AMERICAN UNIVERSITY OF BEIRUT

FRUCTOSE INTAKE AND ITS ASSOCIATION WITH RELATIVE TELOMERE LENGTH: A CROSS-SECTIONAL STUDY AMONG HEALTHY LEBANESE ADULTS

by
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ABSTRACT
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Telomeres are composed of repetitive sequences of six nucleotides (TTAGGG) that cover the ends of the linear chromosomes, and their role is to maintain genetic integrity and provide cellular stability. Shorter telomere length has been associated with increased incidence of diseases and poor quality of life. High consumption of sugar-sweetened beverages (SSB) was suggested to be linked with shorter telomere length, possibly because of their elevated sugar content, and specifically fructose, which may contribute to a biochemical environment that is characterized by high inflammation and oxidative stress. To our knowledge, no data are available on fructose intake and its association with relative telomere length (RTL). Hence, the aim of this study is to (1) assess the intake of dietary fructose (total, added and natural) in a sample of Lebanese healthy adults and (2) investigate dietary fructose (total, added and natural) as a predictor of short telomere length, while adjusting for other potential confounders.

This study is a cross-sectional population-based study carried out on 282 healthy adults aged 18 years and older. Anthropometric and biochemical data were collected, and RTL was measured by amplifying telomere and single copy gene using real-time polymerase chain reaction (PCR). Dietary intake was assessed using semi-quantitative food frequency questionnaire (FFQ) and NutriPro software was used to calculate natural fructose intake (g/day) from fruit and vegetables. Added fructose intake was calculated as 50% of added sugars in food products and total dietary fructose intake was measured by summing up the intakes of added and natural fructose.

The average intake of total dietary fructose was of 51.31 ±35.55g/day which represents 6.58 ±3.71% of the total energy intake (EI). Intakes of natural and added fructose were estimated at 12.28 ±8.59g/day and 39.03 ±34.12g/day (1.78 ±1.41% and 4.80 ±3.56% EI), respectively. Intakes of total sugar and total fructose were found to differ significantly across RTL tertiles. Older age, wider waist circumference and higher LDL levels were statistically significantly associated with shorter RTL. To assess the association between fructose intake (total, added and natural) and RTL, multinomial logistic analyses were performed. No significant associations were found.

This study is the first from the Eastern Mediterranean region to investigate the association between fructose intake and telomere length. It documented high intake levels of fructose but did not find any significant association between telomere length

and fructose intakes (total, added or natural). Larger studies, of longitudinal nature, are needed to better elucidate the relationship between fructose intakes and telomere lengths.

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ABBREVIATIONS

RTL: *Relative telomere length*

WC: *Waist Circumference*

SSB: *Sugar-sweetened beverage*

TRF: *Terminal restriction fragment*

RT-qPCR: *Real time quantitative polymerase chain reaction*

SCG: *Single copy gene*

TL: *Telomere length*

BP: *Base pair*

NHANES: *National health and nutrition examination survey*

MD: *Mediterranean diet*

NHS: *Nurse's health study*

BMI: *Body mass index*

MESA: *Multi- ethnic study of atherosclerosis*

LEAD: *Latino eating and diabetes cohort*

LTL: *Leukocyte telomere length*

KHK: *Keto hexokinase*

F1P: *Fructose -1- phosphate*

GAP: *Glyceraldehyde-3- phosphate*

ChREBP: *Carbohydrate responsive element binding protein*

SREBP: *Sterol regulatory element binding transcription factor 1c*

APOc3: *Apo lipoprotein c3*

VLDL: *Very low-density lipoprotein*

LDL: *Low-density lipoprotein*

HDL: *High-density lipoprotein*

TG: *Triglyceride*

TRL: *Triglyceride rich lipoproteins*

TAG: *Triacyl glycerol*

DNL: *De novo lipogenesis*

ROS: *Reactive oxygen species*

ATP: *Adenosine triphosphate*

AGE: *Advanced glycation end products*

BPA: *Bisphenol A*

EI: *Energy intake*

AUB: *American University of Beirut*

NFSC: *Department of Nutrition and food sciences*

IPAQ: *International physical activity questionnaire*

BP: *Blood pressure*

CAP: *College of American pathologists*

Hba1c: *Hemoglobin a1c*

FFQ: *Food frequency questionnaire*

HFCS: High fructose corn syrup

OR: *Odds ratio*

RCT: *Randomized control trials*

AUBMC: *American university of Beirut medical center*

CHAPTER I

INTRODUCTION

Telomeres play a critical role in maintaining the genome's structural integrity and in protecting chromosomes from end-to-end fusion and degradation (Blackburn, 2000). However, the length of telomeres shortens progressively with age in various tissues and cells. (Harley et al, 1992). Telomere length may also be perceived as a possible biomarker of biological age, showing the cumulative burden of inflammation and oxidative stress (Aviv, 2004). In fact, an increased number of epidemiologic and clinical data suggest that accelerated shortening of telomere length is linked with diseases of aging such as higher risk of coronary heart disease, diabetes, heart failure (Demissie et al, 2006) and osteoporosis (Valdes et al, 2007). Likewise, shorter telomeres were observed in patients with metabolic diseases like type 2 diabetes mellitus when compared to the control subjects. An association was also observed between obesity, increased body mass index and shortened telomere length (Valdes et al, 2005).

Available evidence also suggests that many lifestyle factors may affect telomere length (Slagboom et al, 1994). For instance, smoking and alcohol consumption were associated with shorter telomere length in a dose-dependent manner (Morla et al, 2006), while the consumption of antioxidant rich food such as vegetables, whole grains and fish was linked with longer telomeres (Lee et al, 2015). Moreover, adherence to the Mediterranean diet was linked to positive effects on telomere length, providing a greater insight on diet and its potential impact on the dynamics of telomere length. (Marin et al, 2012). On the other hand, higher consumption of sugar-sweetened beverages (SSB) was

suggested to be linked with shorter telomere length (Wojcicki et al, 2018) (Leung et al, 2014). It was argued that the link between SSB and telomere length may be mediated by the sugar content of these beverages, and specifically fructose, given that high fructose intake may contribute to a biochemical environment that is characterized by high inflammation and oxidative stress (Wojcicki et al, 2018). However, none of the available studies have assessed the specific link between fructose intake and telomere length. The objectives of this study are therefore to assess the intake of dietary fructose (Total, added and natural) in a sample of Lebanese healthy adults and investigate the association of fructose intakes with relative telomere length (RTL) in the study sample.

CHAPTER II

LITERATURE REVIEW

A. Definition, Structure and Function of Relative Telomere Length (RTL)

Telomeres are double stranded, and they are composed of repetitive sequences of six nucleotides (TTAGGG) that cover the ends of the linear chromosomes (Samassekou et al., 2010). They are bound to a protein complex known as human shelterin that is made up of six telomere-binding proteins. The role of telomeres and human shelterin is to maintain genetic integrity, and hence provide cellular stability. Also, they play an important role in protecting the ends of chromosomes from fusion, and they are needed in the process of eukaryotic DNA replication (Palm & de Lange, 2008). Both play a part in avoiding the loss of nucleotide coding during each replication of DNA. This is identified as “the chromosome end-replication problem” which occurs due to the incapability of the cell to fully replicate the lagging DNA strand’s 5’ end (Blackburn, 1991). This problem of replication can cause the telomere to shorten during cell division until it reaches a critical length (Mu & Wei, 2002). Hence, the cell can become senescent after having more than one critically short telomere, and the integrity of the DNA coding can be threatened by any further cell division (Cong et al., 2002) (Shay & Wright, 2011). Therefore, telomeres play a crucial role in preserving our genetic information. (Shammas, 2011)

Telomere length can hence serve as a biological clock that determines the lifespan of a cell and an organism. Telomerases have the ability to extend the length of telomeres and they can be found in certain hematopoietic and germline cells. However,

somatic cells have little or negligible levels of telomerase activity and undergo shortening (Shammas, 2011).

B. Importance of Relative Telomere Length

Assessment of relative telomere length as a biomarker has been shown to be important in risk assessments of various health outcomes, since shorter telomeres are linked to having poor health outcomes and earlier death (Epel, 2012). Moreover, according to some studies, there is an association between shorter telomere length and increased cancer risk (Starkweather et al., 2014). Hence, measuring telomere length can be used as a tool, either individually or in coordination with other biological and health variables, for disease prevention, development of interventions, monitoring of diseases and for further improvement of bio-behavioral theory (Montpetit et al, 2014). Moreover, it is important to measure telomere length to determine biological age and age-related cardiovascular diseases and their progression in a certain population (Zgheib et al, 2018).

C. Methods to measure Telomere Length

There are several methods to measure telomere length, but the two most commonly used ones are terminal restriction fragment (TRF) analysis and polymerase chain reaction (PCR).

1. ***Terminal Restriction Fragment (TRF)***

This method was considered as the first and traditional way to measure telomere length (Moyzis et al., 1988). TRF is seen as the "gold standard" and it is used as a reference or a source for calibration by the other methods that were developed afterwards like the PCR and in situ hybridization-based methods. (Müezzinler et al., 2013). However, the main disadvantage of TRF, especially in the case of large-scale studies, is the fact that large quantities of DNA, ranging between 0.5-5μg/sample, are required. In addition, it requires more labor and time (3-5days/sample). Another drawback is that TRF lengths cannot be readily compared with different studies since, with respect to restriction enzymes, they are not standardized in terms of DNA quality and quantity. Restriction enzymes have other alternatives and depending on the selection of restriction enzyme, the length of individuals' telomere can have a variation of 5% because of the polymorphisms that can be present in the subtelomeric restriction sites and/or in subtelomeric regions. According to previous studies, TRF can have a subtelomeric region that varies between 2.5 and 6 kilo-base-pairs (KB) among individuals who are unrelated. (Levy et al., 1992). This can act as a confounder in determining the true inter-individual telomere length variability.

2. ***Real Time Quantitative Polymerase Chain Reaction (RT-qPCR)***

This method was developed by Cawthon (2002) and then was subject to some modifications by different researchers to have the optimal efficiency of its methodology under laboratory conditions that are slightly different. Currently, it is considered as the only method that is high-throughput and can be used for measuring a large amount of samples. The advantages of the PCR method are the fact that its application is relatively

quick and easy, works at lower costs and requires smaller quantity of DNA (60ng/sample) compared to the TRF analysis method which is the traditional method (0.5-5µg/sample). For the quantification in this method, two factors are taken into consideration. The reference gene and the expression levels of a target sequence. In the case of measuring telomere length, telomeres are the target sequence, and the reference gene is a single copy gene (SCG). Hence, the ratio is T/S which is referred as the telomere copy number (T) to SCG (S). The ratio and the average telomere length are proportional to each other. Although there is rapid adoption of qPCR, comparability and variability matters of the method and the results are still under discussion. The effects that are carried out using different primers and SCG among different studies in addition to the presence of variations while applying the same procedure are still needed to be evaluated (Müezzinler et al., 2013). Aubert et al. (2012) describes in a recent review that every method of measuring telomere length has its own disadvantages, advantages and limitations. Hence, in the process of investigating telomere length, it is important to carefully take into consideration measurement error in addition to other factors that affect telomere length like genetics and environmental factors (Aviv et al.,2011).

D. Unmodifiable Factors that Affect Telomere Length

1. *Age*

According to some studies, age is one of the factors that affects telomere length. It is claimed that telomere length decreases with aging. Degenerative diseases can lead to an accumulation of irreversible mutations that can be caused by aging and hinder cell division. This causes an increase in susceptibility of the cell to morbidity resulting in cell death. The telomeres get shorter in every cell division because the number of cell

divisions that human cells can undergo are limited (Razgonova et al., 2020). A systematic review of longitudinal studies, that had 75 to 510 participants, found that telomere length (TL) decreased with age. In fact, three of them showed that the rate of telomere loss yearly ranged between 32.3 to 45.5 base pairs (BP)/year. (Müezzinler et al., 2013). Moreover, according to Cawthon et al (2003), when telomere length is shorter than the normal average length for a specific age group, it was shown to be linked with a higher incidence of age-related diseases and/or reduced lifespan.

2. ***Gender***

Another crucial factor that affects length of telomeres is gender. Although at birth there is no apparent difference, (Akkad et al., 2006; Mayer et al., 2006), it has been shown that telomere length is longer in females than males at similar ages (Nawrot et al., 2004; Fitzpatrick et al., 2007). The mechanism behind this difference is thought to be mediated via the higher estrogen levels which cause an increase in telomere length by increasing telomerase activity (Kyo et al., 1999). Also, antioxidant effects of estrogen may be involved through several mechanisms, such as preventing the production of free radicals, scavenging free radicals and enhancing detoxification by stimulating some enzymes (Massafra et al., 2000)

3. ***Ethnicity***

Ethnicity is also observed to be affecting telomere length. According to Chen et al. (2011), longer leukocyte telomere length was observed in subjects who were African American compared to those who were White Americans, at ages that were similar. It

also showed that White Americans had higher telomere loss rates. A study done in New Zealand, on a cohort of 4-year-old ethnically diverse children showed that indigenous Māori (Polynesian people who are settled in New Zealand), Pacific and Asian children had longer telomeres compared to children of European origins (Ly et al., 2019). Children with Pacific origins had the longest, followed by Asian and then Māori children. This study suggested that the explanation behind the longer telomeres in the Māori, Pacific and Asian children could be that they could be perceived as a population that are genetically less complex (Ly et al., 2019). Another study done on 800 adults from 11 countries in Europe, that used the qPCR method of measurement and adjusted telomere length for age, also confirmed the presence of differences among various ethnicities (Eisenberg et al, 2011).

4. ***Heredity***

Telomere length is considered as a trait that is complex and is shaped by a mixture of epigenetic, genetic, and environmental determinants (Benetti et al, 2007). Growing evidence suggests that, from the factors that are biological and affect telomere length, heredity brings a crucial role to the table. Telomere length is in fact influenced by several genes (Gatbonton et al, 2006) and the range of heritability is 36% to 90% (Andrew et al, 2006). The heredity's mode has not been clarified yet but some research links it to the X chromosome. However, the study by Nordfjall et al (2005), demonstrated a paternal inheritance pattern. According to this study, telomeres of 49 unrelated families were analyzed for possible gender-linked inheritance, and a statistically significant association was found between telomere pairs of fathers and their sons however no association was observed between mothers and their daughters. A

parent-child comparison was done on a large-scale, and telomere lengths of 962 individuals with ages ranging between 0 and 102 years were investigated (Nordfjäll et al., 2009). The parent-child as well as grandparent-grandchild telomere pairs were analyzed. They found out that a father and his child's telomere length had a highly significant correlation, regardless of the sex of the child. This correlation was also observed in grandparent-grandchildren pairs. However, weaker associations were found for mothers and their children (Nordfjäll et al., 2009). Hence, these findings can indicate that fathers bring a significant contribution to their offspring's' telomere length, but we cannot completely ignore the maternal influence on their offspring (Nordfjäll et al., 2009).

E. Modifiable lifestyle factors and their association with telomere length

Telomere length can also be affected by some lifestyle factors that can be modified by better lifestyle choices and are discussed below:

1. *Smoking and Relative Telomere Length*

Smoking is a factor that can lead to accelerated shortening of telomeres. It has been shown that there is a negative correlation between cigarette dosage and telomere length (Song et al, 2010). A study that investigated 1122 white woman aged 18-76 years found a dose-dependent relationship with smoking where each pack-year (1 pack-year= 7,305 cigarettes) smoked resulted in the loss of 5 base pairs of telomere length (Valdes et al., 2005). A study showed that smoking one pack of cigarette daily for 40 years is equal to 7.4 years of life among women (Valdes et al., 2005). A systematic review of 84

studies showed that smokers had shorter telomere length compared to non-smokers (Astuti et al., 2017). Also, telomeres of those who used to smoke in the past were found to be shorter compared to those who never smoked. Hence, exposure to tobacco smoke may be linked to mechanisms that can shorten telomere length and hence induce age-related diseases (Astuti et al., 2017).

2. ***Stress and Relative Telomere Length***

Stress can play a role in increasing the pace of telomere shortening. When glucocorticoid hormones are released from adrenal glands in response to stress, they can cause an increase in oxidative damage to DNA by reducing the level of antioxidant proteins leading to a decrease in telomere length (Epel et al., 2004). In the review about the theory of stress and telomere biology, Shalev et al. (2013) propose a telomere attrition lifespan model that relates shortened telomeres to stress. According to this model, being exposed to stressors and experiencing reactions of stress over a period of time can cause telomere erosion. Moreover, stress caused by adversity can accelerate telomere shortening from an early age. A systematic review of eleven studies showed that adversity experienced by children from 3 to 15 years of age has an effect on their telomere length leading to an increased erosion of telomeres (Coimbra et al., 2017).

3. ***Physical Activity and Telomere Length***

Physical activity has shown to have some beneficial effects on telomere length. A study that investigated 5823 adults from the National Health and Nutrition Examination Survey (NHANES 1999–2002) cross-sectionally found out that adults who

had higher activity levels had a biologic aging advantage over those who were sedentary (Tucker, 2017). Although the mechanism behind physical activity being the predictor of is not very clear and precise, it is crucial to take into consideration that there is a strong relation between inflammation /oxidative stress and telomere length and cell senescence. (Kordinas et al., 2016). In fact, the mechanisms by which physical activity reduces inflammation and oxidative stress is by the decreased release of reactive oxygen species (Bjork et al., 2012), increased production of DNA-repairing enzymes (Radak et al., 2003), and higher genetic expression of antioxidant proteins (Gomez-Cabrera et al., 2008). Also, the intensity of the exercise has shown to affect telomere length in some studies. A study that assessed 69 adults aged between 50-70 years showed that moderate levels of physical activity is significantly associated with having longer telomeres, compared to low and high levels (Ludlow et al, 2008). The possible cause behind it could possibly be again due to the fact that moderate exercise could impose some enhanced immune response and decreased infection risk compared to both low (sedentary) and high levels (overtraining) of exercise training (Matsubara et al, 2006). Hence, to examine mechanistically the effects of various exercise modalities on telomere length in different clinical populations, further research is required (Arsenis et al, 2017).

F. Dietary Patterns, Food Groups' Intakes, and Relative Telomere Length

Diet may also play a key role in affecting telomere length. Adherence to the Mediterranean diet (MD) was shown to have beneficial effects on telomere length (Marin et al, 2012). The MD is characterized by the habitual use of olive oil and abundant consumption of plant- derived foods like fruits, vegetables, legumes, nuts and

whole grains hence increasing the supply of antioxidants and fiber, which in turn may protect against telomere attrition (Marin et al, 2012). A cross-sectional study done on 217 older subjects reported that higher adherence to the MD was associated with longer telomeres as well as higher telomerase activity, after adjusting for cardiovascular risk factors (Boccardi et al, 2013). Another study further confirms these findings in a sample of 4676 women, who were aged between 42 and 70 years old from the Nurse's Health Study (NHS), where longer telomeres were found in women with greater adherence to the MD, after adjusting for confounders (Crous-Bou et al., 2014). The MD is a complex exposome that has thousands of nutrients and phytochemicals, which may have a positive influence on telomere length. However, it is not clear whether these effects are due to the individual elements of the MD or their unique combination. More studies are needed to further advance our understanding of the link between telomere length and the various constituents of the MD (Davinelli et al., 2019)

There are few other studies that have also examined different dietary patterns and their potential impact on telomere length, such as the prudent diet (a diet characterized by the increased intake of whole grains, seafood, vegetables, legumes and seaweed) and the Western diet (a diet characterized by the increased intake of red meat or processed meat, refined grains and sweetened beverages). Lee and his colleagues (2015) studied the adherence to either of these diet in the past 10 years. They found that adhering to the prudent diet, which shares many similarities with the MD, was positively associated with telomere length. However, no association was found with the Western diet.,

Evidence on the link between telomere length and the consumption of various food groups is still rather limited. The first systematic review of 17 studies, that studied

the relation between food groups and telomere length in human populations, showed that fruits and vegetables had a beneficial effect on telomere length. However, a reverse association was found between telomere length and consuming processed meat, refined cereals, fats and oils (Rafie et al., 2016). Another study showed that a healthier lifestyle with higher consumption of fruits and vegetables in addition to higher exercise and lower body mass index (BMI), was associated with longer telomere length (Mirabello et al, 2009). An increase in the activity of telomerase was observed in a small pilot study done on men who went through comprehensive lifestyle changes and had a diet that included low-fat, unrefined plant-based foods that were supplemented with omega-3, soy, vitamins E and C for 3 months (Ornish et al, 2008). After a 5 year follow up, the comprehensive lifestyle intervention was found to be associated with an increased telomere length (Ornish et al, 2008). Larger randomized control trials would be needed to further confirm these findings (Ornish et al., 2013). The observed positive association between the intakes of fruits and vegetables and telomere length may be exp lained by the lower levels of inflammation and oxidative stress that result from the consumption of these food groups (Chan et al, 2010). In addition, the consumption of other food groups that are also rich in antioxidants such as seeds, nuts, legumes and coffee were associated with having longer telomeres (Lee et al,2015). Antioxidants may in fact protect the DNA from oxidative damage (Ros and Hu, 2013). Moreover, an association between longer telomere length and higher intakes of dietary fiber was observed in a large sub cohort of the Nurse's Health Study (NHS) (Cassidy et al, 2010).

Special attention has been given to the effect of processed meat on telomere length. The cross-sectional associations of dietary patterns, food groups and telomere length were studied among 840 white, black, and Hispanic adults taken from the Multi-

Ethnic Study of Atherosclerosis (MESA). After adjusting for age, other demographics, lifestyle factors, and intakes of other foods or beverages, this study found out that the consumption of processed meat was inversely associated with telomere length, and participants consuming more than one serving of processed meat per week had smaller telomere length compared to non-consumers (Nettleton et al., 2008). This observed association may be due to the fact that processed meat contains high levels of saturated fat, sodium, nitrates and nitrites, cholesterol and iron, all of which could be implicated in accelerating the process of aging (Linseisen et al., 2006). Another more recent cross-sectional study that examined 2846 American Indians from the Strong Heart Family Study, also showed that shorter telomere length was associated with higher consumption of processed meat (Fretts et al., 2016).

Several studies have investigated the association between telomere length and the consumption of sugar sweetened beverages (SSB). Leung et al (2014), investigated the relation between telomere length and the consumption of SSBs, diet soda, and 100% fruit juice in a large sample that is a nationally representative sample of healthy adults from United States. The study population was taken from the National Health and Nutrition Examination Survey (NHANES), and it included 5309 adults aged between 20 and 65 years old. It found that more than 20 % of the population consumed 20 ounces of SSBs per day, which was associated with a shorter telomere length, that can be translated into 4.6 years of aging. In addition, a study that included 1958 Korean adults who were middle-aged showed that higher SSB consumption was correlated with shorter telomere length, when measured after 10 years (Lee et al, 2015). A previous study, that enrolled a cohort of Latino children showed that high intake of SSBs at 3 years of age resulted in an increased attrition of telomeres from 4 to 5 years of age

(Wojcicki et al, 2016). The Latino cohort was taken from The Latino, Eating and Diabetes cohort (LEAD). In another birth cohort from LEAD, that consisted of 61 children, the relationship between beverage consumption (including SSB and 100% fruit juice), obesity, and Leukocyte Telomere Length (LTL) was evaluated at 2-3 years old, in addition to changes in telomere length from birth (Wojcicki et al., 2018). It showed that, by the age of 2-3 years old, high SSB consumption had deleterious effect on cellular health and resulted in shorter telomere lengths. Interestingly, this association was stronger in children who were not obese. Furthermore, although the monthly mean consumption of SSB in the cohort was relatively low (4.7-6.7 servings/month), as well as the consumption of SSBs three or more times per week (11.9%), the findings demonstrated that even a slight amount of SSBs can show a negative effect (Wojcicki et al., 2018). In a cohort of pregnant women, reduced SSB intake was found to be associated with apparent telomere lengthening (Wojcicki, 2016).

G. Fructose intakes and Relative Telomere Length

It was suggested that the link between SSB and telomere length may be mediated by the sugar content of these beverages, and specifically fructose, given that high fructose intake may contribute to a biochemical environment that is characterized by high inflammation and oxidative stress. However, none of the available studies have assessed the specific link between fructose intake and telomere length. It would be therefore important to further understand this link given it public health implications.

Fructose is considered as a natural sugar that can be found in many fruits, but is also frequently used by the food industry, given its high sweetness, which exceeds that of

glucose and sucrose when given in equal amounts (Miller & Adeli, 2008). Consequently, one of the major ways to consume fructose is in the way of sucrose or high fructose corn syrup, that is considered as an added sugar and could be found in commercial soft drinks, juices, and baked goods. Over the last 10 to 20 years, it has been shown that high consumption of high fructose corn syrup, in addition to total fructose, is linked to an increase in obesity and metabolic disorders. This represents a major concern regarding the effects of fructose on humans both on the long and short term (Taskinen et al., 2019).

It is crucial to understand the mechanisms by which fructose may lead to the development of cardiometabolic disease. (Hannou et al., 2018).

1. Metabolism of Fructose in the Intestine and Liver

Fructose has a different metabolism than glucose in the enterocytes and the liver. In the small intestine, ketohexokinase (KHK) metabolizes fructose into fructose-1-phosphate (F1P) (Lee et al,2018). Then, F1P is cleaved into dihydroxyacetone phosphate and glyceraldehyde. The latter gets in turn phosphorylated by a kinase enzyme leading to the generation of glyceraldehyde 3-phosphate (GAP). Then, through gluconeogenesis, GAP and other triose phosphates are resynthesized into glucose or they are converted to lactate or acetyl-CoA which are oxidized or utilized for lipogenesis (Lee et al,2018). In the liver, fructose stimulates the activation of the transcription factors carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element binding transcription factor 1c (SREBP1c) which increase the pathways that trigger fructolysis, glycolysis, lipogenesis, and glucose production

(Hannou et al,2018). This in turn leads to an increase in the production of hepatic glucose and production of lipid intermediates that can influence insulin sensitivity. Also, it can cause an increase in the expression of apolipoprotein C3 (APOC3) and in the release of triglyceride-rich very-low density lipoproteins (VLDL). This increase in the expression of APOC3 increases the concentration of plasma apoC-III which inhibits lipoprotein lipase activity and clearance of lipoprotein remnants from the liver. As an outcome, there will be hypertriglyceridemia and buildup of atherogenic triglyceride-rich lipoprotein (TRL) remnants. (Taskinen et al,2019). Hence, it is important to note that, unlike glucose, only a small portion of fructose that is ingested ends up in the circulation. Also, the steps involved in fructose metabolism are found to be unregulated and they bypass the hormonal control in glycolysis in the liver, in contrast to glucose that is strictly regulated, where insulin plays a major role (Tappy, 2018).

2. *Fructose and Hepatic De Novo Lipogenesis*

For any macronutrient, eating beyond the immediate physiological needs can predict storage that is in the form of fat or glycogen. High fructose intake is associated with both of them. In fact, the characteristics of high fructose input are found to be glycogen synthesis, de novo lipogenesis and triacyl glycerol (TAG) synthesis (Feinman & Fine, 2013). Moreover, it has been shown that dietary carbohydrates, fructose specifically stimulate de novo lipogenesis (DNL) and accumulate liver fat. However, it is still a matter of debate whether this increase is caused by excess energy or fructose intake (Chiu et al,2018). Human studies are limited, but a study that compared between glucose and fructose supplementation in rats for a period of two months showed that, even though consumption of total calories was higher in rats that were glucose

supplemented, metabolic responses caused by fructose were shown to be worse (Sanguesa et al, 2017). Importantly, research has shown that dietary fructose can further upsurge the levels of enzymes that are involved in DNL, since fructose absorption is done via the portal vein and then delivered to the liver in concentrations that are much higher compared to other tissues. Interestingly, contrary to glucose metabolism, metabolites that stimulate hepatic DNL can be generated by the breakdown of fructose (Stanhope & Havel, 2010). Moreover, in a situation of insulin resistance, fructose can initiate lipogenesis since it does not need insulin to be metabolized. Hence, it instantly stimulates sterol regulatory element-binding protein 1(SREBP-1c) as discussed before, which is a major transcriptional regulator of DNL (Malik & Hu, 2015). In addition, fructose can promote hepatic DNL by causing is suppression in hepatic beta-oxidation.

3. *Potential metabolic links between Fructose and telomere length*

Oxidative stress is assumed to be a major cause of telomere shortening. The mechanism behind it is explained by the fact that the production of reactive oxygen species (ROS) produced from exogenous sources (pollutants and UV radiation) and mainly intracellularly as a by-product of aerobic metabolism and production of adenosine triphosphate ATP in the mitochondria, can cause oxidative damage to different biomolecules (Balaban et al, 2005). Hence, it has been claimed that telomeres are sensitive to oxidative damage due to their high guanine content (Kawanishi, et al, 2004). Thus, oxidative stress will lead to an accumulation of DNA damage and aggravate telomere loss (Von Zglinicki, 2002). However, most studies that provide such evidence come from *in vitro* cultured cells and hence the role of oxidative stress as a determining factor of telomere shortening *in vivo* remains less clear (Reichert et al,

2017). But a review of some correlative and experimental studies supports the existence of a connection between oxidative stress and telomere attrition, even though more experimental studies are needed. (Reichert et al, 2017).

In parallel, it is recognized that high intakes of dietary, fructose, which can induce the metabolic syndrome, are associated with increased inflammation and oxidative stress (Demircan et al. 2008). Hyperlipidemia induced by excessive fructose consumption can lead to lipid retention in the hepatic cells, which in turn can trigger the release of ROS and activation of inflammatory cytokines at different intracellular levels (Tsochatzis et al. 2006). Also, the increased accumulation of fat can cause free radical release and oxidative damage in the adipose tissues (Furukawa et al. 2004). Hence, it is claimed that visceral obesity is characterized by a low-grade systemic inflammation, reflected by increased serum inflammatory markers like C-reactive protein and tumor necrosis factor-α (Diehl 2004). In addition, high- fructose diet can induce hyperglycemia and damage glucose tolerance leading to hyperinsulinemia and insulin resistance (Hozayen et al. 2016). Hyperglycemia can in turn lead to the auto-oxidation of glucose or fructose resulting in the production of advanced glycation end products (AGEs) (Guglielmotto et al. 2012). According to Miller and Adeli (2008), AGEs production by fructose is 10x higher than that of glucose, and high levels of AGEs can promote the production of ROS and hence mediate inflammatory pathways (Cheng et al. 2014). Hence, although a possible link may exist between high fructose intake and attrition in telomere length, this link has not been previously investigated in the literature.

CHAPTER III

MATERIAL AND METHODS

The current study's data was based on the cross-sectional survey titled "Assessment of Bisphenol A (BPA) levels and their association with the health status among the Lebanese population". The parent study was conducted on a representative sample of Lebanese adults living in the greater area of Beirut, and it was carried out between March and May 2014. The survey and study protocol were approved by the Institutional Review Board of the American University of Beirut. An informed consent form was signed by all participants prior to enrollment in the study, and they were given the right to withdraw from the study at any time (Appendix I).

A. Participants

Participants were adults from the Greater Beirut area, who were selected using a multistage probability sampling, where the districts of the area were the strata. Then using a systematic random sampling approach, a sample of neighborhoods and then households were chosen randomly within each district. At the household level, the adult individual with the most recent month of birth was chosen to participate in the study, if eligible. Exclusion criteria included participants who were mentally disabled, pregnant or on dialysis. Also, participants who worked in plastic or other chemical companies were excluded due to the fact that they have been exposed to BPA (and hence will not meet the eligibility criteria of the parent study which aimed at evaluating exposure of the general population to BPA).

For the purpose of the current study, participants were selected from the original population (n=501) according to the following criteria:

1. Healthy, with no history of chronic disease

2. Having complete anthropometric, biochemical and dietary data

3. No under or over reporting of energy intake (EI)

In total, 282 participants, aged ≥ 18 years, were included in this study.

B. Data Collection

Participants in the study were invited to visit the American University of Beirut (AUB) after an overnight fast, for the collection of data, which took place at the Department of Nutrition and Food Sciences (NFSC) in the Faculty of Agricultural and Food Sciences. Data collection included sociodemographic, lifestyle, dietary and medical history characteristics, that were collected using a pre-tested questionnaire. Data collection also comprised anthropometric and biochemical assessment, as well as blood pressure measurements. Trained personnel collected the data and administered the study questionnaire in an interview setting (Appendix II).

1. Socio-demographic and lifestyle characteristics

The socio-demographic characteristics were included in the current study comprise age (in years), gender, marital status (married, engaged or single, including divorcees and widowers), educational level, monthly household income (expressed in terms of U.S. dollars), and crowding index. Monthly household income was categorized

into < 600$, 600$ ≤ income ≤ 2000$, > 2000$. Educational level was divided into no schooling or primary school, intermediate school, secondary school or technical diploma and university degree. Lifestyle characteristics included smoking status (current smokers of cigarette or hookah vs past and non-smokers), alcohol consumption (ever vs. never), coffee consumption (ever vs. never) as well as number of hours of sleep per night on weekdays and weekends and physical activity level. Sleep habits were assessed using the Berlin Questionnaire (Kang et al, 2013). Physical activity was assessed using the short version of the International Physical Activity Questionnaire (IPAQ 2005). Physical activity was categorized into 3 categories: low, moderate, and high.

2. *Anthropometric, blood pressure and biochemical measurements*

Regarding the anthropometric measurements, weight was measured to the nearest 0.1 Kg using a calibrated electronic weighing scale (Inbody 3.0, Biospace Co. Ltd, Korea), while the subjects were wearing light clothes without shoes. A portable stadiometer (Seca 213, Germany) was used to measure height and it was recorded to the nearest 0.5cm. The candidates were required to be in a standing position, flat against the measuring board without shoes. BMI was calculated as weight (Kg) divided by square of the height (meters) (Deurenberg , 1992). Waist circumference (WC) was measured at the umbilical level, using a non-stretchable tape meter (Seca 26201, Germany), to the nearest 0.5cm. Percent body fat was estimated using the Bioelectrical Impedance Analysis technique (Inbody 3.0, Biospace Co. Ltd, Alpha-Tec s.a.r.l.).

After a ten-minute rest, sitting blood pressure (BP) was measured using a standard digital sphygmomanometer. It is important to note that BP measurements were taken twice and the average of the two values was used. As for the biochemical measurements, blood was drawn and put into an EDTA tube and 2 chemistry tubes. The whole blood present in the EDTA tube was kept at -80°C for future DNA isolation. While the other two chemistry tubes were centrifuged for plasma, some of which was immediately transported to the laboratory of the American University of Beirut Medical Center certified by the College of American Pathologists (CAP). Remaining plasma samples were stored at -20°C and were delivered to the laboratory as batches depending on the measurements that were needed. The laboratory measurements that were performed included serum triglycerides (TG), high- density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), as well as glucose and hemoglobin A1c (HbA1C) levels. Levels of serum TG, HDL-C and LDL-C were measured using an enzymatic spectrophotometric technique using Vitros 350 analyzer (Ortho-Clinical Diagnostics, Johnson and Johnson, 50–100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP12 4DP, United Kingdom) at the NFSC department. HbA1c was measured using the BioRad Variant Hemoglobin Analyzer at AUBMC.

a. Relative telomere length measurement

Total DNA extraction from leucocytes of peripheral venous blood was done using Qiagen kit (Qiagen, USA) as per manufacturer guidelines, where it was normalized to a concentration of 10ng/ul and stored at -20 °C until analysis. Then RTL was measured by the use of quantitative real-time polymerase chain reaction (RT-qPCR) on CFX384 Touch Real-Time PCR Detection System from BIO-RAD as

described by Cawthon's method 2002 with a few adjustments made by Cawthon in 2009 (Cawthon RM, 2002) (Cawthon RM, 2009). RTL was calculated according to the formula described by Pfaffl 2001 to account for different plate efficiencies (Pfaffl MW, 2001).

3. Dietary intake assessment

A semi-quantitative, culture specific food frequency questionnaire (FFQ) with 82-food items (Appendix II) was used to collect dietary data, in an interview setting. The FFQ referred to the participants' usual dietary intake during the past year. A reference portion size (expressed in household measures such as cups, spoons and plates and/or customary packing size) was provided for each food item included in the FFQ. To help in quantifying the portion size, the standard two-dimensional food portion visual chart, developed by Nutrition Consulting Enterprises was used (Posner et al, 1992).

For data entry, a database application using Microsoft Access (Microsoft Corp., Redmond, WA, USA) was developed. Accordingly, for each beverage or food item, the frequency of consumption as reported by the participant was converted to daily intake. The Nutritionist Pro software, version 1.2 was used to calculate total energy and macronutrients intakes. Energy (kcal) was computed per gram for each food item/beverage on the FFQ list. The participant's daily EI was then computed by summing the respective products of the quantity consumed and the energy per gram value for each food item/beverage (Flegal et al, 1988). The same procedure was used to calculate the daily intake of each macronutrient (Flegal et al, 1990), as well as for total

fructose. Participants reporting abnormally low or high energy intakes were excluded. (<500 or >6000kcal/day) (Derghan et al., 2017).

4. Estimation of added and natural fructose dietary intakes

Data about fructose content was available for 36 food items based on the NutriPro software (i.e., total fructose). However, no data was available on added vs natural fructose, and hence these were calculated based on the following criteria:

- For whole fruits, vegetables, and honey natural fructose was presumed to be equal to total fructose (Hosseini-Esfahani et al, 2011) (Table 1).

- For processed foods and given that the most common form of added sugar is sucrose or high fructose corn syrup (HFCS), the content of added fructose was calculated as 50% of added sugars (Hosseini-Esfahani et al, 2011) (Sun et al, 2011).

The content of natural fructose is shown in Table 1 and that of total and added fructose per food item is shown in table 2.

Table 1 Natural fructose content of food items (per 100g)

Food item	Natural fructose (in 100g)
Citrus orange/grapefruit	2.0795
Peach, plum, prunes	3.07
Strawberries	3.90
Grapes	8.13
Banana/Apples	5.375
Salad, green: Lettuce, mint, cucumber, green pepper, rocket, purslane, etc.	1.133
Tomato, fresh	1.37
Corn/Green peas, fresh	0.05
Corn/Green peas, canned	0.05
Potatoes, baked/boiled/mashed	0.34
Zucchini/ Eggplants, cooked	1.21
Cauliflower/Cabbage/ Broccoli	1.21
Other canned vegetables (Mushroom, palmetto, asparagus, etc.)	0
Legumes: lentils, beans, chickpeas, etc., dried, cooked	0.1
Legumes: canned (beans, fava, chickpeas)	0.1
Wine, red/ white/ blush	0.778
Mustard	0.18

Table 2 Total and added fructose content of food items (per 100g)

Food item	**Total sugar (g / 100g) (Assumed= added sugar)**	**Added fructose (g/ 100g) (assuming 50% of added sugar is fructose)**
Bread, brown	0.82	0.41
Traditional breads (markouk/tannour)	1.77	0.88
Breakfast cereals, regular/sugar coated/ chocolate/ bran	10.50	5.25
Kaak	1.26	0.63
Fruits canned	17.14	8.57
Fruit juice canned	12.42	6.21
Fruit juice bottled	12.42	6.21
Cakes/ Cookies/ Doughnuts/ Muffins/ Croissant/ Biscuits	24.85	12.42
Ice cream	19.16	9.58
Chocolate bar	57.82	28.91
Sugar, honey, jam, molasses, chocolate spread	99.80	49.9
Arabic sweets (Baklava, maamoul, knefe)	1.185	0.59
Soft drink, regular	11.55	5.77
Cocoa/ Hot chocolate	70.38	35.19
Manaeesh, zaatar/ cheese	0.09	0.045
Energy & sports drinks	10.06	5.03
Pizza	1.98	0.99
Canned/ Pre-packed soups	1.54	0.77
Ketchup	22.77	11.38

Added fructose was calculated and then categorized into tertiles, with T1 corresponding to <19.37 g/day, T2 being between 19.37 and 41.71g/day and T3 corresponding to >41.71g/day. Natural fructose intake was also categorized into tertiles, with T1 corresponding to <7.69g/day, T2 being between 71.69 and 13.84g/day and T3 corresponding to >13.84g/day. Total fructose consumption was calculated by adding added fructose and natural fructose intake (Hosseini-Esfahani et al,2011) which was

also categorized into tertiles: T1 corresponding to <30.92g/day, T2 being between 30.92 and 53.41g/day and T3 corresponding to >53.41g/day.

5. Statistical analysis

Statistical analysis was performed using the Statistical Analysis Package for Social Sciences, version 25.0 (SPSS Inc., Chicago, IL, USA). RTL was into first, second and third tertiles, 1st tertile corresponding to <1.12, 2nd tertile being between 1.12 and 1.55, and the third tertile corresponding to >1.55. Sociodemographic and lifestyle characteristics, anthropometric and biochemical measurements, as well as dietary intakes of the total study population and across RTL tertiles were computed as numbers and frequencies for categorical variables, means and standard deviations for continuous variables. The associations of potential predictors with RTL tertiles were first evaluated using univariate ANOVA and Chi-square test, as applicable.

Afterwards, logistic analyses were carried out, where the dependent variable was relative telomere length, to control for confounding effect when assessing the associations of total, added, and natural dietary fructose intakes with telomere length. More specifically, potential predictors for RTL tertiles were assessed by carrying out a multinomial logistic regression model which included socioeconomic, lifestyle, anthropometric, and metabolic variables that were found to be significantly associated with RTL in the univariate analysis at $p < 0.2$ in model 1 and $p < 0.05$ in model 2. Significant predictors were then included in the final outcome analysis. Results of the multivariate analyses are reported as odds ratio (OR). Correlation analysis using Pearson's coefficient (r) was also performed between RTL as a continuous variable and

each of total, added, and natural fructose (in grams). A $p < 0.05$ was considered to indicate statistical significance.

CHAPTER IV

RESULTS

A. Subject characteristics and dietary intakes/ Assessment of fructose intake

1. Socio-Demographic characteristics

The socio-demographic and lifestyle characteristics of the study population according to relative telomere length (RTL) tertiles, are presented in Tables 3 and 4, respectively. Mean age of the study participants was of 41.01 ±13.74 years, with a higher proportion of females compared to males (67.4% vs. 32.6%). Only (9.1%) of the study subjects had a monthly income greater than 2000$ per month. More than half of the participants had an education level up to intermediate, with only 13.9% having accomplished university level.

As for the lifestyle factors, most of the participants were current cigarette or nargileh smokers (68.8%), and almost half had a low level of physical activity (46.5%). Concerning alcohol consumption, the majority of the participants didn't consume alcohol (83.3%). More than half of the population reported sleeping less than 8 hours on weekdays and weekends.

Regarding socio-demographic characteristics, results showed that older age was significantly related to having shorter telomeres (51.1 vs 43.6 vs 31.9% for ages between 40 and 60 years, 10.6 vs 8.5 vs 7.4% for age greater than 60 years). However, no significant differences were found in lifestyle characteristics across RTL tertiles.

Table 2 Sociodemographic characteristics of the study population (n=282) across RTL tertiles.

Sociodemographic Characteristics	RTL Tertiles				
		<1.12 (n= 94)	**1.12-1.55 (n= 94)**	**>1.55 (n=94)**	**P-value[1]**
	Mean ± SD				
Age (years)	41.01 ±13.74	43.26 ±13.52	40.87 ±14.01	38.9 ±13.47	0.09
Crowding Index	1.57 ± 0.87	1.56	1.65	1.49	0.48
	n (%)				
Age (years)					
< 40	138 (48.9)	36 (38.3)	45 (47.9)	57 (60.6)	**0.04**
Between 40 and 60	119 (42.2)	48 (51.1)	41 (43.6)	30 (31.9)	
>60	25 (8.9)	10 (10.6)	8 (8.5)	7 (7.4)	
Gender					
Male	92 (32.6)	31 (33.0)	28 (29.8)	33 (35.1)	0.73
Female	190 (67.4)	63 (67.0)	66 (70.2)	61 (64.9)	
Marital Status					
Married	192 (68.1)	63 (67.0)	63 (67.0)	66 (70.2)	0.86
Not Married[2]	90 (31.9)	31 (33.0)	31 (33.0)	28 (29.8)	
Educational Level					
No school/primary	89 (31.7)	37 (39.4)	29 (31.2)	23 (24.5)	0.19
Intermediate	76 (27.0)	18 (19.1)	28 (30.1)	30 (31.9)	
Secondary/technical	77 (27.4)	24 (25.5)	27 (29.0)	26 (27.7)	
University	39 (13.9)	15 (16.0)	9 (9.7)	15 (16.0)	
Income Level					
<600$	75 (28.5)	23 (27.1)	29 (33.3)	23 (25.3)	0.11
Between 600 and 2000$	164 (62.4)	49 (57.6)	54 (62.1)	61 (67.0)	
>2000$	24 (9.1)	13 (15.3)	4 (4.6)	7 (7.7)	
Crowding Index					
≤ 1 person/room	109 (38.7)	38 (40.4)	35 (37.2)	36 (38.3)	0.90
> 1 person/room	173 (61.3)	56 (59.6)	59 (62.8)	58 (61.7)	

[1] ANOVA test was done to compare for significance of continuous variables and Chi-squared was done for significance of categorical variables

[2] Not married: Single, divorced and widowed

Table 3 Lifestyle characteristics of the study population (n=282) across RTL tertiles.

	RTL Tertiles				
		<1.12 (n= 94)	**1.12-1.55 (n= 94)**	**>1.55 (n=94)**	**P- value[1]**
Lifestyle Characteristics	**n (%)**				
Smoking status					
None- smoker	63 (22.3)	17 (18.1)	19 (20.2)	27 (28.7)	0.15
Current smoker (cigarette or nargileh)	194 (68.8)	68 (72.3)	70 (74.5)	56 (59.6)	
Ex-smoker	25 (8.9)	9 (9.6)	5 (5.3)	11 (11.7)	
Alcohol Consumption					
Yes	47 (16.7)	16 (17.0)	19 (20.2)	12 (12.8)	0.38
No	235 (83.3)	78 (83.0)	75 (79.8)	82 (87.2)	
Coffee Consumption					
Yes	220 (78.0)	73 (77.7)	80 (85.1)	67 (71.3)	0.07
No	62 (22.0)	21 (22.3)	14 (14.9)	27 (28.7)	
Physical activity					
None	42 (14.9)	18 (19.1)	13 (13.8)	11 (11.7)	0.33
Any	240 (85.1)	76 (80.9)	81 (86.2)	83 (88.3)	
Levels of Physical Activity					
Low-intensity activity	131 (46.5)	45 (47.9)	47 (50.0)	39 (41.5)	0.71
Moderate-intensity activity	87 (30.9)	30 (31.9)	25 (26.6)	32 (34.0)	
High-intensity activity	64 (22.7)	19 (20.2)	22 (23.4)	23 (24.5)	
Number of Hours sleep on weekdays					
4 hours or less	31 (11.0)	10 (10.6)	14 (14.9)	7 (7.4)	0.26
5 to 6 hours	72 (25.5)	32 (34.0)	20 (21.3)	20 (21.3)	
6 to 7 hours	77 (27.3)	27 (28.7)	23 (24.5)	27 (28.7)	
7 to 8 hours	59 (20.9)	12 (12.8)	23 (24.5)	24 (25.5)	
8 to 9 hours	29 (10.3)	8 (8.5)	9 (9.6)	12 (12.8)	
9 hours or more	14 (5.0)	5 (5.3)	5 (5.3)	4 (4.3)	
Number of Hours sleep on weekends					
4 hours or less	31 (11.0)	10 (10.6)	12 (12.8)	9 (9.6)	0.14
5 to 6 hours	49 (17.4)	22 (23.4)	15 (16.0)	12 (12.8)	
6 to 7 hours	62 (22.0)	23(24.5)	22 (23.4)	17 (18.1)	
7 to 8 hours	61 (21.6)	12 (12.8)	24 (25.5)	25 (26.6)	
8 to 9 hours	39 (13.8)	10 (10.6)	11 (11.7)	18 (19.1)	
9 hours or more	40 (14.2)	17 (18.1)	10 (10.6)	13 (13.8)	
Sleeping Difficulties					
Never	70 (24.8)	21 (22.3)	21 (22.3)	28 (29.8)	0.28
Rarely/sometimes/ frequently	90 (31.9)	34 (36.2)	25 (26.6)	31 (33.0)	
Almost always	122 (43.3)	39 (41.5)	48 (51.1)	35 37.2)	

[1] Chi-squared was done for significance of categorical variables

2. *Anthropometric, Biochemical and Blood Pressure Characteristics*

Table 5 presents the sample's anthropometric and biochemical measurements as well as blood pressure data according to RTL tertiles.

The results showed mean WC was significantly higher in the shorter telomere length categories (94.82 ±12.40 vs. 90.33 ±13.81 vs. 90.73 ±13.84). Similarly, mean LDL-C was significantly higher in the shorter telomere length categories (116.33 ±37.94 vs. 107.74 ±34.39 vs. 96.85 ±28.88).

Table 4 Anthropometric, biochemical and blood pressure characteristics of the study population (n=282) across RTL tertiles.

			RTL Tertiles		
		<1.12 (n=94)	**1.12-1.55 (n=94)**	**>1.55 (n=94)**	**P- value[1]**
Anthropometric and cardiometabolic characteristics			**Mean ± SD**		
BMI (kg/m2)	28.05 ±5.63	28.56 ±5.55	27.84 ±5.63	27.75 ±5.72	0.56
Waist Circumference (cm)	91.96 ±13.48	94.82 ±12.40	90.33 ±13.81	90.73 ±13.84	**0.04**
Body fat (kg)	27.15 ±11.21	28.32 ±10.82	26.76 ±10.98	26.34 ±11.81	0.44
Blood pressure					
Systolic Blood Pressure (mmHg)	116.71 ±16.65	116.32 ±15.96	118.13 ±19.21	115.66 ±14.51	0.57
Diastolic Blood Pressure (mmHg)	72.89 ±9.63	73.29 ±8.48	72.38 ±11.27	72.98 ±8.96	0.80
Lipid levels					
Triglycerides (mg/dL)	120.79 ±70.99	116.21 ±61.41	127.35 ±79.27	118.81 ±71.42	0.53
LDL (mg/dL)	106.98 ±34.76	116.33 ±37.94	107.74 ±34.39	96.85 ±28.88	**0.001**
HDL (mg/dL)	51.96 ±15.88	54.10 ±16.29	49.86 ±14.14	51.93 ±16.96	0.18
Fasting blood sugar (mg/dL)	98.18 ±13.33	97.66 ±8.84	98.05 ±10.92	98.84 ±18.41	0.82

[1] ANOVA test was done to compare for significance of continuous variables.

3. Dietary Energy and Macronutrient Intakes

Table 6 presents the dietary and macronutrient intakes of the study participants according to RTL tertiles. The average intake of total dietary fructose was of 51.31 ±35.55g/day which represents 6.58 ±3.71% of the total energy intake (EI). Intakes of natural and added fructose were estimated at 12.28 ±8.59g/day and 39.03 ±34.12g/day (1.78 ±1.41% and 4.80 ±3.56% EI), respectively. Intakes of total sugar and total fructose were found to differ significantly across RTL tertiles.

Table 5 Intakes of macronutrients and fructose in the study population (n=282) across RTL tertiles.

			RTL Tertiles			
			<1.12 (n=94)	**1.12-1.55 (n=94)**	**>1.55 (n=94)**	**P-value[1]**
Energy & Macronutrients	**Mean ± SD**	**Median (IQR)**				
Energy (kcal)	3123 ±1290	2913 (1756)	3015 ±1229	3223 ±1203	3131 ±1431	0.54
Carbohydrates (g)	387.38 ±157.81	364.09 (210.2)	373.49 ± 150.25	403.23 ± 156.30	385.40 ±166.70	0.43
Percent Carbohydrates (%)	50.41 ±8.29	50.76 (10.30)	50.37 ±8.23	50.35 ± 8.01	50.51 ± 8.72	0.99
Protein (g)	102.19 ±59.79	91.68 (62.0)	97.38 ± 44.32	102.04 ± 48.69	107.16 ± 80.11	0.53
Percent Protein (%)	13.01 ±3.64	12.37 (3.52)	12.97 ± 3.39	12.53 ± 2.59	13.52 ± 4.62	0.17
Fat (g)	131.19 ±64.25	119.20 (88.4)	126.68 ± 64.76	136.08 ± 57.25	130.82 ±70.40	0.6
Percent Fat (%)	39.09 ±7.87	38.36 (10.11)	38.87 ± 7.88	39.70 ± 8.34	38.69 ± 7.42	0.64
Saturated Fat (g)	36.88 ±20.23	32.59 (25.40)	36.36 ±21.17	38.53 ±18.73	35.76 ±20.82	0.61
Percent Saturated Fat (%)	10.31 ±2.72	10.21(3.37)	10.34 ±2.59	10.49 ±2.70	10.11 ±2.90	0.64
Fiber (g)	28.08 ±11.72	26.27 (14.56)	27.16 ±12.57	29.70 ±11.59	27.37 ±10.89	0.25
Total Sugars (g)	104.80 ±58.47	91.42 (66.23)	98.11 ±54.60	118.50 ±65.93	97.82 ±52.14	**0.02**
Fructose	**Mean ± SD**	**Median (IQR)**				
Total fructose (g)	51.31 ±35.55	41.96 (37.23)	48.06 ±36.02	59.06 ± 38.33	46.82 ± 31.01	**0.03**
Percent total fructose (%)	6.58 ±3.71	5.96 (4.13)	6.48 ± 3.98	7.23 ± 3.94	6.03 ±3.08	0.08
Natural fructose (g)	12.28 ±8.59	10.34 (10.12)	12.04 ±8.21	13.25 ±9.97	11.54 ±7.37	0.37
Percent natural fructose (%)	1.78 ±1.41	1.42 (1.48)	1.88 ±1.63	1.80 ±1.46	1.65 ± 1.10	0.53
Added fructose (g)	39.03 ±34.12	28.34 (36.33)	36.01 ± 34.08	45.80 ±37.80	35.27 ± 29.28	0.06
Percent added fructose (%)	4.80 ±3.56	4.00 (3.75)	4.59 ±3.63	5.43 ± 3.96	4.38 ±2.97	0.10

[1] ANOVA test was done to compare for significance of continuous variables.

4. *Intakes of food groups*

Table 7 shows the participants' intakes of different food groups in grams per day. No significant differences were found between RTL tertiles and food groups.

Table 6 Food groups intakes (in grams/day) across RTL tertiles in the study population (n=282)

		RTL Tertiles			
		<1.12(n= 94)	**1.12-1.55 (n= 94)**	**>1.55 (n=94)**	**P- value[1]**
Food Group	**Mean ±SD**				
Grain and grain products (g/d)[2]	471.71 ±233.98	461.52 ±231.03	470.45 ±242.59	483.16 ±230.13	0.81
Fruit (g/d)[3]	340.15 ±228.24	315.10 ±186.04	268.05 ±269.67	337.31 ±220.53	0.28
Vegetables (g/d)	236.53 ±175.84	247.42 ±176.60	232.47 ±193.82	229.71 ±156.50	0.76
Meat and other protein sources (g/d)[4]	118.16 ±164.97	105.69 ±86.94	107.33 ±102.59	141.46 ±251.63	0.24
Milk and milk products (g/d)[5]	164.37 ±136.01	170.12 ±157.99	180.07 ±129.10	142.92 ±116.27	0.15
Fats and oils (g/d)[6]	67.65 ±50.13	67.07 ±49.41	71.35 ±55.45	64.53 ±45.33	0.64
Sweets, sweetened beverages and desserts (g/d)[7]	470.14 ±544.67	441.93 ±596.89	540.19 ±581.55	429.31 ±440.89	0.30
Savory snacks (g/d)[8]	86.20 ±88.85	79.85 ±96.52	94.10 ±89.06	84.67 ±80.61	0.53
Condiments and sauces (g/d)[9]	4.22 ± 10.42	4.17 ±11.79	3.32 ±6.54	5.18 ±12.02	0.47
Water and other unsweetened beverages (g/d)	1543.02 ±1134.48	1621.26 ±936.56	1551.30 ±1344.05	1456.49 ±1091.50	0.50

[1]ANOVA test was done to compare for significance of continuous variables.
[2] Grain and Grain products include: Whole grains, refined grains, legumes, canned legumes, manaeesh and pizza
[3] Fruit includes: Fresh and canned fruit
[4] Meat and other proteins sources include: Red meat, processed meat, poultry, fish, eggs and organ meats
[5] Milk and milk products include: Full fat and low-fat dairy
[6] Fats and oils include: Nuts, seeds and added fats
[7] Sweets, sweetened beverages and desserts include: Sweets and desserts, soft drinks and sweetened beverages
[8] Savory snacks include: Fries and chips
[9]Water and other unsweetened beverages include: Diet soft drinks, hot beverages, water and alcohol

B. Association of fructose intake with relative telomere length

For the purpose of investigating the association between fructose intake (tertiles) and telomere length (tertiles), we performed multinomial logistic regression analyses. Hence, two models were presented. Model 1 was adjusted for variables that showed an association with RTL in the univariate model at a p-value <0.2, and model 2 was adjusted for variables that were associated with RTL at a p-value<0.05. No significant associations were found.

Table 7 Logistic regression analysis examining associations between total, added and natural fructose (tertiles)and RTL tertiles

		Model 1[1]		Model 2[2]	
		AOR (95% CI)	**P-value**	**AOR (95% CI)**	**P-value**
Total fructose intake tertiles (g/day)	T1	0.95 (0.52-1.72)	0.87	1.08 (0.62-1.90)	0.76
	T2	0.77 (0.43-1.37	0.38	0.83 (0.48-1.44)	0.51
	T3	Reference	Reference	Reference	Reference
Added fructose intake tertiles (g/day)	T1	0.86 (0.46-1.59)	0.63	1.01 (0.57-1.78)	0.97
	T2	1.13 (0.63-2.03)	0.66	1.35 (0.78-2.35)	0.27
	T3	Reference	Reference	Reference	Reference
Natural fructose intake tertiles (g/day)	T1	1.07 (0.60-1.91)	0.80	0.98 (0.57-1.68)	0.94
	T2	0.79 (0.45-1.39)	0.42	0.81 (0.47-1.39)	0.45
	T3	Reference	Reference	Reference	Reference

[1] Model 1: Adjusted for variables that have a p-value <0.2: Age, gender, education status, smoking status, coffee consumption, waist circumference, LDL, HDL, Sleeping Hrs. on weekends, percent protein.
Income was not included in the model given that it was found to be collinear with education
[2] Model 2: Adjusted for variables that have a p-value <0.05: Age, gender, LDL, waist circumference

CHAPTER V

DISCUSSION

Telomeres play a critical role in maintaining the genome's structural integrity and in protecting chromosomes from end-to-end fusion and degradation (Blackburn, 2000). An increasing number of epidemiologic and clinical data suggest that accelerated shortening of telomere length is linked with diseases of aging such as higher risk of coronary heart disease, diabetes, and heart failure (Demissie et al, 2006). Our study showed that shorter telomere lengths was associated with higher age, elevated WC and higher LDL-levels. However, our study did not find any significant association between telomere length and fructose intakes (total, added or natural).

Based on a cross-sectional community-based study conducted in a sample of Lebanese urban adults, our study showed that telomeres' length shortened with age, a finding that is in agreement with that reported by several previous studies where telomere shortening was progressive with aging and was associated with age-related diseases (Cawthon et al, 2003) (Al-Attas et al,2010). Our study also showed a significant relationship between increased waist circumference (WC) and shorter telomere length (RTL). These findings are consistent with those reported by a previous study conducted in 2018 by Batsis et al, amongst 7,827 subjects, based on the National Health and Nutrition Examination Survey 1999–2002. Another study conducted on 2,912 Chinese women aged 40-70 years old, also reported an inverse association between telomere length and waist circumference (Cui Yong et al, 2013). A significant relationship was also found between LDL and RTL tertiles in our study. Consistent with these findings, Koriath et al's (2019) study on 4944 subjects from the population-based

Gutenberg Health Study showed an association between telomere length and higher LDL-C levels in the oldest tertile of age. Another study that examined 305 subjects in Belgium also showed that age and gender-adjusted telomere length was inversely associated with LDL (Nawrot et al, 2010). Other studies, however, did not find such associations (Chen et al, 2019).

Given that available evidence suggests that lifestyle and dietary factors may affect telomere length (Slagboom et al, 1994), the aim of the present study was to investigate the association of dietary intakes, and particularly fructose intake (total, added and natural), with telomere length. This hypothesis was derived from the fact that high consumption of fructose, especially in its added form, can increase oxidative stress and inflammation, both of which can impact telomere attrition (Sheils et al, 2011). The study showed that total dietary fructose intake was of 51.31 ±35.55g/day, a value that exceeds the proposed upper limit for fructose intake (>50g/day) (Livesey, 2009). Moreover, when compared with data reported from other countries, the consumption of dietary fructose in Lebanon appears to be slightly higher. For instance, according to Sun et al 2014, the dietary intake of total fructose in the US was estimated at 48.07 ± 35.73 g/day amongst adults. In Germany, fructose intake was found to range between 8.4 and 40.6g/day and 11–34.8g/day, in men and women respectively (Schulze et al., 2008); and in Finland dietary fructose intake was estimated to range between 6 and 28.8g/day (Montonen et al., 2007).

In our study, we have gone beyond the evaluation of total fructose, and performed an assessment of added vs natural fructose intakes. This was deemed of importance given that natural fructose, which is present in fruits and vegetables, is not hypothesized to contribute to Telomere shortening (Shammas, 2011). In fact, fruits and

vegetables include a wide range of nutrients, phytochemical and antioxidants that may actually prevent or delay telomere lengthening (Garcia-Calzon et al, 2015). On the other hand, we hypothesized that added fructose from added sugar and sweetened foods/beverages that are poor in nutrients, may contribute to the shortening of telomere length. In our study, the average intake of added fructose was of 39.03 (±34.12) g/day, providing approximately 5% of energy intake (EI), while that of natural fructose was of 12.3 g/day (±8.59), contributing 1.8% of EI. Few studies have undertaken such evaluations, which limits our ability to compare our findings with the literature. A study conducted in Iran estimated added fructose intake at 26.9 ± 13.9 and 19 ± 13.7 g/day, in men and women respectively (Hosseini-Esfahani et al, 2011), estimates that are lower than those obtained in the current study.

However, it is worth noting that no significant associations were found in our study between fructose consumption, in any form (total, added and natural), and relative telomere length. The association between telomeres' shortening and dietary intakes is actually disputable. Some studies have reported both an increase and decrease in telomere length, as a result of dietary exposures (Pérez et al, 2017) (Kasielski et al,2016). In agreement with our findings, a recent systematic review and meta-analysis of RCTs that investigated the influence of diet on telomere length, showed that there is no impact of dietary intakes on telomere length (Perez et al, 2017). This systematic review has also highlighted a high heterogeneity in the available studies, in terms of study duration and type of dietary interventions (Perez et al, 2017). In addition, in a cross-sectional study conducted among 840 white, black, and Hispanic adults from the Multi-Ethnic Study of Atherosclerosis, no associations were found between telomere length and sugar-sweetened soda consumption (Nettleton et al,2008). Moreover,

Previous narrative reviews have however reported an association between diet and telomeres' shortening. A review of epidemiological studies and randomized trials regarding nutrients, foods, dietary patterns and telomere length, showed that consumption of saturated fat, meat and meat products, refined flour cereals and sugar-sweetened beverages (SSBs) is associated with shorter telomeres (Freitas-Simoes et al,2016). In addition, a cross-sectional study that examined associations between SSBs and telomere length in a nationally representative sample of healthy adults from the National Health and Nutrition Examination Surveys (N=5,309) (NHANES), found out that SSB consumption was associated with shorter telomeres (Leung et al,2014). The study argued that SSBs were related to shorter telomeres due to their effect on oxidative stress, systemic inflammation, and insulin resistance (Malik et al, 2010). (Shiels et al, 2011). However, in the NHANES study no significant association was found between consumption of non-carbonated SSBs and telomere length. They claimed that this could be due to the large degree of heterogeneity of the sugar content across the beverages (Harris et al, 2011). Also, the study sample had a lower-than-average consumption of non-carbonated SSBs compared to carbonated SSBs, hence they proposed that sugar consumption might affect telomere length only at higher levels of intake (Leung et al, 2014). It is important to note that in Leung et al's NHANES study, where a positive association between SSBs and telomeres was observed, average consumption of SSBs was of 1.5 servings per day (12 ounces/day) which approximately provide 74 grams of added sugars, (Neuhofer et al, 2020). However, in our study sample, the highest tertile of added fructose intake was of 40.71g/day, which is less than the amount reported by the NHANES study. Hence, it might be possible to argue that we needed to have a higher level of added fructose consumption, in order to get significant associations.

The results of this study may be considered in light of the following limitations. First, the small sample size and the cross-sectional design of the study may have prevented us from detecting an association between RTL and Fructose intake. It was in fact not possible for us to take into consideration longitudinal changes in telomere length in relation to diet. However, to decrease possible reverse causation, participants who reported the diagnosis of a chronic disease or metabolic abnormalities that may have affected their dietary habits were excluded from the study. Moreover, it may be possible to argue that although adjustment for demographic and lifestyle factors that were previously shown to be associated with telomere length was carried out, the presence of residual confounding could remain a possibility that might have affected our results. Although every effort was done to measure these covariates accurately, residual confounding could be unavoidable, to some degree (Nettleton et al, 2008).

The method adopted for the assessment of dietary fructose may also be associated with some limitations. In fact, we have used a semi-quantitative food-frequency questionnaire (FFQ) for the collection of dietary data, which, similarly to other dietary assessment methods, may be associated with recall bias and misreporting of intakes (Kushi, 1994). However, despite the limitations of the FFQ, it was shown to be amongst the most suitable tools for dietary assessment in large epidemiological studies, and particularly in the ranking of individuals based on their dietary intakes (Shahar et al, 2003). The FFQ is also an appropriate method for the estimation of dietary intake over a long period of time (Shim, Oh and Kim, 2014). The fact that data collection in our study was conducted in an interview setting may have led to a social desirability bias and misreporting of dietary intakes in a way that is perceived as favorable to the interviewer (Hebert et al., 1995). However, in our study, data collection

was performed by fieldworkers who had received extensive training to reduce judgmental verbal and nonverbal communication in order to minimize any social desirability bias.

Moreover, WC cut off points were not specific to the Lebanese or Middle Eastern population. Cut off values that are applicable to the European population were used and it may not be fully applicable to sample, because lower cut off points were suggested for ethnic Arabs according to some studies (Al-Lawati & Jousilahti, 2008). Finally, the present study was restricted to an urban setting, and thus, findings pertinent to the consumption levels of fructose may not be representative of less urban settings in the country. The choice of Beirut for this study may be explained by the fact that it hosts 40 % of the Lebanese population and is usually considered a melting pot of the country (Nasreddine et al, 2003).

CHAPTER V

CONCLUSION

This study is the first from the Eastern Mediterranean region to investigate the association between fructose intake and telomere length. It documented high intake levels of fructose but did not find any significant association between telomere length and fructose intakes (total, added or natural). Although our findings are in line with those reported by a recent systematic review (Perez et al, 2017), they are to be interpreted with caution, given the small sample size and the cross-sectional nature of our study. Larger studies, of longitudinal nature, are needed to better elucidate the relationship between fructose intakes and telomere lengths.

APPENDIX I

CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Consent to participate in a genetic research study

Assessment of BPA levels and their association with the health status among Lebanese population

Protocol number: IM.HT.03
Investigator: Dr. Hani Tamim
Address: American University Hospital
Hamra Street
Beirut, Lebanon
Phone: (01) 350 000 ext: 5453

Site where the study will be conducted: AUBMC

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You are being asked to participate in a clinical research study conducted at the American University of Beirut. 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 the representative of the contracted company if you need more information or clarification about what is stated in this form and the study as a whole.

The aim of our study is to measure Bisphenol A (BPA) levels in a representative sample from the Lebanese population residing in Greater Beirut, and to assess if it is related to different diseases. We also would like to see if BPA measures change over time in any person. This study will be composed of 2 stages; at baseline and a 2-year follow up. We will be recruiting approximately 500 subjects and study will be conducted at AUBMC whereby this informed consent along with the data collected will be used for this study only.

BPA is a synthetic chemical that interferes with the natural hormones in the body. It can be found in plastic bottles and water containers, baby bottles and toddler cups, plastic ware, the inner lining of food cans and beverages. Humans may ingest BPA if it leaches from the plastic container into the food or drink under certain conditions. Consumption is associated with adverse health effects including heart disease, high blood pressure, diabetes, changes in cholesterol, triglycerides, and thyroid levels. BPA can also affect the expression of DNA material, called 'epigenetic effect.

The CITI certified field workers employed by the contracted company (Information International) will use the direct approaching method to recruit the cohort. They will visit the respondents in their residence to explain the study aims and method of implementation. Then the respondents will be consented and given the details of the date and time of the study. The name, date of birth, availability on week days and telephone number of the potential participant will be recorded for further follow up to specify the exact date for taking them to AUBMC. Each visit will include 10 participants who will complete the procedures described below.

Participating in this study means that you will sit with a certified research assistant who will conduct a survey which includes multiple questions about the demographic and socioeconomic status (Age, gender, location, education, occupation, income), lifestyle (smoking, alcohol, coffee, physical activity),

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health status (medical history and medication), and dietary habits (Food Frequency Questionnaire). Moreover, you will undergo a physical exam to measure weight, height, waist circumference, blood pressure, and heart rate. Moreover, your blood sugar will be checked by a fingerstick, which means a very small prick will be done to your finger to get less than a drop of blood to do the test. You will also be asked to have blood withdrawn for specific genetic testing (DNA methylation) and clinical laboratory tests (including HBA1c, fasting blood sugar, creatinine, lipid profile, TSH, SGPT, GGT, fasting insulin, urinary creatinine, microalbuminuria, 25 OH vit D, Cortisol, leptine, C-peptide, prolactin). Moreover, urine will be collected for measuring BPA levels. These tests will be done free of charge, but will be done at a later time during the study.

During your visit, the duration for completing the procedures is expected to be for around an hour and a half over one day only, divided between 30 minutes for blood withdrawal and urine collection and 60 minutes for filling the surveys for each participant. Your total visit time to AUBMC is expected to be for a maximum of 3 hours, given that there will be other participants undergoing the same process.

After around 2 years from the baseline visit, you will be contacted by phone to be invited to complete the second part of the study (2-year follow-up stage) by visiting the AUBMC and going through the same process as the one described at baseline.

Although any study may be associated with any unforeseeable risk, this proposal has minimal risk. None of the data collection measures bare any long term hazards, and all blood withdrawal will be done under sterile hygienic conditions and the total volume required is 20 cc. Possible side effects include mild pain, bleeding, bruising at the site of the needle insertion. Fainting or light-headedness can sometimes occur, but usually last only a few minutes.

The results of all tests conducted will be freely provided to the participants by calling them and providing them with the results of the test upon its completion. Moreover, the participants will be compensated for travel expenses with 30,000 LBP upon arriving to AUBMC. In addition, we will provide the participants with breakfast the same day.

If you agree to participate in this research study, the information will be kept confidential. Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your information collected.

All data and biological samples collected will be stored in a confidential manner. These measures will all be conducted ensuring there is no breach of participants' privacy. Moreover, the remaining blood and urine samples will be stored securely indefinitely in Dr. Nathalie Zgheib Khoueiry's laboratory at the AUBMC. If you elect to withdraw your consent for the study, your samples will be destroyed.

You may ask that we provide you with the genetic results and explain their significance to you. The information will be kept confidential.

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I would like to know if you would be willing to participate in this study. You have the right to accept or decline participation. Refusing to participate will not involve any loss of benefits offered in the future by AUBMC. Moreover, you are entitled to withdraw from the study at any time without any loss of benefits offered by AUBMC at any time.

I agree to participate in this study and the procedures explained above.

YES .... NO............

I agree to be contacted for future studies

YES .... NO............

I would like to be contacted if the genetic test results are significant

YES .... NO............

Using remaining blood and urine for other future studies

We would like to keep the remaining blood and urine samples for potential use in other future studies. To do so, there might be future collaborators at AUB, at other institutions in Lebanon and/or outside Lebanon. There will be no extra prick. The stored blood and urine samples will be coded (*"Coded" means identifiable, traceable. Blood* and urine *samples that are unidentified for research purposes but can be linked to their source through the use of codes; however, the principal investigators or VMP will be the only ones to have the list linking patients to the codes assigned.)*

I agree to permit the use of the remaining blood and urine sample for future studies

YES .... NO............

Your coded blood and urine samples may be shared with other investigators for related studies. These investigators will not know your identity.

I agree to have my coded blood and urine samples shared with other investigators for related studies.

YES .... NO............

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Patient's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. ____Hani Tamim at 01350000 extension: 5453 or any of his/her designee involved in the study in case of any questions. If I felt that my questions have not been answered, I can contact the Institutional Review Board for human rights at 01350000 extension: 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 the care I might receive at AUBMC. I also understand that my participation may be ended by investigator at anytime. I know that I will receive a copy of this signed informed consent.

______________________________ ______________________________

Name of patient or Legal Representative or Parent/Guardian — Signature

____

Date & Time

______________________________ ______________________________

Witness's Name — Signature

____

Date & Time

Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _________ ________________________ (name of patient, legal representative, or parent/guardian) the purpose of the study and its risks and benefits. I have answered all the patient's questions clearly. I will inform the participant in case of any changes to the research

______________________________ ______________________________

Name of Investigator or designee — Signature

____

Date & Time

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APPENDIX II

DATA COLLECTION FORM

Assessment of BPA levels and their association with the health status among Lebanese population

Name:	**Initials:**	**Study ID number:**
Tel number:		**Date:**

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Demographic Factors:

Date of birth:	**Gender:** □ Males □ Females
Marital status: □ Married □ Single □ Widow □ Divorced □ Engaged	

Socioeconomic:

Have you lived outside Lebanon for the past year: □ No □ Yes	
If yes, where ____________ and for how long ____________	
Which area do you live?	
What do you work?	
What is your income per family:	□ <600$ □ 600- 999.9$ □ 1000-2000$ □ >2000$ □ I don't know/ Not sure □ I prefer not to answer
What is your highest level of education?	□ No schooling □ Primary school □ Intermediate school □ Secondary school □ Technical diploma □ University degree □ I prefer not to answer
What is the total number of individuals living in your house? (Including relatives, family members and maids that frequently live with you on a semi-permanent basis)	
How many rooms are there in your house? (Excluding kitchens, bathrooms, hallways, balconies, and garage)	

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Lifestyle:

Smoking history			
Cigarette	Do you currently smoke cigarettes?	□ No □ Yes	If yes, how many cigarettes/day? Since when?
	If no, are you a previous cigarette smoker?	□ No □ Yes	If yes, when did you stop?
Narghileh	Do you currently smoke narghileh?	□ No □ Yes	If yes, how many narghileh/day? Since when?
	If no, are you a previous narghileh smoker?	□ No □ Yes	If yes, when did you stop?

Alcohol		
Do you currently drink alcohol?	□ No □ Yes	
	If yes specify type? Since when?	How many glasses/week?
Previous drinker?	□ No □ Yes	If yes, when did you stop?

Coffee	
Do you currently drink coffee?	□ No □ Yes If yes how many cups/day?

Physical activity	
During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, aerobics, or fast bicycling for at least 10 minutes (or any activity that take hard physical effort and make you breathe harder than normal)?	--------------- days/week □ None - How much time in total did you usually spend on one of those days doing vigorous physical activities? _____ hours _____ minutes? - How many weeks did you spend doing vigorous physical activities during the last 3 months? --------------weeks
During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or tennis or any activity that take hard physical effort and make you breath harder than normal)? Do not include walking.	--------------- days/week □ None - How much time in total did you usually spend on one of those days doing moderate physical activities? _____ hours _____ minutes? -How many weeks did you spend doing moderate physical activities during the last 3 months? --------------weeks
During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for sport, exercise or leisure.	--------------- days/week □None - How much time in total did you usually spend walking on one of those days? _____ hours _____ minutes? -How many weeks did you spend walking during the last 3 months? --------------weeks
During the last 7 days, how much time in total did you usually spend sitting on a week day? This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.	_____ hours _____ minutes? -How many weeks have you been spending the same time in terms of sitting during the last 3 months? ---------------weeks

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Medical History:

Coronary artery disease:

Do you have any family member who has been diagnosed with coronary artery disease or died suddenly?	□ No □ Yes If yes: specify who	At what age:
Have you been told by a doctor that you had a heart attack?	□ No □ Yes If yes when:	
Did you undergo cardiac catheterization?	□ No □ Yes If yes when:	
Was a stent placed?	□ No □ Yes If yes when:	
Did you have coronary heart bypass surgery?	□ No □ Yes If yes when:	

Hypertension:

Have you been told by a doctor or a health care worker that you have high blood pressure?	□ No □ Yes If yes when:
Have you had your blood pressure measured by a doctor or a health care worker?	□ No □ Yes If yes when? What was it?
Are you taking any treatment for high blood pressure?	□ No □ Yes If yes specify: □ Life style modifications □ Drugs:

Diabetes Mellitus:

Have you been told by a doctor or a health care worker that you have raised blood sugar or diabetes?	□ No □ Yes If yes when:
Have you had your blood sugar measured by a doctor or a health care worker?	□ No □ Yes If yes when? What was it?
Are you taking any treatment for high blood sugar or diabetes?	□ No □ Yes If yes specify: □ Life style modifications □ Drugs:

Dyslipidemia:

Have you been told by a doctor or a health care worker that you have raised cholesterol or triglycerides?	□ No □ Yes If yes when:
Have you had your cholesterol measured by a doctor or a health care worker?	□ No □ Yes If yes when? What was it?
Are you taking any treatment for dyslipidemia?	□ No □ Yes If yes specify: □ Life style modifications □ Drugs:

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Thyroid disease:

Have you ever been told by a doctor or a health care worker that you have thyroid disease?	□ No □ Yes If yes when? What was the disease?
Have you had your thyroid hormones measured by a doctor or a health care worker?	□ No □ Yes If yes when? What was it?
Are you taking any thyroid drug?	□ No □ Yes If yes specify:
Do you have any family history of thyroid disease? (Parents, siblings and grandparents)	□ No □ Yes If yes specify who:

Cancer history:

Have you ever been told by a doctor or a health care worker that you have cancer?	□ No □ Yes If yes when? What was the disease?
Are you taking any chemotherapy or other drug for cancer?	□ No □ Yes If yes specify
Do you have any family history of cance? (Parents, siblings and grandparents)	□ No □ Yes If yes specify the disease: Specify who:

Fracture history:

Did you ever sustain a fracture?	□No □Yes
If yes:	Where? Age at onset? How did it happen? (fall from height, accident…)?

Other diseases:

Have you been told by a doctor or a health care worker that you have any?	
Stroke?	□No □Yes If yes when:
Arthritis?	□No □Yes If yes when:
Chronic bronchitis or emphysema?	□No □Yes If yes when:
Liver disease?	□No □Yes If yes when:

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Do you have any other illnesses?

Dentist visits:

Have you visited any dentist in the past year?	□ No □ Yes If yes when:
Did you have any fillings done in the past year?	□ No □ Yes If yes when:

Medications (if not brought, call the participant later)

Name (brand and generic)	Dose	Date started

Review of system:

Do you have any weight changes during the last 3 months?		□ Stable weight □ Lost weight How many Kgs? □ Gained weight How many Kgs?
For women:	When was your last menstrual period?	
	Are you: □ premenopausal □ postmenopausal	If premenopausal do you have □ Regular mences □ Irregular mences
	Do you have? □ Acne □ Hirsutism	

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Physical Exam Form

Name of the participant: ------------------------ Initials:-------------------- Study ID number: ----------------

	Results النتائج	Healthy ranges النطاقات الصحية
Body weight (kg الوزن		
Height (cm): الطول		
BMI: مؤشر البدانة		*18.5-24.9 kg/m²*
Waist circumference (cm): قياس دائرة الخصر		نساء *<80 cm*, رجال *<94 cm*
Body fat (kg): نسبة الدهون في الجسم		نساء *<32%;* رجال *<25%*
Muscle mass (kg): نسبة العضل في الجسم		نساء *24-30 %;* رجال *33-40%*
Waist to hip ratio: قياس محيط الأوراك		نساء *<0.9,* رجال *<0.85*
Heart rate: قياس نبض القلب		*50-100 bpm*
Blood Pressure – Measurement # 1 قياس ضغط الدم 1		
Systolic blood pressure (mmHg): العالي		*120 mmHg*
Diastolic blood pressure(mmHg): الواطي		*80 mmHg*
Blood Pressure – Measurement # 22 قياس ضغط الدم 2		
Systolic blood pressure (mmHg): العالي		*120 mmHg*
Diastolic blood pressure(mmHg): الواطي		*80 mmHg*

Time of urine collection	
Time of blood withdrawal	

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Sleep Habits and Berlin questionnaires

1- How many hours do you sleep per night on weekdays?					
4 hrs or less	5 to 6 hrs	5 to 7 hrs	7 to 8 hrs	8 to 9 hrs	9 hrs or more

2- How many hours do you sleep per night on weekends?					
4 hrs or less	5 to 6 hrs	5 to 7 hrs	7 to 8 hrs	8 to 9 hrs	9 hrs or more

3- Do you feel that you are not getting enough sleep?				
Never	Rarely (1 / month)	Sometimes (2-4 / month)	Frequently (5-15 /month)	Almost Always (16-30 / month)

4- Do you have Trouble falling asleep?				
Never	Rarely (1 / month)	Sometimes (2-4 / month)	Frequently (5-15 /month)	Almost Always (16-30 / month)

5- Do you wake up during the night and have difficulty resuming sleep?				
Never	Rarely (1 / month)	Sometimes (2-4 / month)	Frequently (5-15 /month)	Almost Always (16-30 / month)

6- Do you wake up too early in the morning and be unable to resume sleep?				
Never	Rarely (1 / month)	Sometimes (2-4 / month)	Frequently (5-15 /month)	Almost Always (16-30 / month)

7- Did your doctor tell you that you have sleep apnea?	
Yes	No

8- Do you snore?		
Yes	No	Don't Know

9- If you snore, your snoring is?			
a. Slightly louder than breathing	b. As loud as taking	c. Louder than talking	d. Very loud-can be heard in adjacent rooms

10- If you snore, how often do you snore?				
a. Nearly every day	b. 3-4 times a week	c. 1-2 times a week	d. 1-2 times a month	e. Never or nearly never

11- If you snore, has your snoring ever bothered other people?		
Yes	No	Don't Know

12- Has anyone noticed that you quit breathing during sleep?				
a. Nearly every day	b. 3-4 times a week	c. 1-2 times a week	d. 1-2 times a month	e. Never or nearly never

13- How often do you feel tired or fatigued after you sleep?				
a. Nearly every day	b. 3-4 times a week	c. 1-2 times a week	d. 1-2 times a month	e. Never or nearly never

14- During your waking time do you feel tired, fatigued or not up to par?				
a. Nearly every day	b. 3-4 times a week	c. 1-2 times a week	d. 1-2 times a month	e. Never or nearly never

15-Have you ever nodded off or fallen asleep while driving a vehicle?	
Yes	No

16- If yes, how often does this occur?				
a. Nearly every day	b. 3-4 times a week	c. 1-2 times a week	d. 1-2 times a month	e. Never or nearly never

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FOOD FREQUENCY QUESTIONNAIRE

Name of the participant: ------------------------------- Initials:--------------------------- Study ID number: -------------------------------

Please think about your eating patterns during ***the past year****. Please indicate your usual intake of each of the following food items per day, week, or month. Please be as precise as you can in your recall.*

Code	Food item	Reference Portion	Serving Size	Day	Week	Month	Rarely/Never
	Examples:						
	Rice, white, cooked	*A side*	*½ A 11*		*3*		
	Cheese, regular	*B side/ Thickness*	*B1 / Th. 2*	*4*			
	Legumes, canned (beans, peas)	*Side A/ Page 4*	*1.5 cups*		*2*		
1	**Bread and Cereals**						
1.1	Bread, white	1 large Arabic loaf					
		1 medium Arabic loaf					
		1 French baguette					
		1 pain de mie/ toast					
1.2	Bread, brown	1 large Arabic loaf					
		1 medium Arabic loaf					
		1 French baguette					
		1 pain de mie/ toast					
1.3	Traditional breads(markouk/tannour)	1 loaf					
1.4	Breakfast cereals, regular/ sugar coated/ chocolate/ bran	Side A					
		Carton (35 g)					
1.5	Kaak	Finger size Small round / Page 13					
1.6	Rice, white, cooked	Side A/ Page 5					
1.7	Pasta/ Noodles, plain, cooked	Side A/ Page 5					
1.8	Wheat/ Bulgur, cooked	Side A/ Page 5					
1.9	Rice/Pasta/Cereals, whole grain	Side A / Page 5					
2	**Dairy Products**						
2.1	Milk, skim/low-fat (0-2%)	Side A					
2.2	Milk, whole-fat	Side A					
2.3	Yogurt, fat-free/low-fat	Side A					
		Bottled ayran					
2.4	Yogurt, whole-fat	Side A					
		Bottled ayran					

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2.5	Cheese, regular / yellow	Side A Side B / Thickness Cube/ triangular portion					
2.6	Cheese, low fat / white	Side A Side B / Thickness Cube/ triangular portion					
2.7	Labneh, regular	Side A					
2.8	Labneh, low fat	Side A					
3	**Fruits and Fruit Juices**						
3.1	Citrus orange/ grapefruit	Side A / 1 medium					
3.2	Peach, plum, prunes	Side A / 1 medium					
3.3	Strawberries	Side A / 10 strawberries					
3.4	Grapes	Side A / 10 grapes					
3.5	Banana/ Apples	Side A / 1 medium					
3.6	Dried Fruits	Raisins= 1 tbsp Dates: 1 portion Apricots: 1 portion					
3.7	Fruit juice, fresh	Side A					
3.8	Fruit juice, canned	1 can					
3.9	Fruit juice, bottled	1 bottle/ carton					
3.10	Fruits, canned	Peach/ apricot = ½ fruit Pineapple = 1 slice					
4	**Vegetables**						
4.1	Salad, green: lettuce, mint, cucumber, green pepper, rocket, purslane, etc.	Side A/ Page 8					
4.2	Dark green or deep yellow (spinach, Swiss Chard, Jew's mellow, carrots..)	Side A/ Page 4					
4.3	Tomatoes, fresh	1 medium / 10 cherry					
4.4	Corn / Green peas, fresh	Side A/ Page 4					
4.5	Corn/ Green peas, canned	Side A/ Page 4					
4.6	Potatoes, baked / boiled/ mashed	Side A / 1 medium					
4.7	Zucchini/ Eggplants, cooked	Side A/5 med. stuffed					
4.8	Cauliflower/ Cabbage/ Broccoli	Side A/ Page 4					
4.9	Other canned vegetables (Mushroom, palmetto, asparagus, etc.)	Side A/ Page 4					
4.10	Vegetable juice, fresh	Side A					
5	**Meat and Meat Alternatives**						
5.1	Legumes: lentils, beans, chickpeas, etc., dried, cooked	Side A/ Page 4					
5.2	Legumes, canned (beans, peas)	Side A/ Page 4					

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5.3	Nuts & seeds: walnuts, peanuts, almonds, sunflower seeds, etc.	Side A/ Page 4 Pre-packed small bag					
5.4	Red meat, beef/ lamb/goat	Side A/ Ground Steak - Side B/ Thickness					
5.5	Poultry	Leg/thigh/breast/wings Side B					
5.6	Fish/ Seafood, fresh	Side B/ Thickness Shrimp: 1 medium Calamari: 1 medium Crab: 1 medium					
5.7	Fish, canned (tuna, sardines)	1 large can/ 1 small can Page 19					
5.8	Eggs	1 medium					
5.9	Organ meats (liver, kidney, brain)	Side B/ Thickness					
5.10	Luncheon meats (mortadelle, turkey, salami, ham, etc.)	Side B/ Thickness Regular slice					
5.11	Sausages, makanek, uncanned	Side B/ Thickness Makanek size					
5.12	Sausages, makanek, hotdogs, canned	Hotdog size Makanek size Side B/ Thickness					
6	**Added Fats and Oils – Salads/ Cooking / Fries**						
6.1	Vegetable oil, corn/ sunflower/ soya	Side A					
6.2	Olive oil (including with thyme)	Side A					
6.3	Olives	5 olives					
6.4	Butter	Side A					
6.5	Ghee	Side A					
6.6	Mayonnaise	Side A					
6.7	Tahini	Side A					
7	**Sweets and Desserts**						
7.1	Cakes / Cookies/ Doughnuts / Muffins/ Croissant / Biscuits	Side B / Thickness Page 14-15-16					
7.2	Ice cream	1 scoop/ 1 stick/ Page 9					
7.3	Chocolate bar	1 medium					
7.4	Sugar, honey, jam, molasses, chocolate spread	Side A					
7.5	Arabic sweets Baklava, maamoul, knefe	Side B					

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8	Beverages						
8.1	Soft drink, regular	Side A / 1 can (330 mL)					
8.2	Soft drink, diet	Side A / 1 can (330 mL)					
8.3	Turkish coffee	Side A					
8.4	Instant coffee / Tea	Side A					
8.5	Cocoa / Hot chocolate	Side A					
8.6	Beer	Side A / 1 bottle					
8.7	Wine, red / white/ blush	Side A					
8.8	Liquor, whiskey/ vodka/ gin/ rum	Side A					
8.9	Water	Side A / Bottle (0.5 L)					
9	Miscellaneous						
9.1	Manaeesh, zaatar/ cheese	1 regular / 1 bouché Page 17- 18					
9.2	French fries	Side A Page 4					
9.3	Potato chips / Tortilla	XS/ S/ M/ L/ XL bag Page 20					
9.4	Falafel, without bread	1 medium falafel					
9.5	Shawarma	1 medium sandwich					
9.6	Burgers (beef, chicken, fish)	1 medium burger					
9.7	Pizza	Side B / Thickness					
9.8	Canned/ Pre-packed soups	Side A / Page 3					
9.9	Ketchup	Side A					
9.10	Mustard	Side A					

10.1. How many times do you ***season your food with a tomato-based sauce*** (tomato, onion, garlic and simmered with olive oil)?

---------------------------------- number of times per day / week / month?

10.2. Do you actually consume chicken or turkey meat ***instead of*** veal, pork, hamburger, or sausage?

------ Yes ------ No

* Are there any other foods/supplements that you regularly consume **[at least once per week]** and that were not mentioned in the FFQ list above?

Food Item	Usual serving size	Frequency of intake per week

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Dietary Habits Questionnaire

1. Do you know what Bisphenol A (BPA) is? ----- No ----- Yes
2. Are you aware of BPA free bottles / plastic containers (Tupperware)? ----- No ----- Yes

		Always (6-7 times/week)	Most of the times (4-5 times/week)	Few times (2-3 times/week)	Rarely (1x/week to 2x/month)	Never	Don't know
3	Do you store foods in plastic containers?						
4	Do you heat foods in plastic containers?						
5	Do you make sure that the plastic containers you use are BPA-free?						
6	Do you heat foods that are wrapped in cling film?						
7	Do you drink bottled water?						
	7.1 From plastic- bottled water: ------------------ cups/day 7.2 From water cooler: ------------------ cups/day						
8	Do you reuse bottled water?						
9	Do you drink from bottles you left in your car?						
10	Do you eat outside home (snacks, restaurants, bars)?						
11	Do you order delivery foods?						
12	Do you purchase soft drinks in cans and/or plastic bottles?						
13	Do you consume canned tomato paste?						

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24-Hours Dietary Recalls

Date: (dd/mm/yyyy) -----/-----/---------- **Day of the week:** ------------------------

Time	Food eaten	Amount	Method of preparation

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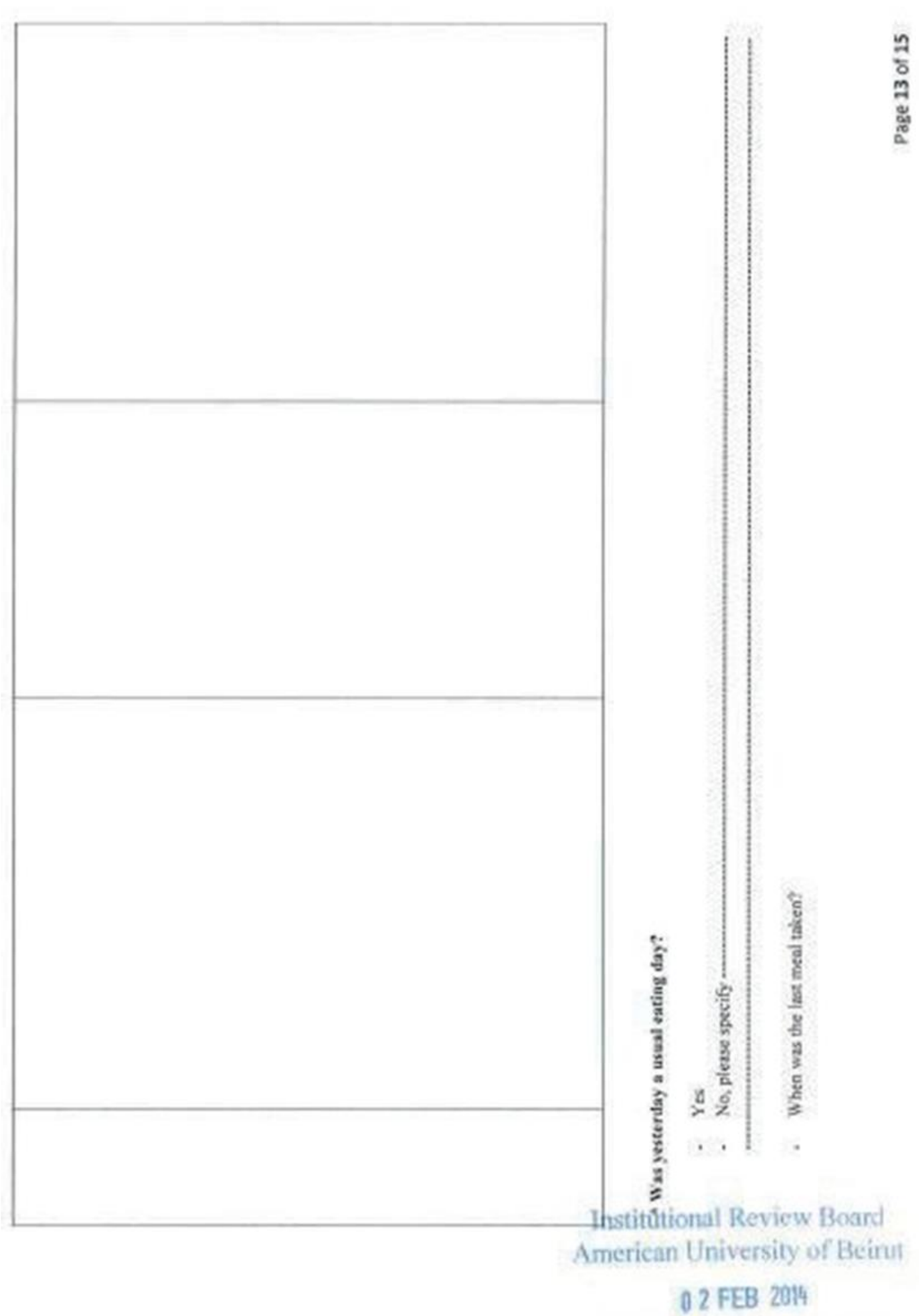

- Was yesterday a usual eating day?
  - Yes
  - No, please specify --

- When was the last meal taken?

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Lab work data collection

Name of the participant: ------------------------ Initials:------------------------ Study ID number:-----------

Test	Unit	Result
Hba1c		
LDL		
SGPT		
Urinary creat		
FBS		
HDL		
GGT		
Spot microalbumin		
Fasting insulin		
Triglycerides		
CRP		
Creatinine		
Total cholesterol		
TSH		
25OHvit D		
Cortisol		
C-peptide		
Prolactin		
Leptin		

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Physical Exam Form

Name of the participant: -------------------------- Initials:-------------------- Study ID number: ----------------

		Results النتائج	Healthy ranges النطاقات الصحية
Body weight (kg	الوزن		
Height (cm):	الطول		
BMI:	مؤشر البدانة		18.5-24.9 kg/m^2
Waist circumference (cm):	قياس دائرة الخصر		نساء <80 cm, رجال <94 cm
Body fat (kg):	نسبة الدهون في الجسم		نساء <32%; رجال <25%
Muscle mass (kg):	نسبة العضل في الجسم		نساء 24-30 %; رجال 33-40%
Waist to hip ratio:	قياس محيط الأوراك		نساء <0.9, رجال <0.85
Heart rate:	قياس نبض القلب		50-100 bpm
Blood Pressure – Measurement # 1	قياس ضغط الدم 1		
Systolic blood pressure (mmHg):	العالي		120 mmHg
Diastolic blood pressure(mmHg):	الواطي		80 mmHg
Blood Pressure – Measurement # 22	قياس ضغط الدم		
Systolic blood pressure (mmHg):	العالي		120 mmHg
Diastolic blood pressure(mmHg):	الواطي		80 mmHg

Time of urine collection	
Time of blood withdrawal	

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