is an indicator of lifestyle attributes and is inversely related to the prevalence, morbidity, and mortality rates associated with hypertension. Vasan et al (2002) estimated the lifetime risk to develop hypertension to be 90% especially that its prevalence increases progressively with age. Prevalence of hypertension is increasing in many countries including developing ones, with the highest prevalence being in the region of the Americas, as shown in figure 1. In India, raised blood pressure increased from 5% in the 1960s to nearly 12% in 1990s, to more than 30% in2008. In Indonesia, the percentage of adult population with raised blood pressure increased from 8% in 1995 to 32% in 2008. In Myanmar, the Ministry of Health reported an increase in high blood pressure prevalence, from 18% to31% in males, and from 16% to 29% in females during 2004–2009 (WHO, Global and Regional Overview, World Health Day, 2013). As for the MENA countries, Sibai et al (2009) and Fahed et al (2011) showed that Syria and Bahrain have the highest prevalence rates of reported hypertension as shown in figure 2. In Lebanon, CVDs account for around 60% of all-cause mortality in persons aged 50 years and older and

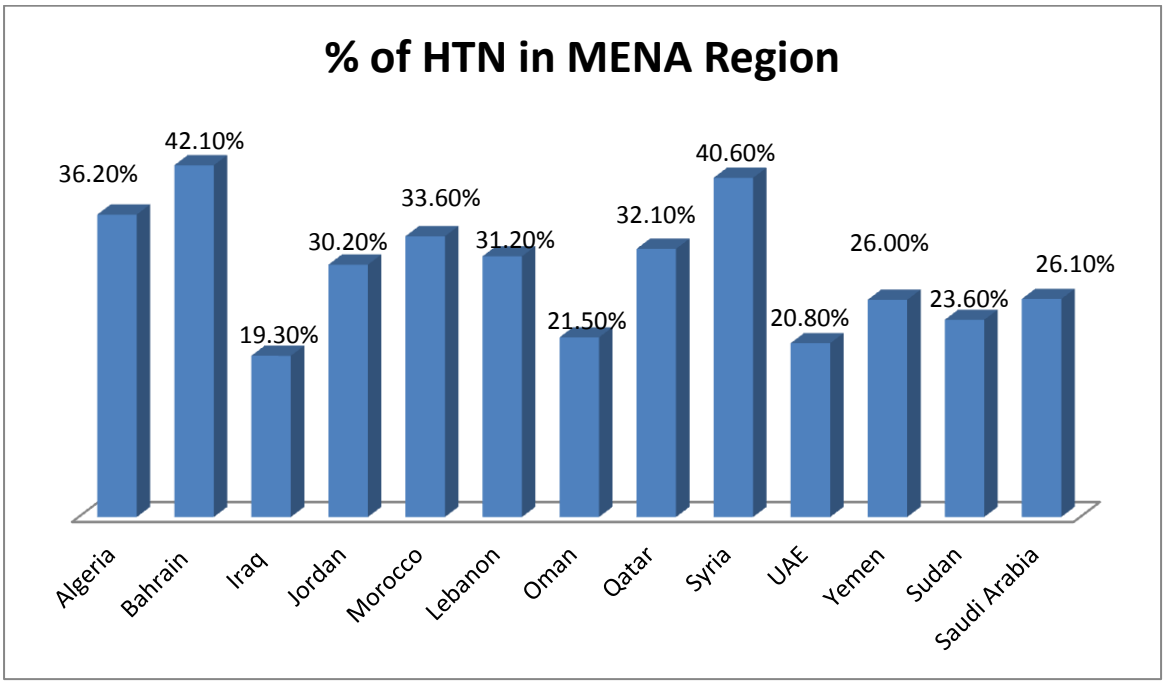
a recent national study showed that, among adults aged 25-64 years, 13.8% were diagnosed with HTN (Sibai et al, unpublished data). According to the Lebanese Ministry of Public Health, hypertension was shown to increase by almost 3-fold in the past decade, and the prevalence of hypertension of individuals aged between 18 to 65 years old was 31.2 % (Sibai et al., 1992). Abolfotouh et al (2001) stated that the highest hypertension prevalence rates in the MENA region were found among illiterate and individuals living in urban areas, whereas lower prevalence rates were found in individuals involved in agricultural work.

Figure 1: Prevalence of HTN in selected parts of the world



Adapted from:*Global status report on non-communicable diseases, 2010.* Geneva, World Health Organization 2011

Figure 2: Prevalence rates of HTN in selected countries of the MENA Region:



Adapted from:*Diet, Genetics, and Disease: A Focus on the Middle East and North Africa Region* (Fahed et al, 2011).

Hypertension is a major risk factor for stroke, myocardial infarction (heart attacks), heart failure, aneurysms of the arteries peripheral arterial disease and is a cause of chronic kidney disease. Even moderate elevation of arterial blood pressure is associated with a shortened life expectancy (Chobanian et al., 2003) and an increase in BP, even if it does not reach the hypertensive range, increases CVD risk (Appel et al, 2006). Cutler et al (1997) reported the presence of a strong positive and continuous correlation between BP and the risk of CVD (stroke, myocardial infarction, and heart failure), renal disease, and mortality, even in the normo-tensive range. This correlation is stronger with systolic than with diastolic BP (Lewington et al., 2002).Stamler et al (1992) estimated that almost a third of BP-related deaths occur in non-hypertensive individuals with a systolic BP of 120 to 139 mm Hg or diastolic BP of 80 to 89 mmHg. Therefore, normalizing BP in the optimal range to control hypertension and preventing age-related increases in BP remain important public health priorities. While studying high blood pressure and CVD mortality risk among U.S.

adults, Gu Q et al. (2008) reported a strong, significant, and independent association of elevated blood pressure with CVD mortality risk, where hypertensive adults <65 years had a 3.86-fold increased CVD mortality risk respectively. Black (2004) estimated the impact of a modest elevation in BP to 130/90 mm Hg as to decrease life expectancy by seven to eight years.

2. Types of HTN and risk factors

Hypertension is classified as either primary (essential) hypertension or secondary hypertension (Carretero & Oparil, 2000). Secondary hypertension may explain5–10% of HTN cases and results from other conditions that affect the kidneys, arteries or heart (Fisher & Williams, 2005; (O'Brien et al., 2007)).Renal disease is the most common cause of secondary hypertension (O'Brien et al., 2007). Similarly, Hypertension can be caused by conditions that affect the endocrine system, such as Cushing's syndrome, hyperthyroidism, hypothyroidism, acromegaly, Conn's syndrome or hyperaldosteronism, hyperparathyroidism and pheochromocytoma (Dluhy & Williams, 2012). Other causes of secondary hypertension include obesity, sleep apnea, pregnancy, coarctation of the aorta, excessive liquorice consumption and certain prescription medicines, herbal remedies and illegal drugs (Lawlor & Smith, 2005).

Inherited or essential hypertension holds a strong genetic component with several family studies demonstrating associations of BP among siblings and between parents and children (Carretero & Oparil, 2000). There is a higher association between BP values amongst biological children compared to adopted children and in identical as opposed to non-identical twins (Carreteto & Oparil, 2013). Primary (essential or monogenic) hypertension is the most common form of hypertension, accounting for the remaining 90–95% of all cases of hypertension (Carretero & Oparil, 2000). In almost all contemporary societies, blood pressure

rises with aging and the risk of becoming hypertensive in later life increases considerably (Vasan et al, 2002). Hypertension results from a complex interaction of genes with environmental factors, but the genetic basis of hypertension are still poorly understood (Lifton et al, 2001). Numerous common genetic variants with small effects on blood pressure have been identified (Ehret et al, 2011), as well as some rare genetic variants with large effects on blood pressure (Lifton et al, 2001).Mutations in at least 10 genes have been shown to raise or lower BP through a common pathway by increasing or decreasing salt and water reabsorption by the nephron (Lifton, 1996). Glucocorticoid-Remediable Aldosteronism is an autosomal dominant form of monogenic hypertension in which aldosterone secretion is regulated by adrenocorticotropic hormone. Glucocorticoid treatment causes BP todecrease and gives the syndrome its name. The genetic mutation that causes GRA has been identified by Lifton (1995).As for Liddle's Syndrome, it is an autosomal dominant form of primary hypertension that results from mutations in the amiloride-sensitive epithelial sodium channel, leading to increased channel activity (Findling et al, 1997). It is characterized by the early onset of hypertension with hypokalemia and suppression of both plasma renin activity and aldosterone (Hansson et al, 1995). Apparent Mineralocorticoid Excess is another autosomal recessive form of monogenic juvenile hypertension that results from a mutation in the renal-specific isoform 11b-hydroxysteroid dehydrogenase gene (Mune et al, 1995). Normally this enzyme converts cortisol to the inactive metabolite cortisone. The enzymatic deficiency allows the mineralocorticoid receptors in the nephron to be occupied and activated by cortisol, causing sodium and water retention, volume expansion, low renin, low aldosterone, and more importantly, a salt-sensitive form of hypertension (Carretero & Oparil, 2013).

Several environmental factors influence blood pressure. Lifestyle factors that lower blood pressure include reduced dietary salt intake (He & Macgregor, 2013), increased consumption of fruits, vegetables, and low fat products (Dietary Approaches to Stop

Hypertension (DASH diet) (He & Macgregor, 2009), exercise (Dickinson, et al, 2006), weight loss (Haslam & James, 2005) and reduced alcohol intake (Whelton et al., 2002). Epidemiological studies suggest that consumption of milk and milk product is inversely related to the risk for hypertension. The association between milk consumption and blood pressure was reported in the analysis of first National Health and Nutrition Examination Survey (NHANES I) (McCarron et al, 1984). Several milk peptides have been shown to have antihypertensive effects in animal and in clinical studies. The most studied mechanism underlying the antihypertensive effects of milk peptides is inhibition of angiotensin-converting enzyme (Abbott et al, 1996). Milk peptides may also have other additional mechanisms to lower blood pressure such as opioid-like activities and mineral-binding and antithrombotic properties (Jauhiainen & Korpela, 2007).

In contrast, weight gain, obesity, and insulin resistance have been suggested as risk factors for HTN (Sorof & Daniels, 2002).Ashley et al (1974) classified obesity, and especially abdominal obesity, as the main “hypertensinogenic” factor. It was estimated in the Framingham study that each 10% weight gain is associated with a 6.5 mm Hg increase in systolic BP. Haffner et al (1992) described the relationship between BP and body fat as not restricted to the morbidly obese but as continuous throughout the entire range of body weight. A direct association between hypertension and BMI has been observed in cross-sectional and longitudinal population studies from early childhood to old age. Rocchini et al (1989) explained that the mechanism by which obesity raises BP is not fully understood, but increased BMI is associated with an increase in plasma volume and cardiac output; both these changes and BP can be decreased by weight loss in both normotensive and hypertensive subjects. Ashley et al (1974) stated that obesity is also the cause of insulin resistance and hyperinsulinemia; Anderson et al (1993) stated that even though insulin has vasodilator effects, it also increases both sympathetic nerve activity and sodium and water retention

which may lead to increases in BP. More recently, Diez (1999) associated insulin-like growth factor I and Mark et al (1999) linked leptin,a neuropeptide that regulates appetite, to also have implications in the pathogenesis of obesity-induced hypertension. Thus, although the mechanisms by which obesity and insulin resistance increase BP remain undefined, it is clear that they do cause increases in BP.

The role of stress in inducing hypertension was investigated with inconclusive evidence on its role in BP regulation and on the role of specific relaxation techniques in hypertension prevention and management (Dickinson et al., 2006). The evidence on the possible role of other factors such as caffeine consumption (Mesas et al., 2011) and vitamin D deficiency (Vaidya & Forman, 2010) is also not conclusive. Recent studies have implicated events in early life (for example low birth weight, maternal smoking and lack of breast feeding) as risk factors for adult essential hypertension, although the mechanisms linking these exposures to adult hypertension remain obscure (Dluhy & Williams, 2012).

A robust body of evidence exists on the role of dietary sodium in blood pressure regulation. Large-scale observational studies have been key to understanding the association of sodium with blood pressure and vascular disease and the most widely cited of these is INTERSALT. First reported in 1988 (INTERSALT, 1988) this study identified significant positive associations of 24-h urinary sodium excretion with systolic and diastolic blood pressure. INTERSALT findings were based on two sets of investigations: 1) in cross-population analyses of 52 population samples from different countries; and 2) at the individual level for 10 079 participants aged 20–59 years. More recently, reviews of observational studies comparing sodium intake and blood pressure levels between populations and reviews of studies comparing sodium consumption and blood pressure between individuals within the same population have confirmed these findings and the effects of sodium on blood pressure were observed amongst both hypertensive and non-hypertensive

individuals (Report of WHO Forum, 2006). Effects were found to be greater amongst older individuals and amongst people with higher baseline blood pressure levels (Law, Frost & Wald, 1991). According to Weinberger (1996), individuals, both normotensive and hypertensive, vary in their blood pressure responses to changes in dietary salt intake, bringing the two terms, “salt-resistant” (SR), and “salt-sensitive” (SS). Most studies have found that, among hypertensive subjects, salt-sensitive individuals far outnumber salt-resistant individuals. Hoffman et al (2008) showed in his one year program study that the SR phenotype protects from obesity-induced elevations in BP. Hence, Mendis (2006) stated that an individual’s BP response to sodium intake, i.e. salt sensitivity, is highly determined by genetic factors, age, body mass, associated disease, and ethnic factors. Johnson and Herrera-Acosta (2002) reported that salt sensitivity is not found in all hypertensive individuals. In fact, there is a long list of conditions for hypertension to be classified as salt-sensitive, such as low birth-weight with nephron under dosing, primary glomerular disease, ageing, obesity, and diabetes. Krotkiewski (1979) et al identified the variables that best predicted sodium sensitivity, which included fasting plasma insulin, plasma aldosterone,and plasma norepinephrine, supporting the hypothesis that BPis sensitive to dietary sodium and that this sensitivity may bedue to the combined effect of hyperinsulinemia, hyperaldosteronism, and increased activity of the sympathetic nervous system.

Only about 10 to 15 percent of the population is considered salt-sensitive, meaning that they are sodium retainers; their metabolic characteristics cause them to retain sodium whether they consume large or even small amounts of salt (Ben & Bursztyn, 2011). They generally have high or even hyperactive adrenal gland function; it is known that when the adrenals are overactive, sodium is retained in the body; these people are also low in the key minerals, which help to protect the body from sodium build-up, such as calcium and magnesium (Kimura, 2008). The term salt resistant or salt-insensitive is used for individuals who lose

sodium from the body, even though they may eat large amounts of salt. Salt insensitivity is usually found in slow metabolic types, as these individuals are frequently found to have adrenal insufficiency. Low adrenal function reduces the body's ability to retain sodium. An extreme example is Addison's disease, which is associated with an almost total lack of adrenal cortical hormone production. Individuals with this condition crave and eat copious amounts of salt, unless they are taking adrenal hormones (Weinberger, 1996).

The mechanisms behind salt-sensitivity are currently not fully understood. Lifton and Geller (2001) recognized 15 monogenetic conditions in which renal handling of sodium is affected and in each, there is a BP consequence. These conditions are divided equally into “salt-losing” and “salt-retaining” states, however, these settings are rare, and provide little data about population-wide causes of hypertension. Nevertheless, they indicate that sodium balance could have a genetic basis, which can in turn influence BP. According to the WHO, interventions to reduce population-wide salt intake have been shown repeatedly to be highly cost-effective (Reducing Salt Intake in Populations Report, 2006).

However, while the response to sodium reduction does vary between individuals, the concept of salt sensitivity is not particularly valid, with the vast majority of individuals likely to achieve blood pressure reductions and health benefits from a reduction in dietary salt consumption (Aldrerman, 2002). Several randomized trials of sodium restriction have confirmed the findings of observational epidemiological studies (Report of WHO Forum, 2006). Systematic reviews and meta-analyses of the results of these trials clearly demonstrate that reducing dietary sodium decreases blood pressure with greater effects in the elderly and amongst individuals with higher baseline blood pressure (Aburto et al, 2013). It is of note that blood pressure lowering effects with sodium reduction were observed amongst individuals with normal blood pressure as well as amongst subjects with high blood pressure and that in both cases the reductions achieved would be anticipated to translate into

significant health benefits (Report of WHO Forum, 2006). Lower sodium consumption results in lower blood pressure and lowering blood pressure is a profoundly effective means of reducing vascular risk (Prospective Studies Collaboration, 1995; Asia Pacific Cohort Studies Collaboration, 2003; Blood Pressure Lowering Treatment Trialists Collaboration, 2003).Evaluation of BP response to sodium reduction was assessed in a 3-year lifestyle dietary intervention trial; BP changes at 18 and 36 months after enrollment were analyzed per 2300 mg/24 h reduction in sodium excretion and resulted in corresponding net decreases in SBP/DBP of 2.0/1.4 mmHg at 18 months, and 1.7/0.9 mmHg at 36 months (Cook et al, 2005).

B. Dietary Sodium

1. Metabolism of Sodium

Sodium, together with potassium, is an essential mineral for regulating body fluid balance (Expert Group on Vitamins and Minerals, 2003).Sodium is the major extracellular cation and along with its associated anions accounts for almost the entire solute concentration in the extracellular fluid. The major adjustment of Na balance is made by the kidneys which have the capacity to eliminating large quantities of Na as well as the capacity to excrete only a few mEq per day (Karppanen & Mervaala, 2006). Sodium is filtered at the glomerulus and reabsorbed along the length of the tubule, normally in accordance to body needs. Approximately, 65% of the filtered Na is reabsorbed by the proximal tubule by active transport. In the ascending limb of the loop of Henle, an additional 25% of the filtered Na is reabsorbed. An additional 4 to 8% of Na is reabsorbed in the distal tubule. The remaining Na is reabsorbed along the collective duct where the final adjustments are made (Tuomilehto et al., 2001). The renal regulation of Na is intertwined with the Extracellular Fluid Volume (ECFV). Thus, an expanded

ECFV results in less reabsorption and more excretion of Na, and a contracted ECFV has the opposite effect. The effects may be accomplished by indirect mechanisms including a volume receptor mechanism and efferent mechanisms such as aldosterone and “natriuretic hormone”, as well as by a sensing apparatus within the kidney such as the juxtaglomerular apparatus (Jurgens & Graudal, 2003).Activation of the renin-angiotensin-aldosterone (RAA) system increases the retention of sodium and water (Karppanen & Mervaala, 2006). The control range of the RAA mechanism is in excellent agreement with the sodium amounts, which can be derived from diets comprising only natural foodstuffs without artificial additions of salt or other sodium compounds. These findings strongly support the view that human beings are genetically programmed to eat foods that contain sodium in amounts that are naturally present but do not contain added salt (Eaton et al, 1997). More than 95% of the ingested salt is absorbed from the gastrointestinal tract.Sodium is absorbed passively from the lumen of the entire length of the intestine (Osanai et al, 2002).Extra-renal loss of salt may become significant only in massive diarrhea and vomiting or prolonged strenuous exercise with profuse sweating (Mervaala, 1995). Otherwise, extra-renal loss of salt is minimal, with sweating accounting usually for approximately 1 mmol (0.058 g) and other extra-renal losses for 0.002 to 0.18 g/d only. Therefore, to maintain the extracellular sodium concentration (≈142 mmol/L) and total body salt content at constant levels, renal salt excretion has to be almost equal to salt intake. Even a small increase in serum sodium concentration after absorption of dietary salt from the gastrointestinal tract triggers thirst and causes fluid intake until the normal serum concentration is restored (Laatikainen et al., 2006). The necessity of sufficient renal salt excretion can be illustrated by the fact that a daily excess in salt intake of 8.3 g (3266 mg sodium) must be accompanied by a 1-L increase in water intake each day to maintain the normal extracellular sodium concentration of 142 mmol/L.

Theoretically, in the full absence of renal sodium excretion capacity, approximately 250 g of salt and 30 L of water would accumulate in the body during 1 month (MacGregor&Wardener, 1998).Salt intakes that exceed 50 mmol (≈3 g) are not able to substantially suppress the level of the sodium-retaining hormone, aldosterone. Therefore, other mechanism(s) than suppression of the RAA system only is needed to excrete sodium and to maintain sodium and water homeostasis when dietary salt intake is excessive (Alderman et al., 1998).Blood pressure serves 2 important functions in the body. One is maintenance of tissue perfusion. The other important and extremely potent function is control of sodium balance, which largely determines the extracellular fluid volume. By increasing the blood pressure level, the body is able to get rid of excess sodium and water through the pressure-natriuresis mechanism (Mervaala 1995, Guyton, 1991). Blood pressure is, in fact, the most powerful physiologic mechanism in the maintenance of sodium and water balance. The development of sodium deficiency and decreased extracellular fluid volume during a prolonged very small sodium intake or losses due to gastrointestinal causes, sweating, or blood loss, can be effectively prevented by decreasing the blood pressure (Appel & Brands, 2006). By lowering the blood pressure, the body is able to prevent renal sodium and fluid excretion completely. In addition to low blood pressure, sodium deficiency, which is highly unusual, can lead to dehydration, and muscle cramps (Mason, 2006). On the other hand, in the case of high salt intake the body is able to effectively prevent salt and fluid accumulation by raising the blood pressure to such an extent that pressure-induced increase in salt and water excretions matches the intakes (Chobanian & Bakris, 2003).

2. Salt intake as a risk factor for HTN & other CVDs

According to the WHO, several investigations, including genetic, epidemiological, and interventional studies, found evidence for a causal relationship between salt intake and CVD (Reducing Salt Intake in Populations Report, 2006). He et al (1999) reported a

significant positive association between sodium intake and the risk for stroke in overweight adults in the United States, and Nagata et al (2004) showed similar results in a Japanese cohort. In a study of Finnish men and women, Tuomilehto et al (2001) document a significant association between urinary sodium excretion and CVD related mortality. The observed association between salt intake and CVDs may reflect the association between salt intake and increased blood pressure which has been documented by several epidemiological studies (Liu et al, 1979; Geleijnse et al 1990; Joossens et al, 1994; Stamler, 1997).

In a recent Cochrane systematic review, He and McGregor (2013) concluded that a modest reduction in salt intake to 5-6 g/day for the duration of four weeks was found to have significant effects on BP levels, at the population levels. This review also revealed a correlation between the degree of salt reduction and the degree of BP reduction, within the daily range of set intake of 3 to 12 g/day. When reducing salt intake from 9.4 g/day to 4.4 g/day, pooled estimates of changes in blood pressure were -4.18 mm Hg for systolic BP and -2.06 mm for diastolic BP. According to the WHO, systolic blood pressure was reduced by 3.39 mmHg with low Na intake, this reduction in systolic blood pressure was greater in studies specifically targeting individuals with hypertension than in studies targeting individuals without hypertension; systolic blood pressure was reduced to a greater degree when usual sodium intake was reduced by ≥ 1/3 compared with a reduction in intake of < 1/3 (Effect of reduced sodium intake on blood pressure, renal function, blood lipids and other potential adverse effects, 2012). In a more recent systematic reviews and meta-analysis, the effects of lowering sodium intakes within current recommendations was summarized as follows; in adults a reduction in sodium intake significantly reduced resting systolic blood pressure by 3.39 mmHg and resting diastolic blood pressure by 1.54 mm Hg; when sodium intake was <2 g/day versus≥2g/day, systolic blood pressure was reduced by3.47mmHg and diastolic blood pressure by 1.81 mm Hg;. In children, a reduction in sodium intake

significantly reduced systolic blood pressure by 0.84 mm Hg and diastolic blood pressure by 0.87 mm Hg (Aburto et al., 2013). 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 reducing salt to total intake levels 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. Interestingly, the combination of sodium and chloride together seems to have the most potent effects on blood pressure. Ganry et al (1993), in a small crossover study, of six normotensive patients (males and females), examined the effect of sodium chloride or sodium bicarbonate on blood pressure regulation.. Sodium loading in the form of bicarbonate had no effect on blood pressure, whereas sodium chloride for the same period significantly increased the blood pressure.

Besides its effects on BP, dietary sodium intake is thought to be correlated to cardiovascular health independently of BP. Salt loading has been shown to decrease NO production independently of changes in BP, which suggests a direct effect of sodium (or the hormonal response to sodium) on the endothelium (Dickinson et al, 2011). According to Jablonski et al.(2012), restricting dietary sodium intake to a level consistent with the DASH diet (less than 2300 mg/day) improves both conduit artery (macro vascular) and resistance vessel (micro vascular) endothelial function in middle-aged/older adults with moderately elevated SBP and also leads to improvements in vascular endothelial function including increased NO and BH4 bioavailability and reduced oxidative stress; the improvements in EDD(Endothelium Dependent Dilation) with dietary sodium restriction remained significant even after statistically correcting for SBP. Dickinson et al (2011), in a randomized crossover study, compared the effects of a low salt diet (LS) with a usual-salt diet (US) on vascular

function as assessed by brachial artery flow-mediated dilatation (FMD). FMD was significantly greater with the LS diet than with the US diet. The changes in FMD were independent of BP. Dickison et al (2011) also studied the endothelial function of 16 healthy normotensive subjects after receiving a meal with added salt and a control low salt meal on two separate occasions in a randomized order. FMD decreased postprandially after both meals; however, the decrease was more evident after the high salt meal. The amount of salt contained in a high salt meal significantly suppressed FMD within 30 minutes; hence, it appears that higher salt intakes have acute adverse effects on vascular dilatation in the postprandial phase. Sodium restriction also showed positive effects on stress-related endothelial dysfunction since salt reduction decreases urinary free cortisol excretion, which might indicate lower cortisol production (Dickison et al, 2011).

In contrast to the previous studies, some recent evidence suggests that very low sodium intake may actually have adverse effects on cardiovascular health, such as unfavorable blood lipids and insulin resistance (McGuire, 2013). A low salt diet was found to be associated with an increase in insulin resistance by activating the renin-angiotensin-aldosterone and sympathetic nervous system (Garg et al., 2010).When comparing a low salt diet (<460 mg/day) with a high salt diet (>3450 mg/day), higher HOMA levels were obtained independent of age, gender, blood pressure, BMI, serum sodium and potassium, serum angiotensin II, plasma renin activity, serum and urine aldosterone, and urine epinephrine and norepinephrine (National Institute of Health, 2011). In a recent Cochrane review, high salt diets (mean sodium intake 4508 mg/day) were compared to low salt diets (mean sodium intake 1633 mg/day) to study their effects on blood pressure, hormones and lipids in people with normal and elevated blood pressure. Sodium reduction (mean sodium reduction 2875 mg/day) resulted in a significant decrease in BP of 1% in normotensives, 3.5% in hypertensives, and a significant increase in plasma rennin, plasma aldosterone, plasma

adrenaline, and plasma noradrenaline, in addition to a 2.5% increase in cholesterol, and a 7% increase in triglyceride (Graudal et al., 2012).

In 2004, the IOM set Dietary Reference Intakes (DRIs) for sodium. The Adequate Intake (AI) was set at 1,500 mg per day for those aged 9 to 50 years, with lower values for younger and older individuals. The Tolerable Upper Limit (UL) for sodium was set at 2,300 mg per day for people aged 14 years and older, with lower values for those less than 14 years of age; the definition of the UL for sodium in particular is problematic since increases in BP continue with increasing sodium intakes without an apparent threshold. Figure 3 illustrates the AIs and ULs for sodium amongst various age groups. In 2003, the WHO set a global target of 5 g or less of salt (<2000 mg of sodium) per day per person. Many governments have launched initiatives to reduce the sodium intake of their populations, such as Finland, the United Kingdom, the European Union, and most recently, the United States.

Figure 3: Sodium AIs and ULs for various life stage groups

Life Stage Groups (years)	**AI (mg/day)**	**UL (mg/day)**
1-3	1,000	1,500
4-8	1,200	1,900
9-13	1,500	2,200
14-18	1,500	2,300
19-30	1,500	2,300
31-50	1,500	2,300
51-70	1,300	2,300
Over 70	1,200	2,300

Adapted from: *U.S National Academies of Science Institute Medicine*, 2005

Sodium intake can be estimated either directly by the measurement of urinary excretion, or indirectly by dietary assessment.

- **Assessment of Salt Intake**

1-Direct Sodium Estimation

a- 24-hour urine collection

24-hour urinary sodium excretion is recognized as the "gold standard" method for assessing sodium intakes in population surveys (Bingham, 1987; Bates, 1991; Hunter, 1998). A 24-hour period is required to capture the pattern of sodium excretion, since there is a marked diurnal variation in sodium, chloride and water excretion (Wesson, 1964). Electrolyte excretion in healthy individuals reaches a maximum at or before midday, and a minimum at early morning. The cycle is independent of activity although severe exercise may produce decreased excretion of sodium and chloride; the cycle may be reversed in night workers, healthy individuals given large doses of cortisone, in Cushing's disease and primary aldosteronism (Elliott & Brown, 2007). Stanbury and Thomson (1951) made an intensive study of the diurnal cycle of electrolyte excretion in healthy people, making more than 80 observations on each of 12 individuals. Glomerular filtration rate (GFR) was calculated by measuring inulin clearance (inulin was injected continuously by intravenous infusion). The GFR, urine volume and mean rates of sodium and chloride excretion fell overnight to about 30% of the midday value for sodium, and urinary concentrations of sodium and chloride increased with the relative oliguria of sleep. Marked differences in electrolyte excretion rates were observed between individuals. The 24-hour urinary excretion method takes no account of electrolyte loss other than via the kidney, and therefore will tend to underestimate true intake. Holbrook et al. (1984) reported that among 28 adults, average urinary excretion of

sodium from seven consecutive 24-hour urine collections was 86% of that estimated from chemical analysis of duplicate diets collected over the same seven-day period. Losses of sodium in the feces are small under normal conditions (Baldwin, Alexander & Warner, 1960) and over a wide range of intakes (Kirkendall et al., 1976), and other losses are thought to be negligible, except those from sweating which in certain circumstances can be considerable (Dahl, 1958). Such losses depend both on the concentration of electrolyte in the sweat – which is under hormonal control related to the amount of sodium in the diet – and on the rate of sweating. However, losses through sweat (which might be expected to vary with ambient temperature and humidity) are minimal in temperate climates (Elliott & Brown, 2007).

The 24-hour urine collection has the advantage that it is not affected by subjective reporting of dietary intakes, though it is subject to several limitations, such as:

- Participant burden is high and therefore rates of incomplete collection/attrition may be high.
- The collection must be complete, with no more than a few drops lost, otherwise the excretion estimate will be biased.
- There is no absolute check on completeness, though good survey technique may help to reduce levels of incompleteness.
- The collection must be accurately timed to avoid over- as well as under-collection and so that minor deviations from a 24-hour collection period can be corrected.

An important consideration is whether sodium intake is being estimated at individual or group level. Liu et al. (1979) and Joossens et al. (1980) showed that, because of the large day-to-day intra-individual variability in sodium intakes in developed countries, a single 24-hour urine collection is insufficient to characterize the sodium intake of the individual, and as many as fourteen 24-hour urine collections may be required. Smaller numbers of collections may be needed in Asian populations such as in China (Lisheng et al., 1987) and in the elderly

(Elliott et al., 1988) because of lower day-to-day variation in sodium excretion in these groups. On the other hand, by including sufficient numbers of people, mean sodium excretion for populations can be estimated from single 24-hour urine collections, with little error about the mean (Elliott & Brown, 2007).

A critical consideration in the use of the 24-hour urine collection method to estimate sodium intakes is to ensure that urine collections are complete.

- Creatinine: 24-hour excretion of creatinine has been used as a standard to exclude urine collections judged to be incomplete with the assumption that the rate of urinary excretion of creatinine is constant (Doyle Schachter et al., 1980; Ljungman et al., 1981). A diet particularly rich in animal proteins is a contributor to creatine, a precursor of creatinine. The intake of meat caused a rapid rise in creatinine excretion, and exercise had no effect (Bleiler & Schedl, 1962). The large intra-individual variability in creatinine excretion and the responsiveness to meat in the diet seriously limit the utility of creatinine as a marker for the completeness of 24-hour urine collections (Edwards, Bayliss &Millen, 1969). Liu et al. (1979) have suggested that the CV% of creatinine excretion might give a measure of the accuracy of 24-hour collections within the group, and Joossenset al. (1980) have proposed a model to standardize 24-hour excretion values of sodium to creatinine at group level based on functions of weight, height, sex, age and social class. However, according to Bingham and Cummings (1983), up to 40% of urine could be lost in a 24-hour period, and yet creatinine excretion measured in the remainder would still fall within the normal (24-hour) range. In population terms, systematic under-collections of urine – of up to 40% – may go undetected by the creatinine method and could result in lower estimates of urinary sodium excretion from survey data.

- p-aminobenzoic acid (PABA): Bingham and Cummings (1983) have proposed the use of PABA as a biomarker for the completeness of 24-hour urine collections as an alternative to the creatinine method.

PABA, a B-complex vitamin, was selected as it was considered to be non-toxic in man; it is thought to be absorbed and excreted within 24 hours and can be readily analyzed (International Agency for Research on Cancer, 1978).The PABA technique relies on the participant taking PABA tablets (3 x 80 mg). Informed consent and ethics committee approval is required (Henderson et al., 2003). The technique relies on the participant taking their tablets as instructed at intervals during the 24-hour period; missing a tablet, or taking the tablets at the wrong times, might result in complete urine collections being falsely rejected. Once absorbed, PABA is metabolized in the liver to p-aminohippuric acid (PAHA) and both PABA and PAHA are acetylated. In a series of experiments in human calorimeters, excretion of PABA in the urine was found to be dose dependent. At a dose of 240 mg (80 mg three times a day with meals), mean recovery of PABA over a 24-hour period was 93% in 33 individuals. The range between minimum and maximum values was 15% of the mean, while 70% for creatinine excretion was calculated. Bingham and Cummings (1983) concluded that in the general population, collections containing more than 85% (205 mg) of the administered dose can be considered complete. The use of PABA in validation studies has proved to be effective, however, its wider use in population studies was found to be problematic (Bingham, 2002). A number of drugs, such as sulphonamides, folic acid, paracetamol, phenacetin and frusemide might mistakenly produce high PABA recovery rates (Bingham & Cummings, 1983). Jakobsen, Pederson & Ovesen (2003) observed that PABA recovery declined with age, at a rate of approximately 1% per year from age 30 onwards, such that in older individuals complete urine collections might be falsely rejected (false negative). They also raised the possibility that incomplete collections might be falsely accepted (false

positive). In the 2000-2001, the British National Diet and Nutrition Survey initially used the PABA method to validate 24-hr urine collections. However, during data collection, a subject exhibited acute allergic reaction towards the PABA tablets, leading to the termination of the procedure. Hence, the convenience and safety of the PABA technique for population surveys have not been assessed (Henderson et al., 2003).

Overnight and spot (casual or single) urine collections have been proposed as low-burden alternatives to the 24-hour collection (Watson & Langford, 1970), as fewer voids are required, and the participant does not have to continue the collection during daily activities.

b- Overnight urine collections:

Collection of overnight urine is more readily achieved than 24-hour urine collections, and a higher rate of compliance is likely in large epidemiological surveys (Pietinen et al., 1976). Before going to sleep, the participant is required to empty his or her bladder and discard the urine. The collection then begins and the time is recorded; all urine voided during the night, and the first void in the morning upon rising, constitute the overnight collection. The total volume of urine collected and the time that the collection is completed are recorded, so that sodium excretion may be calculated and corrected to an 8-hour base (Dyer et al., 1987).

While the overnight urine collection method has distinct advantages over the 24-hour urine collection in terms of feasibility for large population surveys and participant burden, there are large individual differences in the rate of electrolyte excretion at night compared to the day, and also in the proportion of solute excreted overnight compared to the full 24 hours. Dyer et al. (1987) compared 24-hour, daytime and overnight rates of sodium excretion in 107 hypertensive adults, and observed a higher average rate of sodium excretion at night than during the day, a reversal of the usual pattern whereby more sodium is excreted during the

day (Pietinen et al., 1976). Overnight sodium excretion was also higher in women than men. These observations raise questions as to whether estimates of sodium intake from overnight urine collections might be biased according to, for example, the mix of hypertensive and normotensive individuals, percentage of men and women, or black and white individuals in the sample (black individuals exhibit a slower excretion rate of Na at night).

c- Spot (casual) urine collections:

A single voiding of the bladder is all that is required for a spot urine collection. Sodium concentration and ratios to creatinine and potassium are obtained from laboratory analysis. Time of day of the collection should be standardized to minimize error introduced by diurnal variation in urinary solute excretion (Widdowson & McCance, 1970). Walker et al. (1979) reported significant correlations between Na:Cr ratios in spot urine and 24-hour urine collections. For 18 individuals with normal blood pressure the correlation coefficient was 0.62; for 37 hypertensive individuals the coefficient was 0.56. Moore et al. (1979) reported a significant correlation between 24-hour urinary sodium and the Na:Cr ratio of the second morning void (r = 0.84) in eight individuals under 30 years of age with essential hypertension. Milne et al. (1980) reported, in a study of 97 men, significant correlations (r = 0.25 to 0.52) between sodium excretion in urine samples at different times of the day and in 24-hour collections of urine. They commented that spot urine specimens might be useful in differentiating between individuals with large differences in electrolyte excretion. More recently, in an analysis of the 10 079 men and women from 52 population samples of the INTERSALT Study, Elliott et al. (1992) found the ratio of sodium to creatinine as assessed by spot urine to be positively correlated with sodium excretion from an independent 24-hour collection (r = 0.82 between population samples and r = 0.37 between individuals). Khaw et al. (2004) reported similar estimates of mean sodium excretion based on spot urines and repeated 24-hour urine collections.

Itoh et al 1992 compared the values estimated from the second morning voiding (SMU) with the 24-hr urine samples and confirmed that the SMU specimens provided similarly accurate information for estimating the 24-hr urinary Na excretion. Good agreements between the estimated mean of the 24-hr urinary Na was observed for either a single day or a 3 day collection period. Kawasaki et al (1991) have developed mathematical equations using the method of the forward stepwise regression analysis, to predict 24-hr urine creatinine excretion (24HUCrV) by means of individual body weight, body height, and age, all of which are easily obtained. Using this equation, the completeness of the urine collection can be evaluated by representing the actual excretion as a ratio of estimated excretion (Kawasaki et al, 1992). This method was also applied to a large number of subjects in a field survey (Kawasaki et al, 1990; Kawasaki et al 1992), and found that even in a single urine collection, the SMU specimen overcomes some of the disadvantages associated with the 24-hr urine collection and provides a reliable estimate of 24-hr urinary Na excretion.

Tanaka et al (2002) developed a simple method to estimate 24-hr urinary Na (24HUNaV) excretion from Na/K ratio of a spot urine specimen collected at any time. This method is very useful to estimate the population level of Na or salt intake for epidemiological surveys and public health activities aimed at reducing blood pressure levels in a community, and to compare their usual intake. Hence, a formula to estimate 24-hr urinary Na excretion, using the Japanese data items of the INTERSALT study was developed. In this process, the participants were asked to provide both, a casual spot urine specimen and a 24-hr urine collection sample.

The method developed by Kawasaki et al is actually used in some epidemiological studies; however, collecting SMU from all subjects is usually very difficult. In order to resolve the disadvantage, this method was built up having no restriction on collection time. Kawasaki et al (1992) developed separate formulas for each of men and women, because of

the amount of muscle that differs with gender. However, Tanaka et al (2002) developed a single formula for both men and women, after clarifying separate formulas and finding fair consistency in the single formula estimation of urine Cr excretion. This method is mostly used for estimating the populations mean of Na excretion, comparing different groups, populations, and annual trends. However, this method is not suitable for individual subjects, for estimating personal Na intake, or alteration of Na intake before and after the intervention or education. In such cases, other methods, such as the one reported by Kawasaki et al or 24-hr urine collection, should be used (Tanaka et al, 2002).

d- New markers for Na intake:

Chloride Titrator Stick: The advantage of this method is that chloride concentration can be estimated rapidly in situ. Sodium chloride is not however the only potential source of chloride, and it has been noted that estimates may be biased for individuals consuming potassium chloride or alkalizers. Though there has long been interest in this method (Sloan, Beevers & Baxter, 1984; Tochikubo et al., 1986, Minetti et al., 1992; Brungel, Kluthe & Furst, 2001), the titrator stick has a relatively narrow calibration range (Brungel, Kluthe & Furst, 2001) and is probably not precise enough for dietary assessment of sodium intake, nor for monitoring changes in population sodium intakes.

2. Indirect Sodium Estimation

Intake of Na can be assessed by several dietary assessment tools which are briefly described below

a- *Dietary Assessment Methods*

i. Food Record

A food record is a written record of all foods and beverages and the amounts of each food/beverage consumed over one or more days. The record also collects information on food

preparation and any additions to food, such as salt or condiments. The amounts consumed can be weighed using a weighing food scale or assessed via household tools such as cups and tablespoons, or estimated using food models and pictures (Trabulsi& Schoeller, 2001).Nutrient intakes can be estimated by summing the total amount of each food portion consumed, converting it to a gram weight, and multiplying it by the nutrient composition obtained from a food composition database or other data source. This method can be conducted using a paper and pencil, computer, voice recorder, or other tool. Because foods and beverages are measured and recorded at each occasion, the food record method has the potential to provide accurate information (Thomson & Subar, 2001). However, food records have limitations. First, literacy and a basic knowledge of the types of food, serving sizes, and preparation techniques are often necessary. Because respondents are asked to record all foods and beverages as they are consumed, some respondents may misrepresent the type and/or amount of food they consumed (Gersovitz et al, 1978). In addition, subjects may change their dietary habits due to difficulty in recording complex dishes (Watson et al., 2009) or may choose to report "healthier foods" thus increasing reporting bias (Bliss, 2004; Trabulsi& Schoeller, 2001).

Due to the large amount of effort required to collect and process multiple days of food records or recalls, these methods are seldom used for estimating dietary intake in large scale epidemiological research(Willet 1998, 121). Instead, they are used to describe mean intakes for smaller groups as well as in validating food frequency questionnaires, which are currently, the primary method for estimating intake on a larger scale (Willet 1998, 67).

ii. 24-hour Recall

The 24-hour recall (24-HR) is an interview aiming to collect information about what the subject has eaten and drank over the past 24 hours. Even though it can be self-reported, it is best carried out by a skilled and trained dietary interviewer in order to ask the proper

sequence of open-ended neutral questions and probe on forgotten foods, food preparation methods, ingredients of composite dishes, brand names of commercial products as well as supplemental intake (Thompson&Subar 2001, 8). This method is typically conducted in person or via telephone. Typically, data from multiple 24-hours including weekdays and weekends are averaged together (Trabulsi&Schoeller, 2001) especially that reporting a single day's intake may not be representative of the individual's overall dietary intake (Block, 1982).

This method relies on the subject's short term memory. The strength of the 24-hour recall is its ability to collect detailed, qualitative information about the food consumed (Trabulsi&Schoeller, 2001). It is quick, inexpensive, and non-intrusive and does not exert heavy burden on the subject, especially if accompanied with practical food portions options (Biroet al. 2002). Since this method does not require literacy from the subject and is not burdensome, it is more likely that the subjects agreeing to participate in the study are representative of the general population (Thompson&Subar 2001, 8).However; this method is subject to potential drawbacks especially that subjects may have incomplete knowledge and memory of their food intake. Because the 24-hour recall method relies on the memory of the subject, daily fluctuation in dietary intake due to intra-individual variation between days may increase. Thus, a single 24-hour recall is not considered a reliable indicator of an individual's usual intake. Rather, taking multiple recalls by a trained interviewer is needed to compensate for day to day variation and accurately estimate intake (McPherson et al., 2000; Willet 1998, 56).

Currently, the United States Department of Agriculture's (USDA) Multiple Pass Food Recall (MPR) is being used in order to attenuate the 24-hour recalls' limitations (Moshfegh et al., 2008; Raper et al., 2004). It's a 5-step interview with:

- An initial “quick list” in which the subjects list all the foods and beverages they have consumed over the past 24 hours without interruption.
- The interviewer then reminds the subjects of a forgotten food list often omitted in 24-hour recall reporting.
- The interviewer then asks about the time and meal of reported foods
- The interviewer probes about the foods and beverages eaten: their portion sizes, way of preparation, brand names and supplemental intake.
- And last, a final review for the whole 24-hour recall.

iii. Food Frequency Questionnaire (FFQ)

The food frequency approach requires subjects to report the usual frequency of consumption (per day/week/months or rarely/never) of each food from a list of different food items. Food items are listed using generic names and often, foods with similar nutritional qualities are grouped together for ease of use. A FFQ list can range between 40 to 200 food items, with longer FFQs demonstrating a higher validity compared with shorter ones (Willet, 2001). The inclusion of ethnic foods in FFQs is more likely to improve the accuracy of assessing nutrient intakes among specific ethnic population groups (Khokharet al., 2009). Usually, information is collected on the frequency of intake and sometimes on the portion size, but not the method of cooking or food combinations (Thompson & Subar 2001, 9). FFQs that do not request information on portion size are known as qualitative or non-quantitative. FFQs with open-ended responses as well as portion sizes estimated using weights, measures or food models are considered quantitative as opposed to semi-quantitative FFQs where portion sizes are standardized for foods that are consumed in typical portions and response answers are limited to the number of portions consumed (McPherson et al., 2000; Serdulaet al., 2001).

The underlying principle of the FFQ is to cover a longer period of dietary recall (typically the previous year), as compared to 24-hour recalls and food records, which are both considered as short-term dietary assessment methods. Overall, nutrient intake estimates are derived by summing all foods reported, their portion size x frequency x nutrient content per portion in order to estimate a daily intake of nutrients or food groups (Bliss, 2004). FFQs are minimally burdensome, cost-effective, and easy to administer and process as well as being suitable for large population studies (Willet & Hu, 2006; Zulkifi& Yu, 1992). They can be self-administered as well as interviewer-based as they do not affect or change subjects' eating behaviors associated with reporting bias. FFQs allow to compare average dietary intakes of different population groups as well as to rank individuals within a group (Block, 1982; Willet 1998, 65-66).

FFQs aiming to assess dietary intake by nutrient and/or food groups have been validated for more than 20 years in countries such as Japan, Brazil, Greece, Germany, USA, Australia, to name a few (Cade et al., 2004; Kolodziejczyket al., 2012; McPherson et al., 2000). Because FFQs should be tailored and validated specifically in the population under study, many countries have validated various FFQs depending on the target population. Validated FFQs to solely assess salt intake have not developed yet in the MENA region, and as shown in figure 4, only two previous experiences have been documented in the literature (Charlton et al, 2007; Pavadhgul et al., 2009).

Figure 4: Validated FFQs to assess salt intake

Reference	**Questionnaires compared**	**Population**	**Correlation coefficient for validity**
Charlton et al., 2007	FFQ versus: b- 3 24-hr recall c- 3 24-hr urine samples	324 men and women (20-65 years of age) from Cape Town City Council offices.	• Spearman correlation for FFQ/repeated 24-hr recall: 0.683

			• Spearman correlation for FFQ/urinary Na: 0.173 • Sensitivity : 12.4% • Specificity : 93.9%
Pavadhgul et al., 2009	FFQ versus 3 day food record	170 undergraduate students (17-20 years of age) from the dormitory of Mahidol University	• Spearman correlation : r= 0.71 (p < 0.001) • Difference in mean Na: p=0.298

Dietary assessment of Na intake is prone to numerous errors including reporting errors, inaccurate or incomplete food composition tables, coding errors, and sampling bias (Bingham, 1987). Specific sources of error include difficulties in estimating the amount of sodium chloride added during cooking, at restaurants, and at the table; variation in the proportion of salt added during cooking that is retained by the food; plate losses; differences in sodium content of manufactured foods; and variation in sodium concentration of local water supplies (Bingham 1987; James et al 1987). As a consequence of the above sources of error, sodium estimates based on the food diary, weighed records, food–frequency questionnaire or 24-hour recall approach may tend to underestimate sodium intakes as compared with intakes estimated from duplicate diets or 24-hour urine collections. Schachter and colleagues (1980) compared estimates of sodium intake from duplicate food portions (with sodium content measured by flame photometry) and 3-day food records with those from urinary sodium excretion measured over the same period (three consecutive 24-hour collections) in nine adults. The highest estimate of sodium intake was obtained from the duplicate portions. Mean urinary sodium excretion was on average 5% lower than intake

assessed by duplicate portions, while intake assessed by food records was on average 6.3% lower than the urinary estimate. Similarly, Clark and Mossholder (1986) estimated sodium intakes among eight adolescent girls using the duplicate diet method and compared chemically analyzed values against urinary excretion from 24-hour urine collections, and calculated estimates from food composition tables. Discretionary use of salt from the saltshaker was estimated separately. For each participant, urinary sodium excretion was higher than that calculated from food tables. Urinary sodium excretion comprised on average 83% of total sodium intake estimated from chemical analysis of sodium in the duplicate diets plus discretionary sodium. In a study of 55 middle-aged adults, Caggiula et al. (1985) compared sodium intakes estimated from a six-day food record with urinary excretion from a single 24-hour urine collection. On average, mean 24-hour urinary sodium excretion was higher than sodium intake estimated from food records by approximately 40%. More recently, Espeland et al. (2001) estimated sodium intake from repeated 24- hour dietary recalls (including discretionary use of sodium) and Khaw et al. (2004) from a seven-day diary (excluding discretionary sodium), in comparison with urinary sodium excretion from repeated 24- hour urine collections. Both studies found that sodium intake was underestimated by the recall/diary methods in comparison with urinary sodium excretion, on average by 22% and 16–17% respectively. However, not all studies that carried out such comparisons have found that sodium intake is underestimated by dietary intake methods versus urinary sodium excretion. Pietinen (1982) reported that urinary sodium excretion (from three consecutive 24-hour collections) represented 93% of sodium intake estimated from food records kept for four consecutive days. The study concluded that food consumption data collected in nutrition surveys could be used for estimating sodium intakes where accurate data on the sodium content of local foods are available. In the INTERMAP study, similar estimates of sodium intakes were obtained from the average of four 24-hour

dietary recalls and two 24-hour urine collections, for samples from Japan, United Kingdom and USA (Stamler et al, 2003).

Each dietary assessment method has its advantages and limitations and none is capable to measure food intake without error. Therefore, tests of validity are necessary to understand the relationship between what the method actually assesses and what it intends to measure. This is important in order to properly interpret the assessment results (FAO, 2008).Using a non-validated assessment method or dietary assessment tools with low validity can attenuate true associations between diet and disease, generating false-negative conclusions (Day et al., 2001). Due to the absence of a true gold standard in dietary assessment methods, it is significantly complicated to validate such tools (Block, 1982). Validation studies are in fact comparison studies in which one method's validity is determined based on its agreement with a 2nd assessment method, considered to be more accurate (Rockettet al., 1997). For example, when validating a FFQ, the food record is considered to be the most ideal validation standard. However, when cooperation, motivation or literacy of the subjects is limited (Willet 1998, 131) or when subjects are of low-income status (Vucic et al. 2009), multiple 24-hour recalls may be the best alternative. Evaluating intake through a 5-step Multiple Pass Food Recall (MPR) technique has been shown to accurately assess energy and macronutrient intake (Conway et al., 2006) and to have stronger correlation with Doubly Labeled Water (DLW) than other dietary assessment methods (Blanton et al., 2006).

The validity of measurements of dietary intake in free living individuals is difficult to assess because all methods rely on information given by the subjects themselves, which may not be correct. In an attempt to determine objective measures of validating dietary assessments, the search has begun for objective measures using biological specimens that closely reflect food intake (Black et al., 1991). A biological marker for food intake is a

marker in any “specimen” that gives a predictive response to a given dietary component. “Specimen” in this context can be blood, urine, feces, hair, nails, etc… (Livingstone & Black, 2003). Biomarkers, such as urine samples, do not rely on self-reports of food intake and thus random measurement errors of the biomarker are not likely to be correlated with the errors of the dietary assessment method (McKeown et al., 2001). The underlying assumption of a biomarker applied to validate a measure of intake is that it responds to intake dose-dependently (Willett, 1998).However, all markers are not perfect. There are limitations, such as cost, availability, relevant time period, etc… (Gunnar, 2006).

Another important aspect to consider when using dietary assessment tools in research studies is reproducibility (also known as reliability or precision). This refers to the ability of the tool to reproduce the same measurements when administered to the same subjects within specific time frame at different time-points, keeping in mind that conditions of administration are never identical (Bountziouka&Panagiotakos, 2010; Willet 1998, 101).

D- Overview of Dietary Salt Intake in Various Parts of the World:

Unfavorably high sodium intakes remain prevalent in most parts of the world, and its sources vary largely between countries (Brown et al, 2009). The INTERSALT study provided standardized estimates of sodium intakes from 52 population samples in 32 countries based on data from 24-h urinary collection (INTERSALT Cooperative Research Group, 1988). As shown in figure 5, lowest values of sodium excretion were found among the Yanomamo Indians of Brazil: 0.8 mmol/d (18 mg/d) in men and 1.0 mmol/day (23 mg/d) in women. Three other remote population groups had 24-hour urinary sodium excretion at or below 60 mmol/d (1.38 g/d): Xingu Indians of Brazil, Papua New Guinea Highlanders and the Luo in rural Kenya. Highest values of urinary sodium excretion were recorded in Tianjin, China, and were 259 mmol/d (5.95 g/d) in men and 233 mmol/d (5.35 g/d) in women. Values over 200 mmol/d (4.6 g/d) in men were also found in Canada, Columbia, Hungary, Ladakh (India),

Bassiano (Italy), Poland, Portugal and the Republic of Korea (Elliott & Brown, 2006). The INTERMAP Study of macro- and micronutrients and blood pressure (Stamler, 2003a), as shown in figure 6, included men and women, aged 40–59 years, from 17 randomly selected population samples in China (3 samples), Japan (4 samples), United Kingdom (2 samples), and USA (8 samples). As in the INTERSALT study, the highest mean values of urinary sodium excretion were found in China, reaching as high as 299 mmol/d (6.88 g/d) in men and 253 mmol/d (5.82 g/d) in women in the Beijing sample, northern China. In the USA, mean 24-hour urinary sodium excretion for the eight samples were all in the range of 180–190 mmol/d (4.14–4.37 g/d) for men and 130–150 mmol/d (2.99–3.45 g/d) for women. For the four Japanese samples, mean 24-hour urinary sodium excretion ranged from 195–220 mmol/d (4.49–5.06 g/d) in men and 160–200 mmol/d (3.68–4.60 g/d) in women. Averaged 24-hour urinary excretion values for sodium in the two United Kingdom samples were 161 mmol/d (3.70 g/d) in men and 127 mmol/d (2.92 g/d) in women. Canada's assessment of sodium consumption has been based on its national dietary survey. A 2004 usual intake baseline was estimated from the Canadian Community Health Survey Cycle 2.2 (2004), developed at Iowa State University. The mean sodium consumption in Canada is estimated at 3100 mg/day, which does not take into account sodium added at table. The primary source of sodium information comes from the Canadian Nutrient File (CNF).

As for China, A nutrition survey in 2002 indicated that there had been no significant change in salt intake since 1982. However, the Chinese diet is traditionally high in salt.

More recently, Elliott & Brown (2006) suggested that most populations appear to have mean sodium intakes well in excess of 100 mmol/d (2.30 g/d), and in many (especially the Asian countries) in excess of 200 mmol/d (4.60 g/d). As shown in figure 7, sodium intakes in men are greater than those in women, most likely reflecting the higher food consumption (energy intake) among men. Sodium intake in adults appears to be slightly

lower above the age of 50 years than at younger ages. In 2010, global mean sodium intake was 3.95 g/day, which is nearly twice the WHO recommended limit of2 g/day and equivalent to 10.06 g/day of salt (Powles et al., 2010). As shown in figure 8, between 1990 and 2010 modest increases in sodium intakes were identified.

Independent of CVD risks, high dietary Na intake has been correlated with an increased risk for osteoporosis. Goulding (1930) suggested that Na intake could affect bone mass in animals. Breslau et al. (1937) concluded that sodium chloride increases urine calcium excretion and Wasler (1967) showed that Na and Ca competed for the same reabsorption mechanism in the proximal renal tubule. Urine calcium rises by from 0.5 to 1.5 mmol for every 100 mmol of Na ingested; hence, bone mass suffers if absorbed Ca is less than the amount needed to offset this loss (Nordin et al., 2014). The mechanism behind this bone resorption is that high dietary Na leads to an increase in the extracellular fluid volume, which results in an inhibition of Ca reabsorption in the distal renal tubule (Ginty et al., 2007). Therefore, calciuria is partly due to salt induced volume expansion leading to an increase in the glomerular filtration rate, as well as to the competition between Na and Ca ions (Heaney &Creighton, 2006). When urinary Ca excretion rises, serum Ca ion drops which signals an increase in serum parathyroid hormone release, activating renal synthesis of vitamin D and increasing bone resorption in order to normalize Ca ion serum levels (Shortt & Flynn, 2009).

Figure 5: Mean dietary intake of sodium according to the INTERSALT study:

	Salt Intake (Grams/day)	
Country	**Males**	**Females**
Yanomamo	0.2	0.2
Xingu Indians (Brazil), Papua (New Guinea) and Luo (rural Kenya)	1.1	1.1
Southern China	4	3.7
Japan	5.1	4.6
Canada, Colombia, Hungary, India, Italy, Poland, Portugal and South Korea	4.6	-

Adapted from: INTERSALT Results (1985-1987)

Figure 6: Mean dietary intake of sodium among participating men and women from four countries in the INTERMAP study:

	Salt Intake (g/day)	
Sample	**Male (40-59 years)**	**Female (40-59 years)**
United Kingdom	1.5986	1.6180
Southern China	1.3037	1.3175
Northern China	2.3115	2.3138
United States of America	1.6499	1.6778
Japan	2.2710	2.3613

Adapted from: Stamler et al, 2003

Figure 7: Mean dietary intake or urinary excretion of sodium for adults around the world; 1988–present:

Country (survey year)	**Reference**	**Age (year)**	**Measurement**	**Sample Number**	**Mean Sodium**
Australia (1995)	Beard et al., 1997	18–70	Single 24-h urine collection	87 men 107 women	3910 mg/d 2714 mg/d
Brazil (1999-2000)	Pavan et al., 1997	22–89	Unspecified dietary questionnaire	370 individuals	3937 mg/d
Canada (1999-2000)	Institute of Medicine 2004, citing Health Canada	≥19	Unspecified dietary method	18 214 individuals	3120 mg/d
China (1985-2000)	Liu et al., 2001	48-56	Single 24-h urine collection	775 Han 510 Uygur 204 Kazaks 125 Tibetans	4439 mg/d 3990.5 mg/d 4901.3 mg/d 5835.1 mg/d
France (NS)	du Cailar et al., 2004	14–40 41–70	Two 24-h urine collections	438individuals 417individuals	3312 mg/d 3381 mg/d
Italy (NS)	Pavan et al., 1997	22–89	Unspecified dietary questionnaire	370 men and women	4331 mg/d
Japan (1985-1994)	Kawamura et al., 1997	30–65	Single 24-h urine collection	132 men 70 women	5313 mg/d 4347 mg/d
Nigeria (1994)	Kaufman et al., 1996	>45	Single 24-h urine collection	144 men	2566.8 mg/d

Figure 8: Age- standardized estimated sodium intakes (g/day) in 1990 and2010; persons aged 20 and over in selected Arab countries:

Country	1990	2010
Lebanon	2.60 (2.34–2.88)	3.13 (2.78–3.54)
Bahrain	4.40 (4.03–4.82)	5.38 (4.85–5.91)
Syria	3.80 (3.22–4.46)	4.18 (3.53–4.92)
Algeria	3.91 (3.32–4.59)	4.28 (3.59–5.00
United Arab Emirates	3.40 (2.73–4.16)	3.67 (2.97–4.47)
Yemen	3.27 (2.76–3.86)	3.37 (2.82–4.05)
Saudi Arabia	2.98 (2.47–3.54)	3.20 (2.63–3.78)
Qatar	3.53 (2.87–4.28)	4.21 (3.34–5.17)
Morocco	3.96 (3.34–4.66)	4.31 (3.67–5.06)
Libya	3.74 (3.14–4.45)	4.24 (3.55–5.01)
Kuwait	3.66 (3.31–4.05)	3.88 (3.46–4.31)
Iraq	3.46 (2.94–4.09)	3.76 (3.19–4.46)
Egypt	3.63 (3.10–4.22)	3.68 (3.13–4.32)
Tunisia	4.12 (3.51–4.91)	4.43 (3.72–5.23)

Adapted from: Powles et al., 2013

E- Sources of Dietary Sodium

Sodium occurs naturally in most foods. The most common form of sodium is sodium chloride, which is table salt. Milk, beets, celery, and drinking water also naturally contain sodium, although the amount varies depending on the source. Sodium is also added to various food products. Some of these added forms are monosodium glutamate, sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate in addition to sodium chloride (salt) (Aronow et al, 2011). Salt may be added for flavor enhancement and to extend

the shelf life of certain foods. Various forms of sodium, including sodium chloride or salt, are used as preservatives to inhibit the growth of food-borne pathogens. Sodium is also used to bind ingredients, enhance color and serve as a stabilizer (American Heart Association, 2013).Salt is added to most canned foods, some frozen vegetables, fast foods, smoked and cured meats, and pickled foods. It is used in most cheeses, sauces, soups, salad dressings and many breakfast cereals. It is also found in many other ingredients used in food processing. Many commercially prepared condiments and seasonings are also high in sodium, which is why the food industry in developed countries is working to decrease their sodium content (Mahan et al, 2012).

Differences between developed and developing countries with respect to dietary sources of salt exist. In Europe and North America, a large proportion of the sodium ingested is added as sodium chloride in food manufacture and foods eaten away from home (James et al 1987; Mattes et al, 1991). Hence, sodium is usually “hidden” in foods and individuals are unaware of the amount of salt they consume (Laatikainen et al, 2006). Based on the National Food Survey Data for 2000, in the United Kingdom, cereals and cereal products including bread, breakfast cereals, biscuits, and cakes contribute about 38%of estimated total intake, followed by processed meat and meat products (21%), and other foods such as soups, pickles, sauces, and baked beans(13%). As for the United States, similar data is available. Bread, ready-to-eat cereal and cakes, cookies, quick breads, and doughnuts contribute up to 17% of sodium intake; ham, beef, poultry, sausage, and cold cuts about 13%, milk and cheese up to 9%; condiments, salad dressing, and mayonnaise about 5%; other foods including potato chips, popcorn, crackers, and pretzels, margarine, hotdogs, pickles, and bacon a further 23-25% (Cotton et al, 2004). Sodium is also a major interest for the Canadian population. According to the Canadian Community Health Survey (2012), the major contributors to dietary sodium intake are commercially prepared foods, and the main food group contributing

to high dietary sodium intake is bread, which include all commercial breads, muffins, buns, biscuits, rolls, and similar bread products (14%) (Sodium Working Group, 2010). As for the Asian countries, a different picture with respect to dietary sources of sodium is apparent. According to data from the 2002 Chinese Health and Nutrition Survey, a large proportion of sodium in the diet comes from sodium added in cooking (75%), and from other sources, including soy sauce (8%) (Zhao et al, 2004). In Japan, the main sources were soy sauce, fish and other sea food, soups and vegetables (66% in total) with a further 10% being contributed by salt added during cooking (Campbell et al, 2006). The situation of the Middle East and North Africa (MENA) region is of special concern because of the rapid nutrition transition and very high prevalence rates of non-communicable diseases (Fahed et al., 2012). However, limited data exist on dietary salt intake in countries of the region (Kelishadi et al., 2012). A study done on young Iranian children revealed large amount of sodium consumption; the mean ± SD of urinary Na was 4,075 ± 660 mg/day without significant difference according to gender and living area (Kelishadi et al., 2012). Based on a national study in Lebanon in 2008-2009, the mean sodium intake amongst adults is estimated at 2870 mg/day, where 59.7% of the population is consuming above the WHO recommendation (2000 mg/day). The main food contributors being bread products (25%), processed meats (12%), followed by cheese and labneh (10%) (Sibai et al., 2008, unpublished data).

CHAPTER III

METHODS

A. Study Design

This study aimed at developing and validating a Food Frequency Questionnaire (FFQ) for the assessment of dietary sodium intake in Lebanese adults. In a cross-sectional design, the FFQ was validated against urinary sodium excretion. In addition, dietary sodium estimates provided by the FFQ were compared to those provided by repeated 24 hour dietary recalls.

B. Study Participants

A convenience sample of 100 healthy male and female individuals was recruited. A sample of the order of 100 subjects would be 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).

In order to be eligible to participate, individuals must be aged between 19 and 55 years. Individuals with reported kidney disease or Diabetes were excluded from the study.

C. Ethics Approval

The study was reviewed and granted approval by the Institutional Review Board (IRB) of the Social and Behavioral Sciences at the American University of Beirut (AUB). All documents used in data collection (both English and Arabic versions) were approved by the IRB. Written consent was obtained from participants, prior to their enrollment in the study.

All participants were interviewed at the Department of Nutrition and Food Sciences, American University of Beirut.

D. Development of the FFQ

A 47-item semi-quantitative questionnaire was developed for the purpose of the study.

1. The Food List

For a food item to be included in the FFQ, it must fulfill the following 3 general characteristics:

- It has to be used reasonably, with sufficient frequency, by an appreciable number of individuals in the studied population
- It has to have substantial content of the nutrients or food groups under study
- The use of the food must vary from one person to another, thus showing discrimination in dietary intake (Willett 1998, 76).

In the present study, the development of the FFQ food list was based on dietary data that was collected as part of the national survey of household living conditions which was conducted on nationally representative sample of Lebanese adults (n= 2048 adults) in 2008-2009 (Lebanese Ministry of Social Affairs, 2004). During this survey, a single 24-hour dietary recall was obtained from each participant. The data provided by this 24-hour dietary recall was used in the present study in order to identify the food items that are the biggest contributors to sodium intake among Lebanese adults and which should consequently be included in the FFQ. 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). In addition, closely related food items were clustered together. The final FFQ food list amounted to a total of 47 food items categorized into 8 food groups (Appendix 1):

Breads and Starches – Milk and Dairy Products – Meats, Poultry, Fish, and Eggs – Vegetables and Vegetables based dishes – Legumes – Salty Snacks – Sweets – Soups, Condiments, and Sauce.

2. Portion Size

A reference portion, expressed in household measures or grams, was specified for each food item included in the FFQ (Nasreddine et al, 2006).

3. Frequency Response

As per the approach recommended by Block et al (1986), the FFQ was based on an open-ended format to assess the frequency of consumption of food items per day, week or month. This format may provide enhanced precision in reporting, given that the frequency is continuous, rather than categorical (Block *et al*., 1986).

E. Data Collection

1.Dietary Intake Data

The study subjects were enrolled in the study for a period of 4 weeks. The developed FFQ was administered in a face to face interview. In order to assist subjects in portion size estimation and in order to decrease inter-subject variability in serving size assessment, a two-dimensional food portion visual chart was used (Two-dimensional food portion visual chart). This chart has been developed by Nutrition Consulting Enterprises and validated for use in adult men and women aged 20 to 70+ years as part of the Framingham Heart Study (Posner *et*

al., 1992). In addition, common household measurements (such as teaspoons, tablespoons and cups) as well photographs of common food portions for pre-packed/Lebanese food items (such as Arabic breads, kaak, burger bun, yogurt packs, wrapped processed cheeses, chocolate bars, and chips bags) were also used.

During the same interview, a 24 hour dietary recall was also administered using the USDA Multiple Pass Food Recall (MPR) approach (USDA Food Surveys, 2013). Two additional 24-hour recalls were collected from study participants, 2 and 4 days after the initial interview.

F. Anthropometric Measurements

After explaining the study, its protocol as well as signing the consent form, anthropometric measurements were obtained from study participants. Height and weight were measured in light clothing without shoes, using standardized techniques and calibrated equipment. Body Mass Index (BMI, in$\frac{\mathrm{kg}}{\mathrm{m}^2}$) was calculated.

G. Collection of Urine Samples

Subjects were asked to provide spot urine samples (particularly the second morning voiding) as recommended by Kawasaki *et 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 collected at the Department of Nutrition and Food Sciences at the Faculty of Agriculture and Food Sciences at the American University of Beirut and frozen without preservative at –20°C.Urinary sodium was measured by ion-selective electrodes (ISE) on the Cobas 6000 instrument at AUB-MC. Urinary creatinine was also measured at AUB-MC by the enzymatic method using Cobas 6000 instrument and Roche CREP-2 products.

H. Assessment of dietary sodium intake:

1. Dietary Na estimates based on the FFQ

For each of the FFQ food items, absolute amounts of Na per serving size were determined based on the "Food Composition Tables for Use in the Middle East" (Pellet and Shadarevian, 1970) and the Nutritionist Pro software, version 1.2., which allows for the addition of recipes for Lebanese traditional food items. Individual sodium intake ($In_{FFQ:}$ mg/day) was computed by summation of the respective products of the quantity consumed (grams/day) and the sodium per gram value for each food item.

2. Dietary Na estimates based on repeated 24-hour recalls

Based on the data provided by the 3 dietary recall, the subject's average daily dietary sodium intake (In_{recall}) was calculated using the Nutritionist Pro software, version 1.2.

3. Dietary Na estimates based on Na urinary excretion

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) was used. This method required 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 is based on the application of 2 consecutive steps using 2 consecutive equations:

Step 1:
Adult 24 hour urine creatinine excretion (24HUcrV) can be 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 24h UcrV will allow 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 √XNa
Where XNa = SUNa/SUcr x predicted 24HUcrV.

SUNa = spot urinary sodium concentrations
SUcr = spot urinary creatinine concentrations

It is important to note that 24-hour urinary sodium excretion is 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)

I. Reliabilty of the FFQ

To assess the reliability of the FFQ in assessing sodium intake, the FFQ was re-administered one month after the initial interview (first FFQ = FFQ-1, second FFQ = FFQ-2). Test-retest correlations were in fact shown to be higher when the time interval between the administrations is less than one month versus 6 or more months (Cade *et al*., 2002). Subjects were encouraged to maintain their regular dietary intake and dietary advice from the research dietitian was not provided.

J. Statistical Analysis

Analysis was done using Statistical Package for Social Sciences 19.0 (SPSS for Windows, Version 19.0, Chicago: SPSS Inc.).

Means and standard deviation were calculated for all continuous variables in the anthropometric questionnaire. Categorical variables were taken as frequencies. Validity and reliability statistics were performed on total number of portions per food groups/day.

1. Validity Statistics

i. Mean Difference and Correlation Coefficients

Mean of FFQ-1, of FFQ-2, of FFQ-Average, of the 3 24-hr recalls, and of the Urine Kawasaki were calculated. Mean differences were also calculated as was the percent mean difference.

Although it is useful to compare means for the above methods, it is also important to provide data on the associations between the intakes measured by these methods (Willet 1998, 120). Therefore, Spearman's correlation coefficient and agreement between FFQs and 24-hr recalls, FFQs and Urine Kawasaki, FFQ-1 and FFQ-2, 24-hr recalls and Urine Kawasaki were performed to validate this FFQ. Spearman's correlation coefficients were computed for food group estimates between the methods. A realistic correlation that might be accepted for validity ranges between 0.5-0.7 with an acceptable value of at least 0.4. Correlation coefficients below 0.4would seriously attenuate levels of validity (Willett 1998, 132). Correlation coefficients measure the strength of relation between the methods but not the agreement (Bland &Altman, 1986). Thus, the Bland-Altman statistical method was also administered.

ii. Bland-Altman Method

In order to compare a new measurement technique with an established one, it is necessary to measure the agreement between the methods across the range of intakes (Bland & Altman, 1986). In a study conducted by Serra-Majem *et. al* (2010) on evaluating the quality of dietary intake validation studies, Bland-Altman statistical method earned the study an extra score of evaluation given that it analyzes the standard deviation of the difference between FFQs and 24-hr recalls, especially that it was not influenced by between-person variation (Bland & Altman, 1999). In addition, in a review of 210 agreement studies, 85 % of

the studies adopted the Bland-Altman method as an agreement measure (Zaki*et al*., 2012). The Bland-Altman method used the differences between observations on the same subject and graphically represented them against the average of both methods per subject. The use of the mean of the differences between the methods is able to estimate the existence of bias of one method relative to the other and the use of the standard deviation of the differences can estimate the variability of these differences. The methods have sufficient agreement when the differences are close to zero, thus lying in a narrow band around zero (Bland & Altman, 1986). The use of the Limits of Agreement (LoA) enabled to asses to what extent the methods agree/disagree. If the limits are wide, then an overall bias may be present (Bountziouka&Panagiotakos, 2010). The limits of agreement (LoA) are 2 values that 95% of the differences should lie between.

LoAs are calculated as $mean\ difference - 1.96 \times standard\ deviation$ and $mean\ difference + 1.96 \times standard\ deviation$. 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 less than 0.05 were considered statistically significant.

2. Reproducibility Statistics

i. Intraclass Correlation Coefficient

The Intraclass Correlation Coefficient (ICC) is used to describe how strongly units in the same group resemble each other. It differs than other correlation coefficients in that it analyses data structured as groups, rather than paired ones. ICC estimates the differences within the subjects and not those among the observers (Bountziouka & Panagiotakos, 2010). However, the ICC ignores ordering variances and rather treats the two administered FFQs as random instruments not specific methods (Zaki *et al.* 2012). Therefore, weighted

kappa statistic was administered.

ii. Kappa Test

The Kappa test is used to measure the agreement between 2 or more observers in assigning the data into different categories. In this study, the Weighted Kappa (κ w) was used instead of Kappa in order to take into consideration how close and how different the ratings of the FFQs, 24-hr recalls, and Urine Kawasaki methods were. In this study, Kw was used to evaluate how well the FFQ can categorize individuals into tertiles of the food portion and food percentage intake when compared with the MPRs, and urine Kawasaki categorization, which is considered to allocate individuals in a "golder" manner (Bountziouka & Panagiotakos, 2010).Kappa values were categorized for strength of agreement as suggested by Landis& Koch (1977): Values of Kappa from 0.40 to 0.59 are considered moderate, 0.60 to 0.79 substantial and 0.8 outstanding.

Percent agreement was calculated as tertiles:

[Number of subjects in quartile 1 + number of subjects in quartile 2 + number of subjects in quartile 3] / n=87

K. Methodological Choices

1. Use of a FFQ

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 a range of 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). FFQs rely on recall from 'generic' memory, which may be more easily recalled than 'episodic' memory, but because of the importance of cultural sensitivity, all FFQs require some adjustments and validation when used for a select cultural group (Coultson M, 2008). 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 population (Charlton et al., 2008). Unlike other methods, the FFQ can be used to circumvent recent changes in diet by obtaining information about individuals' diet as recalled about a prior time period and because the costs of data collection, processing, and the respondent burden have traditionally been much lower for FFQs, they have been adopted as a common way to estimate usual dietary intake in epidemiological studies (Thompson & Subar., 2008).

2. Use of a biochemical marker (urinary Na excretion sample) as gold standard

One spot urine sample was taken from every subject during the first interview and analyzed for sodium intake according to the Kawasaki equation (Kawasaki et al., 1993). Because the random measurement errors of urinary sodium are unlikely to be correlated with random errors of the dietary assessment methods, this biomarker was used to independently determine the validity of the test method (McKeown et al., 2001).Biochemical indicators of dietary intake have great intuitive appeal as the gold standard with which to assess the validity of dietary questionnaires. The fundamental advantage of using a biochemical indicator is that measurement errors are essentially uncorrelated with errors in dietary questionnaires (Yamamoto et al., 2001).

3. Use of multiple pass food recall

In this study, sodium estimates provided by the FFQ were additionally compared to those provided by the Multiple Pass Food Recall (MPR). MPRs have been shown to accurately assess energy and macronutrient intake (Conway et al. 2006) in adults. Findings from Rhodes et al. (2013) study in the U.S. population suggested that the USDA MPR is a valid measure for estimating Na intake in adults at the population or group level; the mean reporting accuracy was 0.93 for men and 0.9 for women. Another study by Conway et al. (2003) concluded that the USDA 5-step multiple-pass method effectively assessed mean nutrient and energy intake within 10% of mean actual intake on the previous day.

L. Reproducibility of the FFQ

FFQs can reflect the past month, past few months or the past year depending on the researcher's line of interest (Willett 1998, 82). When comparing FFQ recalls for the past year intake, Fraser *et al.* (1998) found that FFQs asking about intake over the previous months had slightly higher correlations. For the purpose of this study, a time frame of 1 month was chosen to be less burdensome on the subject as well as to eliminate any true changes in intake (Brown &Ogden, 2004).The reproducibility of the FFQ was assessed 4 weeks apart since it is the period of time that is found to be not too early for subjects to recall their FFQ-1 answers and at the same time, not too distant so that it reflects variation in response and true change in the subject's dietary intake (Willett 1998, 105).

CHAPTER IV

RESULTS

A. Descriptive Characteristics of Study Participants

Eighty-seven subjects (37 males; 50 females) completed the study. Table 1 shows the characteristics of the study population. According to gender, mean BMI, height, weight, and creatinine were significantly higher in men compared to women. The mean age was 26.84 ± 7.75 years with no significant difference between genders. Table 2 illustrates sodium intake as calculated by FFQ1, FFQ2, sodium urinary excretion, and 24 hour recall (average of the 3 MPRs). Mean sodium intakes were found to be significantly higher in men compared to women using all the methods under investigation. The percentage of individuals consuming sodium at a level exceeding the WHO limit (2000 mg/d) is shown in table 3. The majority of the study population was found to exceed the WHO upper limit for Na intake. Based on the FFQ data, the proportion of males exceeding the WHO upper limit was found to be significantly higher compared to females.

Table1. Characteristics of the study population by gender:

	Total (n=87)	**Male (n=37)**	**Female (n=50)**	**p-value**
	Mean ± SD			
Age (years)	26.84 ± 7.75	26.19 ± 6.46	26.80 ± 7.90	0.697
BMI (kg/m$^{2)}$	23.31 ± 3.47	24.98 ± 3.15	21.97 ± 3.05	0.000
Height (m)	1.71 ± 0.090	1.79 ± 0.07	1.66 ± 0.06	0.000
Weight (kg)	68.94 ± 14.74	79.79±12.22	60.70 ± 10.46	0.000
Creatinine (mg/L)	1678.55±1016.16	2062.73±1158.47	1396.57±814.02	0.04
Urinary Sodium (mmol/L)	142.17±62.50	154.81±64.11	132.88±60.93	0.114

Table 2. Sodium intake (mg/d) as estimated by the FFQ, repeated 24-hr recalls, and urinary sodium excretion in a sample of Lebanese adults (n=87):

Methods	Total (n=87)	Male (n=37)	Female (n=50)	p-value
	Mean ± SD			
FFQ 1 [a]	4791.39±2087.72	5511.86±2164.87	4246.47±1892.81	0.006
FFQ 2 [b]	4448.41±1963.85	4962.33±1821.88	3931.40±1722.16	0.010
Average FFQ	4618.26±1898.02	5237.09±1894.06	4091.26±1690.27	0.005
Urinary Excretion (Kawasaki)[c]	4573.20±1372.69	5014.44±1456.71	4255.52±1243.13	0.014
24h-recall [d]	3395.04±1282.90	3786.11±1387.24	3124.10±1143.77	0.022

a: Food Frequency Questionnaire administered at first interview
b: Food Frequency Questionnaire administered one month after the first interview
c: Na Urinary Excretion using the Kawasaki Equation (Kawasaki et al., 1991)
d: Average of 3 repeated 24-hour recalls

Table 3. Percentage of individuals exceeding the WHO limit for sodium consumption (2000mg/d) based on the FFQ, repeated 24-hr recall, & urinary sodium excretion:

	Total (n=87)	Male (n=37)	Female (n=50)	p-value
	(%)			
FFQ1[a]	87.1	94.3	82.0	0.074
FFQ2[b]	87.1	97.1	80.0	0.009
Average FFQ	87.1	97.1	80.0	0.009
Urinary Excretion (Kawasaki)[c]	70.2	79.4	64.0	0.121
24-hr Recall [d]	78.6	82.9	75.5	0.415

a: Food Frequency Questionnaire administered at first interview
b: Food Frequency Questionnaire administered one month after the first interview
c: Na Urinary Excretion using the Kawasaki Equation (Kawasaki et al., 1991)
d: Average of 3 repeated 24-hour recalls

B. Validity of the Questionnaire

Mean difference and percent mean difference were calculated for the FFQs vs. urinary excretion, and FFQs vs. repeated 24-hr recalls, as shown in Table 4. Significant differences between total means of all methods were observed, except when comparing

FFQ1 to urinary excretion (0.365). As shown in Table 4, the lowest mean difference was observed between average FFQ and urinary Na excretion (0.98%; 45.06 mg/d). The largest mean difference and the largest percent mean difference were obtained when comparing FFQs to repeated 24-hour recalls.

Table 4. Mean Difference and Percent mean difference in Na intake as estimated by FFQ, repeated 24-hr recall, & urinary excretion in the study population (n=87):

Methods	Mean difference ± S.D	Percent mean difference (%)	p-value
FFQ1 vs Urinary Excretion [e]	218.19 ± 2236.68	4.77	0.365
FFQ 2 vs Urinary Excretion [*e]	-128.26 ± 2150.48	-2.80	0.000
FFQ_Avg vs Urinary Excretion *[e]	45.06 ± 2074.50	0.98	0.000
FFQ1 vs Repeated 24-hr recall *[f]	1403.66 ± 1844.26	41.34	0.000
FFQ2 vs Repeated 24-hr recall *[f]	1053.37 ± 1871.34	31.03	0.000
FFQ_Avg vs Repeated 24-hr recall *[f]	1228.52 ± 1721.36	36.19	0.000

a: Food Frequency Questionnaire administered at first interview
b: Food Frequency Questionnaire administered at second interview
c: Na Urinary Excretion using the Kawasaki Equation (Kawasaki et al., 1991)
d: Average of 3 repeated 24-hour recalls
e: Percent mean difference = (FFQ1 –Urine)/ Urine *100
f: Percent mean difference = (FFQ1- Recall)/ Recall*100
*: Significant difference between means

For each method, consistency of agreement was examined across the range of Na intake. This was achieved by estimating the regression slope of differences (β) between the FFQ, mean 24-hour dietary recall, and spot urine sample.

The Bland and Altman approach was used to compare the FFQ against the reference methods; urinary Na excretion and 24-hour recall. Based on this approach, the difference between 2 methods in measuring Na intake can be evaluated by plotting this difference against the average value of the 2 methods. Accordingly, the difference in dietary Na obtained by urinary Na excretion and 24-hour recall and by the FFQ under investigation was

compared with the average value of Na intake estimates provided by each of the methods under consideration. This difference was plotted and analyzed using simple linear regression. The hypothesis that the slope was equal to zero was tested in each case. This is equivalent to testing whether the 2 methods have the same error variance. Mean difference, and limits of agreement between the various methods were determined. The wider the limit of agreements relative to the mean value is, the lower the level of agreement between the methods. A one-sample Student's t test was performed to assess 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 FFQs (FFQ1 & FFQ2) versus 24-hour recall were significantly different from zero, indicating that the FFQ and the 24-hour dietary recall are not interchangeable. Mean difference between FFQs and urinary excretion was not significantly different than zero, indicating that the two methods are compatible. However, the slopes for the FFQ1, FFQ2, and FFQ average were positive and significantly different from zero. This indicates that the FFQ tend to overestimate Na intake particularly at high levels of intake. A positive slope also indicates that at high levels of intake, there is a poor agreement between the FFQ, 24-hour dietary recall, and urinary excretion.

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 5. The widest limits of agreement were observed for FFQ1 versus urinary excretion. This suggests that the low correlation obtained between the FFQ and urinary excretion, even though the mean difference was lowest and not significantly different than zero, is probably due to the presence of outliers and extreme values (Osborne & Overbay, 2004).

Table 5. Differences between each of the methods for estimating Na intake based on the Bland Altman approach:

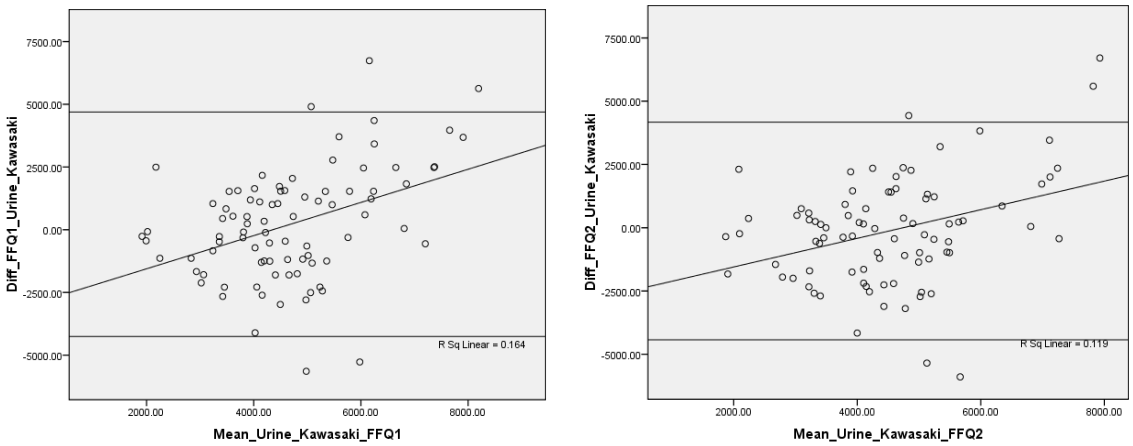
Difference	Mean	Standard	Limits of	Slope [b]	p-value [c]

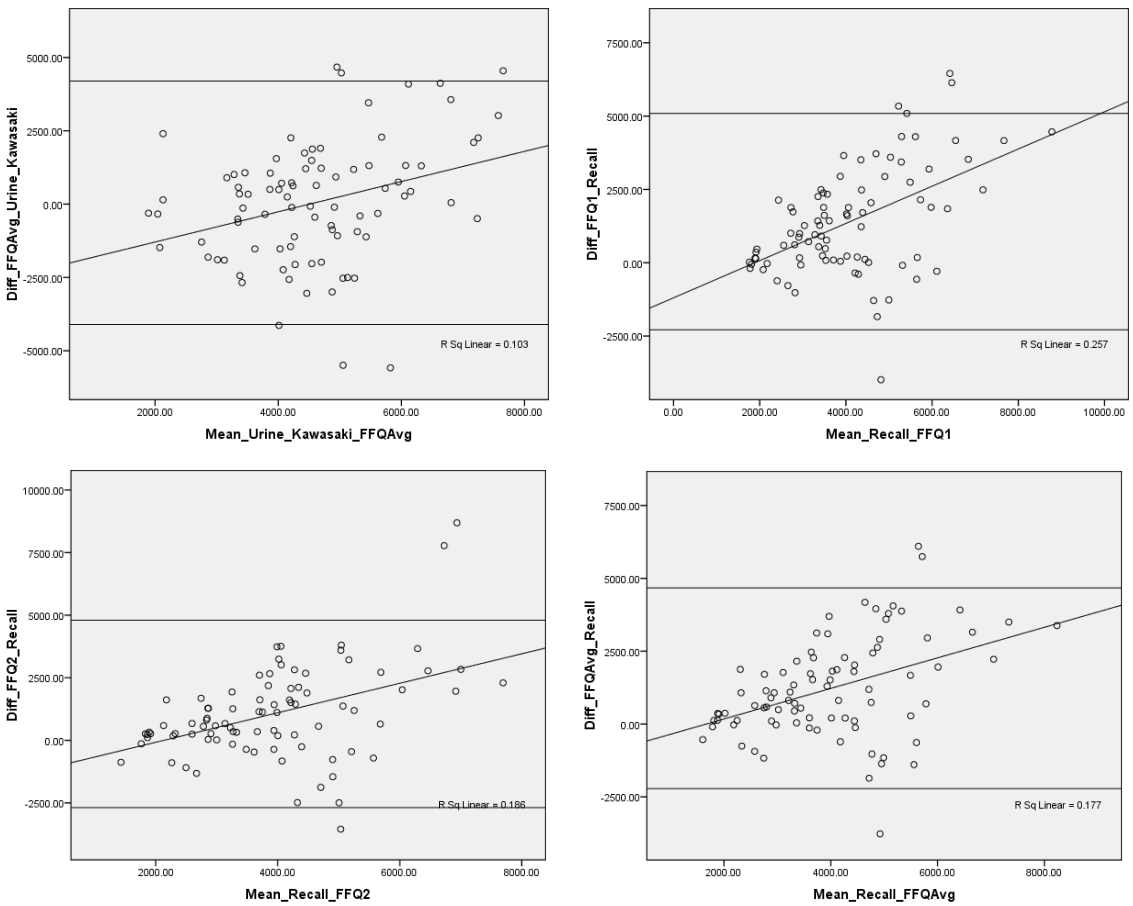
	Difference	Deviation	Agreement [a]		
FFQ1 vs Urinary Na Excretion	218.19	2236.68	(-4255.16 ; 4691.54)	0.661	0.000
FFQ2 vs Urinary Na Excretion	-128.26	2150.48	(-4429.22; 4172.71)	0.566	0.001
FFQAvg vs Urinary Na Excretion	45.06	2074.50	(-4103.95; 4194.06)	0.515	0.002
FFQ1 vs 24-hr recall [d]	1403.66*	1844.26	(-2284.85; 5092.18)	0.634	0.000
FFQ2 vs 24-hr recall [d]	1053.37*	1871.34	(-2689.31; 4796.06)	0.589	0.000
FFQAvg vs 24-hr recall [d]	1228.52*	1721.36	(-2214.20; 4671.23)	0.524	0.000

*: 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: Statistical significance of β
d: Average of 3 repeated 24 hour recalls

The Bland-Altman plots (Figure 11) graphically represent the difference between the FFQ and the other 2 methods (24-hour dietary recall and urinary Na excretion) and the LoA in assessing Na intake, thus showing any extreme or outlying observations. For most methods, the regression line was positive, indicating that the FFQ tended to overestimate the intake when compared to the 24-hour dietary recall and urinary Na excretion.

Figure 9. Bland Altman charts for Na intake as predicted by the FFQ, the mean MPRs, and urinary excretion:





Spearman correlation coefficients between Na intake estimates derived from the FFQ, 24-hour dietary recall, and urinary Na excretion were computed (Table 6). Spearman coefficients were all statistically significant. The values ranged between 0.215 for FFQ1 & urinary excretion, and 0.549 for FFQ1 & repeated 24-hr recall.

Table 6. Association between Na intake estimates derived by FFQ and Na intakes derived by 24-hour dietary recall and urinary Na excretion as examined by Spearman and ICC:

	Spearman	p-value	ICC	p-value
FFQ1 vs Urinary Na Excretion	0.215	0.046	0.332	0.032
FFQ2 vs Urinary Na Excretion	0.213	0.049	0.332	0.033
FFQ Avg vs Urinary Na Excretion	0.224	0.037	0.357	0.022
FFQ1 vs 24-hr recall [a]	0.549	0.00	0.498	0.000
FFQ2 vs 24-hr recall [a]	0.484	0.00	0.467	0.000
FFQ Avg vs 24-hr recall [a]	0.526	0.00	0.512	0.000

a: Average of 3 repeated 24 hour recalls

C. Association of Na intake estimates derived by the FFQ, 24-hour dietary recall, and urinary Na excretion

Intraclass correlation coefficients were calculated between Na intake estimates derived from the FFQ, urinary excretion, and repeated 24-hr recalls, as shown previously in Table 6. The ICC was significant for all methods, ranging from 0.332 for FFQ1 vs. urinary excretion to 0.512 for FFQ Average vs. repeated 24-hr recall**.**

Kappa agreement between the different methods (Table 6) was computed to compare classification of data for Na consumption into tertiles. Percent agreement in the same tertiles was also calculated.

The percent agreement ranked highest for 24-hour recall vs FFQ1. The percent of individuals that were correctly classified into the same tertile, was also highest for the same methods stated above (52.33%).

Table 7. Agreement in classification of Na intake in tertiles:

	Standard Error	Confidence Interval*	% Agreement
Urinary Na Excretion vs.FFQ 1	0.084	-0.04 ;0.28	37.93
Urinary Na Excretion vs FFQ2	0.086	-0.02 ;0.31	41.38
Urinary Na Excretion vs FFQ_Avg	0.086	-0.02 ;0.31	41.38
24-hr recall [a] vs FFQ1	0.079	0.23 ;0.54	52.33
24-hr recall [a] vs FFQ2	0.085	0.10 ;0.43	47.67
24-hr recall [a] vs FFQAvg	0.08	0.17 ;0.49	48.83

*:Kappa value +/- 1.96 (Standard Error)
a: Average of 3 repeated 24-hour recalls

Agreement in the classification of Na intakes above or below the WHO limit (2000 mg/d) is shown in Table 7. The percent agreement, the values were between 76.19% for urinary excretion vs. repeated 24-hr recall and 92.94% for repeated 24-hr recall vs. FFQ1.

Table 8. Agreement in classification of Na intake above or below the WHO limits (2000 mg):

	Standard Error	Confidence Interval*	% Agreement
Urinary Na Excretion vs FFQ1	0.12	0.01; 0.50	81.18
Urinary Na Excretion vs FFQ2	0.12	0; 0.44	78.82
Urinary Na Excretion vs FFQ_Avg	0.1	0; 0.38	80
24-hr recall [a] vs FFQ1	0.15	0.29; 0.88	92.94
24-hr recall [a] vs FFQ2	0.15	0.25; 0.84	91.76
24-hr recall [a] vs FFQ_Avg	0.16	0.18; 0.81	91.76

*: Kappa value +/- 1.96 (Standard Error)
a: Average of 3 repeated 24-hour recalls

D. Reproducibility of the questionnaire (Comparison between FFQ1 & FFQ2)

All previous analysis was also performed to compare FFQ1 to FFQ2 in order to determine its reliability (table 8). The mean difference was statistically different than zero, as examined by the t-test. Percent mean difference between the FFQs was positive (7.87%). As for Spearman and intraclass correlation a value of 0.866 and 0.860 respectively were shown and found to be significant. The limits of agreement between both FFQs were narrow, suggesting that 95% of the difference between FFQ1 & FFQ2 lies between -2445.5 & 3146.08. As for the agreement between the FFQ in classifying subjects into tertiles of Na intake (Table 9), the kappa value was high (0.689), “substantial agreement”. It was of 0.5343, “moderate agreement” when looking at the classification above or below the WHO limit (2000 mg/d).

Table 9. Analysis of the comparison between FFQ1 & FFQ2

	FFQ1 vs. FFQ2	p-value
Spearman	0.866	0.00

ICC	0.86	0.000

Table 10. Weighted kappa analysis between FFQ1 and FFQ2

	Kappa*	**Standard Error**	**Confidence Interval**	**% Agreement**	**Rank**
FFQ1 vs. FFQ2 according to tertiles	0.689	0.059	(0.57; 0.80)	73.56	Substantial Agreement
FFQ1 vs. FFQ2 according to WHO limit	0.5343	0.17	(0.21; 0.86)	93.02	Moderate Agreement

*: Kappa value +/- 1.96 (Standard Error)

CHAPTER V

DISCUSSION

Dietary assessment of sodium has recently gained a lot of attention given that sodium intake has been associated with increased risk of cardiovascular diseases (WHO, 2007). Evidence shows a direct relationship between sodium intake and hypertension; blood pressure rises with increased sodium intake in the general population (Sodium Reduction Strategy for Canada) (Ritz, 2010, Savica et al., 2010), and salt intake has been shown to correlate directly with blood pressure in different population groups (CD frost et al.1991, Savica et al., 2010; Brook et al., 2001; Sharma, 2002; Appel et al., 2003). 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 (Dietary reference intakes for water, potassium, sodium, chloride and sulfate. The national academies press 2004). Available studies suggest that sodium intakes around the world are well in excess of physiological need (i.e. 230-460 mg/day) (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 lack of valid 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. 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). The use of FFQs for the assessment of dietary sodium has been proposed in the literature, but none has yet been developed and validated for the Lebanese population (Charlton et al., 2008).

A. Major Findings of the Study

In the present study, the reliability and validity of a short FFQ was evaluated for the assessment of dietary Na intake against urinary Na excretion and 3 24-hour dietary recall in a sample of Lebanese adults.

Our findings indicate that the developed FFQ is reliable with acceptable validity in assessing dietary Na intake. The results of this study are similar to previous Na-based FFQ validation studies in adults (Charlton et al., 2008; Na & Lee, 2012).

B. Findings on Relative Validity of the FFQ

To be able to detect associations between diet and disease, correlations coefficients of the FFQ when validated against the 3 MPRs need to be at least 0.3-0.4 (Cade et al., 2002). In this study, the Spearman correlation coefficient between dietary Na intakes estimated by the FFQ and dietary Na intakes estimated by the 24-hour dietary recall was of 0.549, which is lower but comparable to 0.683, which is the correlation obtained in the study of Charlton et al. (2008) amongst South Africans. A spearman coefficient of 0.598 was found between a FFQ and a 3 day food record to assess Na intake in Korean adults, which is also similar to the results in this study (Na & Lee, 2012). Although the correlation between the FFQ and 24-hour dietary recall was significant, the mean difference was highest and significantly different from zero. This suggests the presence of a correlation of error, probably due to the fact that both dietary assessment methods are retrospective, hence relying on the subject's mem-ory.

As For urinary Na excretion, the association with the FFQ reported by Spearman was of 0.224, which is slightly higher than the one reported by Charlton et al. (2008) by repeated 24-hour urinary Na excretion.

In this study, the mean of ICC obtained for Na intake was 0.479 (range: 0.332 – 0.86). ICC is sensitive to the amount variability between subjects in the study, and ranges from 0 to +1, with values close to +1 indicating that there is no variance due to individual's replies (Shrout & Fleiss, 1979). Hence, high levels of ICC suggest a reliable tool (Bountziouka & Panagiotakos, 2010).

Mean difference between the FFQ and urinary Na excretion was not significantly different than zero indicating good agreement (Bland & Altman, 1999). However, the slope was positive and significantly different than zero indicating a tendency for the FFQ to overestimate intake at higher levels of Na dietary intake.

C .Findings on the Reproducibility of the FFQ

In order to evaluate reliability, the FFQ was administered twice, one month apart. As per Cade *et al*. (2002) and Willet (1998, 110), a reliability coefficient ranging between 0.5 and 0.7 is considered acceptable. In similar validation studies, both Spearman and ICC correlations were used to assess reliability between the 2 FFQs. In this study, the spearman correlation between FFQ1 & FFQ2 was calculated to be 0.866. However, given that Spearman correlations consider only correlations or 'relative positions' of ratings whereas ICC correlations take into account the agreement and differences in ratings in addition to correlations between raters, ICC is considered a more appropriate reproducibility test (Shrout & Fleiss, 1979). In this study, the ICC obtained between FFQ1 and FFQ2 was 0.86, indicating a high level of reproducibility, since an ICC approximately close to 0.5 is the minimum correlation for an assessment tool to be considered reliable (Cade *et al.*, 2002).

Moreover, the weighted kappa statistic was used in evaluating the reliability of this FFQ as it could represent the agreement in classification of tertiles of Na intakes between the 2 FFQs. A kappa value of 0.689 indicating a substantial agreement between FFQ1 and FFQ2 was documented. As for this FFQ's ability to consistently classify individuals, on average, it was able to classify 73.56% of the subjects into the same tertiles as the second FFQ. This indicates that this FFQ can be also used to properly rank individuals based on their Na intake. Therefore, this FFQ had good reliability when measuring Na intake.

D. Comparison of Intake with Upper Limit

When comparing Na intake estimates derived from the 3 methods under investigation with the dietary guidelines provided by the WHO for adults between the ages of 19 and 55 years, our study sample exceeded the recommendations of Na, where 87.1% of the study population exceeded the WHO limit according to the FFQ, 70.2% according to the urinary Na excretion, and 78.6% according to the repeated 24-hr recalls. A bigger percentage of male exceeded the WHO limit, when compared to females, which is similar to other studies where the Na intake in men seems to be higher than that of women (Sasaki et al., 1998; Pavadhgul et al., 2009). According to our study, sodium intake (mg/d) in Lebanese adults is well in excess of the recommended 2000 mg/day, ranging from 3395.04 mg/day according to the repeated 24-hr recalls to reach 4618.26 mg/day according to the FFQ, which is close to the value obtained from Powles et al. (2010), of 3130 mg of Na per day.

‘

E. Strengths, Limitations and Potential Biases

A major strength of the study is tailoring this FFQ specifically to Lebanese population and their food culture. Because the research team developed this FFQ by reviewing data that was collected between 2008 and 2009 as part of the national dietary and anthropometric

survey, and by choosing the food items that are the biggest contributors to Na intake, this aided in directly targeting what Lebanese adults are eating such that the list was inclusive of all their particular dietary habits. Both the frequency section and the portion section in the FFQ were designed to minimize any burden on the participants. The assessment of portion size relied on standard portion sizes, portion sizes commonly used in the Lebanese market and households as well as the 2-D visual poster (Nutrition Consulting Enterprises, Framingham, MA). This may have helped in decreasing errors pertaining to perception and conceptualization of portion intakes.

Furthermore, data collection was conducted by a research nutritionist, trained to follow the same protocol in the interview-administered FFQs, increasing uniformity between the 2 questionnaires (Block et al., 2006). The nutritionist was also trained to follow the 5 steps in multiple pass recalls, increasing accuracy in food recalling (Raper et al., 2004). The research team avoided collecting data on days on which subjects changed their regular dietary habits, such as Ramadan, Christmas, Lent, and official holidays. In addition, the research team controlled the data collection of the 3 MPRs by ensuring that the days were considered as 'regular intakes' of the subjects, and that the 3 MPRs represented 2 weekdays and 1 weekend day.

This study has possible limitations to consider such as relying on 24-hour recalls instead of dietary records (DR) as means of validation. However, even though DRs are considered the gold standard in FFQ validation studies especially that they have the fewest correlation errors with FFQs, pilot-testing the DR in a Lebanese sample showed high burden, low commitment, low motivation and under-reporting. Therefore, the standard validation protocols were adapted in this study to the Lebanese context by using the MPRs. Using the 5-steps MPR probed on possible forgotten foods as well as the time of intake and activities done during the day. This aided in minimizing under-reporting due to memory recall.

Another limitation to consider is the use of single spot urinary excretion specimen, instead of the 24-hour urinary excretion. Because of the difficulty of 24-hr urine collection, using spot urine Na/Cr ratio may be as accurate as 24-hr urinary sodium measurement, with the advantage of being more practical to the participating subject (El Bokl et al., 2009). Measurement of 24-hr urine has major drawbacks; it is cumbersome and inconvenient. Many subjects do not conform to it, and among those who agree to participate, many perform the collection incorrectly (Mann & Gerber., 2010); as many as 30% submit under collections, thus underestimating actual Na intake (Stine et al., 2004). The variability of Na intake also affects 24-hr collections, and in fact three to eight 24-hr collections, an obviously unrealistic option, have been suggested as necessary to provide a representative estimate of sodium excretion (Liu et al., 1979; Liu & Stamler., 1984; Langford & Watson., 1973). Hence, a preferable method, from the perspective of practicality and convenience, would be assessment of Na excretion from spot urine samples (Stine et al., 2004). Kawasaki and colleagues, using the second morning void on the day the 24-hr urine collection was completed, reported a correlation coefficient of 0.53, and among patients who provided three 24-hr collections, the correlation coefficient increased to 0.82 (Kawasaki et al., 1993). The second morning void was also applied to a large number of subjects in a field survey (Kawasaki et al., 1990) (Kawasaki et al., 1992), and found that even in a single urine collection, the second morning void specimen overcomes some of the disadvantages associated with the 24-hr urine collection and provides a reliable estimate of 24-hr urinary Na excretion. In summary, the second morning void is suggested to provide a satisfactory alternative to 24-hr urinary Na excretion in adults especially in extensive epidemiological investigations but also in clinical studies (Kawasaki et al., 1992).

Another limitation to consider is relying on the Food Composition Tables for Use in the Middle East (Pellett & Shadarevian, 1970) for Lebanese foods and their nutritive value, a

reference that has not been updated since the 1970's, and on the USDA's food and nutrient database for foods of international nature. However, when reviewing various nutrient databases, the USDA's has been found to be as the most comprehensive and regularly updated of its kind (Merchant & Dehghan, 2006).

In addition, this study started with 100 interested participants, 13 of which dropped out mainly due to lack of interest. The 87 participants who were initially enrolled in this study showed great interest in knowing more about their Na dietary habits and health, leading to a potential selection bias. However, respecting the autonomy of study participants and the "voluntary" aspect of their participation, this bias could not be avoided. Another bias to consider is the study effect or the risk of participants changing their dietary intake which could not be ruled out (Altman, 1991). In order to avoid such dietary changes, the research team always reminded the participants not to change regular dietary habits, gave no dietary recommendations until the study ended, asked questions in an objective non-leading manner and conducted the 3 MPRs without giving subjects prior notice, thus avoiding any intentional intake adjustments and recall bias.

F. Conclusion and Recommendations

This study represents the first attempt to examine the validity of a short FFQ developed to measure dietary Na intake in the Lebanese population. This study's results indicated an acceptable validity with a substantial reproducibility for the assessment of Na by the FFQ. This FFQ might also allow the ranking of individuals by Na intake so that characteristics of those with high, medium and low intakes can be compared.

This FFQ is relatively inexpensive, easy to administer and not time consuming, with an average of only 25 minutes needed to conduct one questionnaire. This FFQ also eliminates possible burden that food records and or urine collection impose on study participants when

assessing Na intake. This FFQ will be a useful tool to assess dietary Na intake, not only in Lebanon, but also in neighbouring countries whose diet is comparable. These countries, however, could possibly have slight differences in dietary habits which may require adaptation of this FFQ to suit those habits.

This FFQ can reflect long-term dietary intakes needed to assess diet-disease relationships in the context of epidemiological studies, thus it may be used in future research studies. It can also be used by dietitians and health care professionals to screen for Na intake in Lebanese adults. This is crucial especially that early detection of high sodium intake and therefore early intervention to decrease this Na intake may reduce the development of diet-related disease, such as hypertension. It can also be useful for policy makers and governmental use in order to address clear and practical nutritional messages and monitor population health intervention programs.

APPENDIX I

PARTICIPANT CONSENT FORM
(ARABIC VERSION)

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دراسة حول العادات الغذائية لتحديد نسبة تناول الملح والصوديوم عند البالغين في لبنان

استمارة موافقة

الباحث: د. لارا نصرالدين

العنوان: الجامعة الأميركية في بيروت

الحمرا

بيروت، لبنان

الهاتف: 01-350000 مقسم 4547

المكان: قسم التغذية و علوم الغذاء، كلّية العلوم الزراعية و الغذائية، الجامعة الأميركية في بيروت.

يطلب منك أن تشارك/ي في دراسة بحثية تجري في الجامعة الاميركية في بيروت. يرجى أخذ الوقت الكافي لقراءة المعلومات التالية بدقة قبل أن تقرر/ي اذا اردت المشاركة. لا تترددي في طرح الاسئلة إذا كنت بحاجة لمزيد من المعلومات أو توضيح حول الاستمارة أوالدراسة ككل.

تهدف هذه الدراسة الى ايجاد أداة عملية تمكننا من تحديد نسبة تناول الشخص البالغ للملح و الصوديوم في لبنان.

سيتم ادراج 100 شخص أصحاء في هذه الدراسة. سيتم، الإعلان عن هذه الدراسة لاستدراج المتطوعين عبر ملصق سيتم وضعه في مختلف أقسام المركز الطبي و مختلف الكليات في الجامعة الأميركية في بيروت. سيقوم فريق البحث بتعريفك عن الدراسة وشرح أهدافها وسوف نزوّدك بأيّة معلومات أو شرح إضافي قد تسأل عنه. سيتم أخذ موافقتك الخطّية على المشاركة بعد الاطّلاع على كافة شروط المشاركة وذلك في غرفة تتمتّع بالخصوصية في المركز الطبي في الجامعة الأميركية في بيروت أو قسم التغذية وعلوم الغذاء، في الجامعة الأميركية في بيروت. ستنجز هذه الدراسة في هذه المراكز فقط

لتحقيق هذا الهدف، تم تحضير استمارة حول العادات الغذائية و تناول المأكولات. ستستعمل هذه الاستمارة في الدراسة لنتمكن من معرفة ما اذا كانت هذه الاستمارة دقيقة و قادرة على تبيان مستوى تناول الملح و الصوديوم عند البالغين.

. إذا وافقت/ي أن تشارك/ي في هذه الدراسة ، سيطلب منك:

1- الاجتماع مع فريق البحث الذي سوف يعطيك المعلومات الكافية عن الدراسة و يطلعك على كيفية تجميع البول.
2- تجميع البول على فترة 24 ساعة. لا يجب تجميع العينة الأولى في الصباح و لكن يطلب منك تجميع كل العينات اللاحقة لغاية صباح اليوم التالي. يتوجب عليك احضار العينة و تسليمها لفريق البحث في اليوم التالي.
3- بعد تسليمك لعينة البول مباشرة'، سوف يطلب منك ملء استمارة تسمح لنا بتقييم نسبة تناول مأكولات معينة و ذلك من خلال مقابلة ستتّم في المركز الطبي في الجامعة الأميركية في بيروت أو قسم التغذية وعلوم الغذاء، في الجامعة الأميركية في بيروت و تحتاج حوالي 20 دقيقة من وقتك. خلال هذه المقابلة، سيتم أخذ طولك و وزنك أيضا.
4- بعد ملء الاستمارة سوف يطلب منك تزويدنا بعينة واحدة جديدة من البول

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5- سيتم تخزين عينات البول في قسم التغذية وعلوم الغذاء حيث سيتم حفظها هناك في مختبر التغذية وعلوم الغذاء (غرفة 422) على درجة حرارة 20 - مئوية لمدة أقصاها 3 أشهر وذلك من أجل تحليل نسبة الملح فيها. سيتم التخلص من عينات البول فوراً بعد صدور نتائج التحاليل ولن تستعمل من أجل أي فحوصات أو دراسات أخرى.
6- الإجابة عن أسئلة متعلقة بالطعام و الشراب الذي تناولتها في الساعات ال 24 الماضية
7- السماح لنا بالإتصال بك بعد يومين عبر الهاتف ، لنسألك عن الأطعمة و المشروبات التي تناولتها في الساعات ال 24 الماضية

مشاركتك في هذا البحث هي طوعية بالكامل، ولكن مهمة جدا بالنسبة لنا. من خلال المشاركة في دراستنا سوف تساعدنا على انشاء و اثبات أداة بحثية لتحديد نسبة تناول الصوديوم و الملح عند البالغين في لبنان. و ذلك مهم جدا للأبحاث العلمية حيث يعتبر الصوديوم أحد العوامل الغذائية التي تساهم في زيادة مخاطر الاصابة بارتفاع الضغط و أمراض القلب و الشرايين.

سوف يتم تزويدك بنتيجة الاستمارة الغذائية و فحص البول. كما سيتم دفع مبلغ و قدره 50,000 ل.ل كبدل نقل مقسمة على الشكل التالي:

1- بدل نقل 1 (الزيارة الأولى،ارشادات حول الدراسة): 25,000 ل.ل
2- بدل نقل 2 (الزيارة الثانية، يوم المقابلة، تسليم عينة البول لفريق البحث): 25,000 ل.ل

سوف نزودك أيضاً بإرشادات غذائية من أجل تحسين عاداتك الغذائية و نمطك الحياتي

لا توجد أية مخاطر متوقعة من خلال مشاركتك معنا في هذه الدراسة. ولا توجد اية فوائد مباشرة متوقعة لك على السواء. الرجاء الملاحظة ايضا بأن رفض مشاركتك في هذه الدراسة لن يشمل اية خسارة في الفوائد.

إذا وافقت بأن تشارك في هذه الدراسة البحثية، سيتم الحفاظ على سرية المعلومات في خزينة مقفلة بالمفتاح. اضافة إلى ذلك ، فإن جميع مقدمي الرعاية الصحية بما في ذلك طبيبك الخاص لن يكون لهم حق الوصول إلى المعلومات التي قدمتها في هذه الدراسة.

يمكنك الانضمام إلى هذه الدراسة حتى إذا كنت لا تسمح لنا باستخدام بقايا من عينات البول في بحوث مقبلة. يرجى الإشارة إلى اختيارك بعلامة على الخط المناسب أدناه:

_____ **أسمح** بحفظ وإستخدام بقايا من عينات البول في بحوث مقبلة

_____ **أسمح** بتخزين بقايا من عينات البول لكن اطلب أن يتم الاتصال بي للتصريح بإذن لاستخدام هذه العينات في بحوث مقبلة (رقم الهاتف:----------)

_____ **لا أسمح** بحفظ وإستخدام بقايا من عينات البول في بحوث مقبلة

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APPENDIX II

PARTICIPANT CONSENT FORM
(ENGLISH VERSION)

Validation of a Food Frequency Questionnaire to Assess Sodium Dietary Intake from 24-hour Urine Sodium Excretion in Lebanese Adults

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Consent Form

Principal Investigator: _____
Address: American University of Beirut Medical Center (AUB-MC), Department of _____
Hamra
Beirut, Lebanon
Phone: 01-350000 (ext ____)

You are being asked to participate in a research study conducted at the American University of Beirut Medical Center-Department of _____ . Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask us if you need more information or clarification about what is stated in this form and the study as a whole.

The purpose of this study is to validate a short questionnaire for the purpose of assessing the daily/weekly intake of salt and sodium in Lebanese adults.

A total of 100 healthy individuals aged between 19 and 55 years will be recruited for this study. Recruitment of participants will be based on the use of advertisements and flyers which will be placed or distributed in various departments of AUB and AUB-MC. Participants who are interested in joining the study will be briefed about the study objectives and procedures by a trained research assistant. The participant will be provided with any additional information he/she might be inquiring about. Individuals who accept to participate in the study will be invited into an allocated private room at AUB-MC or at the Nutrition and Food Science Department (AUB) and written informed consent will be obtained from them, after they have read all the terms and conditions for participation. Data collection and interviews with participants will only take place at these specified sites.

This study will allow us to know whether the questionnaire that we are using for the assessment of salt intake provides accurate estimates in adults.

If you decide that you want to take part in this study, you will be kindly asked to:

1. Meet with the research team where you will be given all the information about the survey and the procedures involved. A trained researcher will give you the instructions for the 24 hour urine collection procedure as well as all the necessary equipment.

2. Collect the 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. During this period, 24 hour urine samples should be stored at room temperature. The following day, samples should be submitted to the research team at AUB for storage.

3. After submitting the 24 hour urine sample to the research team, you will be asked to fill a short check-list questionnaire which allows us to assess your dietary intake of specific foods. This will be done in

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an interview setting and will not take more than 20 minutes of your time. During this interview, your height and weight will also be measured and your BMI calculated.

4. After filling the checklist, you will be asked to provide one additional urine sample (spot sample).

   All urine samples (24 hour urine samples and urine spot samples) will be stored at -20°C in the department of Nutrition and Food Science room 422, AUB. Urine samples will be kept for a maximum period of 3 months and will not be used for other tests or in other studies. Destruction of urine samples will take place after obtaining the results of the above-mentioned tests.

5. Answer questions related to your food and beverage intake in the past 24 hours (24-hour dietary recall).

6. Allow us to re-contact you, by phone, 2 and 4 days after the interview in order to ask you about your food and beverage intake in the past 24 hours (24-hour dietary recall).

Your participation in this research is completely voluntary but it is very important to us. By participating in this study, you will be helping us to develop a valid tool for the assessment of dietary sodium intake in Lebanon. This is very important in research as well as in clinical settings since sodium is one of the dietary factors that increase the risk of hypertension and cardiovascular disease.

The results of the dietary assessment and of the urine analysis will be communicated to you. You will also be paid the sum of 50, 000 L.L. as a transportation fee, divided as follows:

Transportation 1 (First visit, instruction for the 24h urine collection): 25, 000 L.L.
Transportation 2 (Second visit, submission of the 24h urine sample, interview day): 25.000 L.L

An individualized dietary consultation will be provided to you.

There are no expected risks to you for helping us with this study. There are no expected direct benefits either. Also note that refusal to participate in this study will involve no loss in benefits.

If you agree to participate in this research study, the information will be kept confidential under lock and key. In addition, all of your healthcare providers including your physician will not have access to the information you provided in this study.

You may join this study even if you do not allow the use of your left-over samples for future research. Please indicate your choice on the appropriate line below:

_____I **PERMIT** the storage and use of my left-over samples for future research

_____I **PERMIT** the storage of my leftover samples but request to be contacted to seek permission of use for future research (telephone number: ----------)

_____I **DO NOT ALLOW** the storage nor the use of my left-over samples for future research

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Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _____ _____ (name of interviewed subject), the purpose of the study and its risks and benefits. I have answered to all the subjects' questions clearly.

I will inform the participant in case of any changes to the research study.

_____ _____
Name of Investigator or designee Signature

_____ _____
Date Time

Participant's Consent:
I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily give my consent to be a part of this research study and I know that I can contact Dr. _____. at 01-350 000 ext._____ or any of his/her designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at 01-350 000 ext. 5445. I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits. Refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled. I know that I will receive a copy of this signed informed consent.

Signature of Participant _____
Date _____
Time _____

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APPENDIX III

FOOD FREQUENCY QUESTIONNAIRE

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الجامعة الاميركية في بيروت

تقييم نسبة تناول الصوديوم بين الراشدين اللبنانيين

رقم المشترك (ID): .....

التاريخ : .....

العمر : .....

الجنس: .....المستوى التعليمي:

a. المرحلة إبتدائية أو أقل
b. المرحلة المتوسطة
c. المرحلة الثانوية
d. دبلوم تقني
e. المرحلة الجامعية
f. لا أريد الاجابة

الوزن(kg): .....

الطول(m): .....

BMI (kg/m^2): .....

Spot urinary sodium concentrations:

Spot urinary creatinine concentrations

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المأكولات	نموذج الحصّة	عدد الحصص المستهلكة عادةً من المشارك	تواتر الاستهلاك				Code#
			في اليوم	في الأسبوع	في الشهر	نادراً أبداً/	
الخبز والنشويات							
خبز (خبز أبيض، أسمر، قمح كامل)	1 رغيف وسط (70) 1 رغيف كبير(120غ) خبز فرنجي 16 سم (68غ)						
كعك	-1 كعك أصبع (15g) -3 كعك دائري صغير(15g)						
خبز مرقوق	1/2 رغيف كبير (53g)						
كرواسان	1 صغير (15غ) 1وسط (57g) 1 كبير (120غ)						
المعجنات (كريب ، كيك "muffin"، بريوش)	- 1/2 كريب(57g) - 1 بريوش(57g) - 1 كيك "muffin" (57g)						
منقوشة (زعتر, جبنة, كشك)	1 قطعة = 159غ						
أطباق مكونة من معكرونة / مغربية	1 كباية = 186غ						
أطباق مكونة من أرز (أرز بلحمة ، كبسة, مدردرة)	1 كباية = 185غ						
أطباق مكونة من برغل (برغل ببندورة ، برغل بتفين)	1 كباية						
أطباق مكونة من البطاطا (كبة بطاطا ، بطاطا مشوية /مسلوقة ، بطا طا مقلية)	1 بطاطا وسط = 1 صحن وسط بطاطا مقلية = 20 قطعة = 100غ (1 قطعة = 5غ)						
الفطائر (فطاير جبنة/ سلك / لحمة ، لحم بالعجين ، سمبوسك ،	1 قطعة (23g)						

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رقاقات)							
بيتزا	1 شرحة						
الحليب ومشتقاته							
جبنة (بيضاء ، صفراء, مصنعة)	1 شرحة = 22غ						
لبنة	1ملقعة كبيرة = 27غ						
لبن أو أطباق مكونة من لبن	1 كباية = 208غ						
حليب	1 كباية						
أطباق مكونة من الكشك	1 كباية						
اللحوم و بدائلها							
اللحوم الباردة (هوت دوغ - frankfurter ، مقانق ، مو رتدلا ، جانبون)	1- frankfurter (50g) 2- هوت دوغ(50g) 5- مقانق(50g) 2- شرائح مو رتدلا أو جانبون(50g)						
اللحوم المطبوخة (همبرغر لحمة ، شاورما، كبة، كفتة)	90 g (3oz)						
الدواجن المطبوخة (همبرغر دجاج ، شيش طاووق دجاج بانيه ، دجاج مقلي broasted-)	90g (3 oz)						
أطباق مكونة من اللحمة (ستيك ،لحمة مشوية، لحمة نيه ، لحمة غنم مطبوخة,لحمة خنزير)	90g (3 oz)						
أطباق مكونة من الدجاج (دجاج مسلوق أو مشوي)	90g (3 oz)						
السمك (سمك بانيه أو المعلب)	90g (3 oz)						
البيض	1						
الخضار							
السلطات فتوش ، تبولة ، سلطة خضراء, سلطة) (ملفوف	1 كباية = 182غ						
يخانة الخضار (ملوخيه ،سبانغ، باميه،فاصوليا، بزيلا) **أو لوبيا بالزيت**	1 كباية = 243غ						
المحاشي (ورق عنب، ملفوف محشي ، كوسى محشي)	1 ورق عنب = 16غ 1 ملفوف محشي = 35غ 1 كوسى محشي = 69غ						

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الخضار المعلبة (بزيلا ، ذرة، هليون)	1/2 كباية						
الحبوب							
أطباق مكونة من الحبوب (مجدرة ، مسبحة، بليلة، فول مدمس، عدس بزيت، حمص مسلوق)	1 كباية = 265غ						
فلافل	(قطعة 3) 90g						
بطاطا شيبس	1 كيس 500ل.ل. = 40غ 1 كيس 250ل.ل. = 25غ 1 كيس 1500ل.ل. = pringles (big) = 150غ						
حبوب الذرة- الفشار (Pop corn)	1 كباية = 11غ كيس ميكرويف = 4 كاسات Movies large container = 20 cups -Movies medium container = 15 cups -Movies small container = 10 cups						
البزورات و الترمس	1/2 كباية = 160غ						
الحلويات							
كنافة (بلا كعك)	قطعة(111g)						
البسكويت والكوكيز (cookies)	-1بسكويت(40g) -4 cookies (64g)						
لوح شوكولا	قطعة (60غ)						
كيك	قطعة (100غ)						
صفوف ، أقراص تمر	قطعة(45g)						
الشوربات والصلصات والتوابل							
الشوربات	1 كباية = 274غ						
زيتون، كبيس, مكدوس	**زيتون = 3غ** **كبيس = 30غ** **مكدوس = 59غ**						
زعتر يابس	1 ملقعة صغيرة = 16غ (زعتر و زيت)						
توابل و صلصة البندورة (خردل، كاتشب، صلصة بندورة معلبة، رب البندورة)	1 ملقعة كبيرة = 15غ						

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صلصة الصويا	1 ملقعة صغيرة = 18غ						
مايونز، مرقة و كريمة	1 ملقعة كبيرة = 14غ						
أطباق مكونة من الطحينة (بابا غنوج ،حمص بالطحينة، طرطور)	1 ملقعة كبيرة او كوب						
ملح	رشة = 0.25غ 1 ملعقة صغيرة = 6غ 1 ظرف = 1غ						

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APPENDIX IX

FOOD FREQUENCY QUESTIONNAIRE

Assessment of Sodium Dietary Intake in Lebanese Adults

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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/m^2): .....

Spot urinary sodium concentrations:

Spot urinary creatinine concentrations:

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Food-Frequency Questionnaire

Code #	Food item	Proposed serving size	Portion usually consumed by individual	Frequency of consumption			
				Per day	Per week	Per month	Rarely or Never consumed
Bread and Starches							
	Bread (White/Whole bread)	-1 med pita (70g) -1 large pita (120g) -French baguette 16 cm (68g)					
	Kaak	-1 finger kaak (15g) -3 round kaak (15g)					
	Markouk	½ loaf (53 g)					
	Croissant	-1 med (57g) -1 large (120g) -1 mini (15g)					
	Other Pastries (crepe, brioche, muffin)	-1/2 crepe (57g) -1 brioche (57g) -1 English muffin (57g)					
	Manaeesh (zaatar, cheese and Kishk)	1 piece= 159g					
	Pasta based dishes & moughrabieh	1 cup= 186g					
	Rice based dishes (Riz bi lahmeh, kabseh, mdardara)	1 cup= 185 g					
	Burgol based dishes (burgol bi banadoura burgol bi dfin)	1 cup= 19 g					
	Potato and potato-based dishes (French fries, potato baked, grilled, yakhnet potato, potato kebbe)	1 medium potato = 1 medium plate of French fries = 20 items = 100g (1 item = 5g)					
	Traditional pies (lahm bi 3ajin cheese rolls fried, sambousik meat, fatayer silk)	1 piece = 23g					
	Pizza	1 slice = 103g					
Milk and Dairy products							

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	Cheese (white, yellow processed)	1 slice = 22 g					
	Labneh	1Tbsp = 27g					
	Yogurt and yogurt based dishes (Laban, frozen yogurt)	1 cup= 208g					
	Milk	1 cup=244g					
	Kishk based dishes	1 cup=253 g					
Meats, Poultry, Fish and Eggs							
	Cured meats (hotdogs, ham, mortadelle, sausages, makanek, sujuk)	- 1 frankfurter or sujuk (50g) - 2 hot dogs (50g) - 5 makaneks (50g) -2 slices of ham or mortadelle (50g)					
	Ready prepared meat (kibbe, kafta, shawarma, hamburger patty)	90 g (3oz)					
	Ready prepared poultry (taouk, chicken burger patty, chicken breaded, Chicken flour coated, Chicken batter fried)	90g (3 oz)					
	Meat based dishes (Steak, Lamb cooked, Pork cooked, raw meat)	90g (3 oz)					
	Poultry based dishes (Stewed, grilled & roasted chicken)	90g (3 oz)					
	Fish (canned, fish fingers)	90g (3 oz)					
	Egg	1					
Vegetables and Vegetables based dishes							
	Salads (fattouch, Tabouleh, coleslaw, green salad)	1 cup=182g					
	Vegetables ragouts (mouloukhieh, green peas ragout, spinach ragout, okra ragout, loubieh bi zieit)	1 cup =243g					
	Stuffed vegetables (stuffed zucchini, stuffed	-1 piece of stuffed cabbage (35g)					

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	grape leaves, stuffed cabbage)	-1 piece of stuffed grape leaves (16 g) -1 piece of stuffed zucchini med (69g)					
	Canned vegetables (peas, corn, asparagus)	0.5 cup					
Legumes							
	Legumes based dishes (foul moudamaas, mjaddara, adas bi zeit, balila , msabaha, chickpeas cooked)	1 cup=265g					
	Falafel	90g (3 pieces)					
Salty Snacks							
	Potato chips	-1 bag 500l.l = 40g -1 bag of 250l.l = 25g -1 bag of 1500l.l = Pringles (big) = 150 g					
	Pop corn	-1 cup = 11g -Microwave bag = 4 cups -Movies large container = 20 cups -Movies medium container = 15 cups -Movies small container = 10 cups					
	Roasted nuts, seeds and lupin (salted)	1 cup=160g					
Sweets							
	Kneffeh (without kaak)	1 piece (111 g)					
	Biscuits and cookies	1 biscuit (40 g) 4 cookies (64 g)					
	Chocolate bars	1 bar chocolate (60g)					
	Sfouf, akras tamer	1 piece (45 g)					
	Cakes	1 piece (100g)					
Soups, Condiments and Sauces							
	Soups	1 cup=274g					
	Olives, pickles, makdouss	-1 olive (3 g) -1 pickle-cucmber (30g) -1 makdouss (59g)					
	Dry thyme	1 Tbsp=16g (thym and oil)					
	Condiments and tomato sauces (ketchup, mustard,	1 Tbsp=15g					

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	tomato paste canned, tomato sauce canned)						
	Soya sauce	1 Tbsp=18g					
	Cream, gravies, and mayonnaise	1 Tbsp=14g					
	Tahini-based dishes (tarator, tahini, hummos bi thineh, baba ghanouj)	1 Tbsp or 1 cup					
	Table salt	1 pinch = 0.25 g 1 tsp = 6g 1 sachet = 1 g					

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