Effects of Physical Activity on the Performance of 24-h Urinary Sucrose and Fructose as a Biomarker of Total Sugars Intake

by

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ABSTRACT

Urinary sucrose and fructose has been suggested as a predictive biomarker of total sugars intake based on research involving UK adults. The purpose of this study was to determine the association between total sugars consumption and 24-hour urinary sucrose and fructose (24uSF) in US adult population and to investigate the effect of physical activity on this association. Fifty seven free-living healthy subjects 20 to 68 years old, participated in a 15-day highly controlled feeding study, consuming their habitual diet, provided by the research metabolic kitchen. Dietary sugars were estimated using Nutrition Data System for Research (NDSR). Subjects collected eight 24-hour urine samples measured for urinary sucrose and fructose. Physical activity was assessed daily using a validated 15-day log that inquired about 38 physical activities across six domains; home activities, transportation, occupation, conditioning, sports and leisure. The mean total sugars intake and added sugars intake of the sample was 112.2 (33.1) g/day and 65.8 (29.0) g/day (9.7%EI), respectively. Significant moderate positive correlation was found between 15-d mean total sugars intake and 8-day mean 24uSF ($r = 0.56$, $p < 0.001$). Similarly, added sugars were moderately correlated with 24uSF ($r = 0.56$, $p < 0.001$), while no correlation was found between naturally-occurring sugars and 24uSF ($r = 0.070$, $p < 0.001$). In a linear multiple regression, total and added sugars each explained 30% of variability in 24uSF (Adjusted R^2, p value; total sugars: 0.297, 0.001; added sugars: 0.301, $p < 0.001$). Physical activity had no effect on the association between dietary and urinary sugars in neither the correlation nor the linear regression analysis. 24uSF can be used as a biomarker for total and added sugars consumption in US adults, although its predictability was weaker compared to findings involving UK adults. No evidence was found showing that physical activity levels affect the association between 24uSF and total sugars intake in US adults. More detailed investigation through future feeding studies including subjects with wide range of sugars intake and of different ethnic/racial backgrounds are needed to better understand the characteristics of the biomarker and its uses.

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LIST OF ABBREVIATIONS

24HDR – 24-Hour Dietary Recalls

FFQ – Food Frequency Questionnaires

24uSF – Sum of Sucrose and Fructose in 24-h Urine

PA – Physical Activity

MET – Metabolic Equivalent Task

RMR – Resting Metabolic Rate

NHANES - National Health and Nutrition Examination Survey

WHO – World Health Organization

SSB – Sugar-Sweetened Beverages

FDA – Food and Drug Administration

HFCS – High Fructose Corn Syrup

RCT – Randomized Controlled Trial

25(OH)D – 25-Hydroxyvitamin D

PLP – Pyridoxal-5`-Phosphate

FCT – Food Composition Tables

NMR – Nuclear Magnetic Resonance

GC-MS – Gas Chromatography Mass-Spectrometry

LC-MS – Liquid Chromatography Mass-Spectrometry

EPIC – European Prospective Investigation into Cancer

USDA – United States Department of Agriculture

LAS – Low Added Sugars

HAS – High Added Sugars

CARB – Carbohydrates and Related Biomarkers Study

DONALD - Dortmund Nutritional and Anthropometric Longitudinally Designed

NPAAS - Nutrition and Physical Activity Assessment Study

WHI – Women’s Health Initiative

4DFR – 4-Day Food Records

AF – Attenuation Factor

OPEN – Observing Protein and Energy Nutrition

GLUT 5 – Glucose Transporter Protein

GLUT 2 – Glucose Transporter Protein

CDC – Centers for Disease Control and Prevention

ACSM – American College of Sports Medicine

PAGA – Physical Activity Guidelines for Americans

DHHS – Department of Health and Human Services

MPA – Moderate-Intensity Physical Activity

VPA – Vigorous-Intensity Physical Activity

FR – Fructose Drink

tAUC – Total Area Under Curve

TG – TriGlycerides

VLDL – Very Low-Density Lipoprotein

HDL – High-Density Lipoprotein

LDL – Low-Density Lipoprotein

BW – Body Weight

% EI – As a Percentage of Energy Intake

POTABA – Potassium Aminobenzoate

PABA – Para-Amino Benzoic Acid

HbA1C – Hemoglobin A1C

BMI – Body Mass Index

NDSR – Nutrition Data System for Research

HPLC – High Performance Liquid Chromatography

UV – Ultra Violet

ANOVA – Analysis of Variance

SQRT – Square Root Transformed

SD – Standard Deviation

IQR – Interquartile Range

NHIS – National Health Interview Survey

GL – Glycemic Load

CHAPTER 1

INTRODUCTION

1.1 Background

Measurement errors due to misreported sugars intake has proved to be an ongoing obstacle to evaluate the role of sugars intake in disease risk.[1] Data obtained by self-report dietary assessment instruments, such as 24-hour dietary recalls (24HDR), food frequency questionnaires (FFQ), and food diaries, are subject to random and systematic measurement error.[2,3] Sugars come from diverse food sources, i.e., from unprocessed food (naturally-occurring sugars) and highly processed foods (added sugars). As a result, total sugar intake from foods is very difficult to measure.[4] The issue is further obscured by underestimation of total energy and sugar intake by under reporters.[5] The acknowledgement of limitations of the traditional dietary assessment instruments has prompted the need for the development of an unbiased measurement tool for sugars intake.[4]

Twenty-four hour urinary sucrose and fructose has been developed as a predictive biomarker for total sugars intake based on a few highly controlled feeding studies conducted in the UK.[6,7] In a study of 13 participants consuming their usual diet for 30 days under highly controlled conditions, 30-day mean total sugars intake was significantly correlated with the sum of sucrose and fructose in 24-h urine (24uSF) ($r = 0.84$, $p < 0.001$).[6] These studies showed no effect of BMI[7] or physical activity[6] on the association between 24uSF and total sugars. Although some effect of sex and age was detected,[8] 30-day mean total sugars intake was the main predictor of 30-day mean 24uSF, explaining 72% of the variability in excretion.[6] The sugars content of a US compared to UK diet differs. The main caloric sweetener in the US is the high fructose corn syrup, whereas in Europe, it is sucrose from sugar beet.[9] The difference in chemical composition of the two sweeteners could lead to variation in the biomarker level and could affect the performance of the biomarker. In a recent randomized cross-over feeding study conducted in the US, total sugars intake explained 16.3% of the variation in 24uSF.[10] Authors suggest that other factors besides sugars intake might be responsible for the variation in urinary sugars excretion.[10] Yet, this study did not use

urine preservative, which could have caused sugars degradation in urine over the 24-h collection period, and collected a single 24-h urine per participant. It is therefore necessary that this biomarker is more carefully investigated in the US population under a US diet.

Physical exercise has been shown to affect the hepatic metabolism of fructose, suggesting that regular practice of physical activity may attenuate the obesogenic effects and de novo lipogenesis induced by fructose intake.[11-13] As physical activity has the potential to alter fructose metabolism, it is possible that it can alter the excretion of fructose in urine. Since fructose is one of the biomarkers of sugars intake measured in urine along with sucrose, it is possible that physical activity impacts the urinary fructose excretion and therefore the association between sugars biomarker and intake. Tasevska et al[6] showed no effect of physical activity on the association of the urinary sugars biomarker with sugars intake in the original feeding study, however, the study was conducted under a UK diet, and the assessment of physical activity was limited.

1.2 Purpose of the study

The purpose of the study is to investigate the association between 24uSF and total sugars consumption, and the effect of physical activity on this association in the US adult population. Examining the association between the sugars biomarker and sugars intake, and identifying any determinants of the biomarker other than diet is critical for assessing the validity of the biomarker and its future applications.

This study was a highly controlled feeding study including free living healthy men and women between the age of 18 and 70 years. Over the 15-day feeding period, participants consumed their habitual diet prepared in the study metabolic kitchen, and collected eight 24-h urines measured for sucrose and fructose. Physical activity (PA) was assessed by a PA-log completed by participants daily during the 15-day feeding period. The PA log enquired about hours and minutes participants engaged in six different PA domains; home activities, transportation, occupation, conditioning, sports and leisure, over a 24-hour period.

1.3 Research Aims and Hypothesis

Aim 1 - To investigate the association between 24uSF biomarker, and total sugars consumption, in participants consuming their usual diet under controlled conditions.

Hypothesis 1: Urinary sucrose and fructose will be strongly associated with total sugars consumption.

Aim 2 - To investigate the effects of physical activity on the association between urinary sucrose and fructose, and total sugars consumption, in participants consuming their usual diet under controlled conditions.

Hypothesis 2: Physical activity has no effect on the association between urinary sucrose and fructose, and total sugars consumption.

1.4 Definition of Terms

BMI: Body mass index measured as weight in kilograms divided by height in meters squared.

Obesity: BMI ≥ 30kg/m^2

Dietary Biomarker: A compound in any biological specimen such as urine, blood, hair, etc. that is indicative of nutrient or food intake.[14]

Monosaccharide: Monosaccharides are simple sugars, building blocks of disaccharides, oligosaccharides and polysaccharides. Glucose, fructose and galactose are monosaccharides.

Disaccharide: Disaccharides are two monosaccharides bonded together. Principal disaccharides are sucrose (glucose and fructose), and lactose (glucose and galactose). Other less common disaccharides include maltose (two glucose units) and trehalose (two glucose units).

Sucrose: A disaccharide composed of one molecule of glucose and one molecule of fructose, known as “table sugar”. Naturally, it is found in fruits and vegetables. Commercially, it is extracted from sugarcane or beets.

Fructose: A monosaccharide that is part of sucrose, but also found as free fructose. Naturally, free fructose occurs in fruits and honey, while as an added sugar occurs in high fructose corn syrup along with glucose.

Total sugars: Total sugars is the sum of monosaccharides (glucose, fructose, and galactose) and disaccharides (sucrose, maltose, lactose).

Added sugars: Sugars and syrups added to foods during processing or preparation or at the table.[15]

PA log: A self-reported physical activity assessment instrument used by participants to log their activities over a 24-hour period.

24-h urine collection: Urine collected over an entire 24-h period, starting with the second morning urine of the day and finishing with the first morning urine of the following day.

Feeding study: Study in which participants are provided with diets of a certain composition prepared in a metabolic kitchen, and all intake is carefully measured, over the entire study period.

Metabolic Equivalent Task (MET): One MET, which is the energy expenditure of sitting quietly or Resting Metabolic Rate (RMR), is the equivalent to 3.5 ml of oxygen uptake per kilocalorie per kilogram of body weight per hour.[16]

Low activity level: Less than 600 MET-minutes/week[17]

Moderate activity level: 600 – 2999 MET-minutes/week[17]

High activity level: Minimum of 3000 MET-minutes/week[17]

1.5 Limitations of the study

- Noncompliance with the feeding study and incomplete 24-hr urine collections could impact data collection and results.

1.6 Delimitations of the study

- The study population included healthy non-diabetic men and women recruited from Phoenix Metropolitan Area between the age of 18 - 70 years, hence the results may not be applicable to a different population.
- We used self-reporting instrument for assessment of PA level, rather than accelerometer, which is an objective measurement tool for PA.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Dietary Sugars

2.1.1. Sugars consumption in the United States

Historically, there has been an upward trend in the total sugars consumption of the general US adult population, rising from 110 g/d in the time period 1971 to 1975 based on National Health and Nutrition Examination Survey (NHANES I) to 126 g/d in 1988 – 1994 (NHANES III)[18] and 130 g/d over the period 1999 – 2006.[19]

Total sugars comprise of naturally occurring sugars and added sugars that are sugars added during the preparation or processing of foods. At present, there are no guidelines for total sugars consumption. However, guidelines to limit added sugars consumption have been put forth by a number of authorities, since foods high in added sugars are sources of empty calories and reduce the intake of other nutrient rich foods, when compared to the foods (fruits, vegetables, dairy, etc.) rich in naturally occurring sugars.[20] According to the 2015 - 2020 Dietary Guidelines for Americans, the recommended limit of energy from added sugars is 10%.[21] The World Health Organization (WHO) strongly recommends reducing the intake of added sugars in both adults and children to less than 10% of their total energy intake, and to less than 5% to reduce the incidence of dental carries.[22] Based on the NHANES data from 2005 to 2010, US adults consumed 13% of their total energy from added sugars.[23] NHANES 2011-2012 survey data shows that consumption has remained high in the subsequent years at 14%.[24] Men consumed more calories from added sugars than women, and the consumption decreased with an increase in age and income for both genders. However, in terms of percent of total energy from added sugars, women consumed more added sugars than men (13.2% and 12.7%, respectively), although the difference was not statistically significant.[23] Added sugars intake of men and women ranged from 14.1% and 14.5% among those aged 20-39 years to

10.7% and 11.2% among those aged >60 years, respectively.[23] Less educated men and women and those not in a domestic relationship were more likely to consume more added sugars.[25]

In general, the added sugars intake of non-Hispanic blacks was higher compared to intake of non-Hispanic whites and Mexican-Americans. Non-Hispanic black men consumed 14.5% of their total energy intake from added sugars, while non-Hispanic white men and Mexican-American men consumed 12.8% and 12.9%, respectively. Similarly, added sugars intake of Non-Hispanic black women was higher (15.2%) compared to added sugars intake of their non-Hispanic white (13.2%) and Mexican-American counterparts (12.6%).[23]

According to NHANES 2005-2010 report, majority of added sugars came from foods (67%) rather than beverages (33%).[23] Soft drinks and soda were the top individual sources of added sugars in the American diet, contributing to a third of added sugars intake (33%), followed by candy, sugars, and sugary foods (19.5%), cakes, cookies, quick bread, pastries, and pies (14.4%), fruit drinks (11%), and milk desserts (5.4%).[26] In men, 6.9% of their energy intake came from sugar-sweetened beverages (SSB), including soft drinks, soda and fruit drinks, while women consumed 6.1% of their energy from SSB.[27]

Foods and beverages that are major sources of added sugars predominantly contain fructose and sucrose as sweeteners. Total fructose is the sum of free fructose and fructose from sucrose.[28] The total fructose consumption of US adults based on NHANES III survey data from 1988 – 1994[29] was 51 grams/day and 48 grams/day based on the NHANES 1999-2006.[19] The average nationwide consumption of sucrose among US adults was 54 grams/day according to NHANES III 1988 – 1994 report.[18]

2.1.2. Definition of sugar terms

Added sugars, according to Food and Drug Administration (FDA), is defined as sugars and syrups added to foods during preparation and processing of foods such as jams, cakes, pies, fruit juices and other desserts and bakery products, or sugars added at the table.[15] WHO uses the term “free” sugars” for added

sugars and defines the term as “all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus the sugars that are naturally present in honey, syrups and fruit juices”.[22] Naturally-occurring sugars are sugars naturally present in foods, such as fruits, vegetable, and dairy, and not added during processing, preparing, or at the table. Total sugars are defined as the sum of all monosaccharides (glucose, fructose, and galactose) and disaccharides (sucrose, maltose, lactose), both added and naturally-occurring in foods and beverages. The aim of this study focuses on examining the urinary sugars as a biomarker of “total sugars” consumption.

2.1.3. Dietary sources of sugars

Sugars are naturally occurring sweeteners, and sucrose, fructose, and glucose are the ones most commonly used.[30] Sucrose, also known as table sugar, is a disaccharide molecule that consists of the monosaccharides glucose and fructose. Sucrose is naturally present in sugar beet, sugarcane, fruits and to a lesser extent in honey and vegetables. It is commercially extracted from sugarcane or sugar beets for production of table sugar, a sweetener.[9] Fructose is naturally present in fruits, vegetables and honey. Fructose is also present in the widely used caloric sweetener high fructose corn syrup (HFCS). HFCS is produced from corn with different fructose-to-glucose ratios.[30] HFCS-42 was initially produced in 1967 and contained 42% fructose and 58% glucose, followed by the most commonly used HFCS-55, which consisted of 55% fructose and 45% glucose.[31] According to US Department of Agriculture (USDA) 2017 data, 67% of HFCS used in the food industry was HFCS-55, and the remainder was HFCS-42.[32] While historically sucrose was the main caloric sweetener used, around the year 1975, the use of sucrose progressively decreased, while the use of HFCS increased, especially in SSB.[33] HFCS has conveniently replaced sucrose in the United States, due to its low cost, enhanced sweetness and flavor, freezing point depression and extended shelf life.[29,34,35] Other sweeteners that are added to foods include honey, molasses, and other syrups.[33] SSBs include regular soda, fruit drinks (which includes sweetened bottled water, fruit juices and nectars with added sugars), sports drinks, energy drinks, sweetened coffee and tea, and other beverages

including horchata and sugarcane beverages. SSB do not include diet drinks, 100% fruit juice, alcohol or flavored milk.[27]

2.1.4. Sugars consumption and disease risk

SSBs are known to be the major source of calories and added sugars in the American diet,[26] and to promote lower satiety and an inadequate compensation of energy intake.[36-38] With each additional serving of SSB per day, the risk of coronary heart disease increased by 16% (RR: 1.16, 95% CI: 1.10 – 1.23)[39] and risk of hypertension increased by 8% (RR: 1.08, 95% CI: 1.04-1.12),[40] based on a pooled analyses of four and six prospective cohort studies respectively. A systematic review and meta-analysis based on 30 randomized controlled trials (RCTs) and 38 cohort studies on dietary total sugars and body weight was conducted by Te Morenga et al.[41] Based on the RCTs, decreased sugars intake was associated with reduced body weight (0.80 kg, $p < 0.001$), and an increase in dietary sugars was associated with significantly greater body weight (0.75 kg, $p < 0.001$), when compared to no increase in dietary sugars. Findings from the cohort studies showed greater odds of being overweight (OR:1.55, 95%CI: 1.32-1.82) in those with higher intakes of SSB compared to those with lower intakes after a 1-year follow up.[41] Research also shows that as the use of sweeteners increased, there was a rise in weight gain and obesity trends after a 10-week intervention of sucrose and artificial sweeteners, in 41 men and women.[42] After 10 weeks, the sucrose group showed an increase in body weight and fat mass by 1.6 and 1.3 kg, respectively, whereas body weight and fat mass decreased in the artificial sweetener group by 1.0 and 0.3 kg, respectively, with significant between-group differences in body weight ($p < 0.001$) and fat mass ($p < 0.01$).[42] Epidemiological evidence suggests that added sugars and/or SSB intake are also associated with unfavorable lipid profile,[43] insulin resistance,[44] and risk of fatty liver,[45] type 2 diabetes,[46] cardiovascular disease,[47] metabolic syndrome,[48] visceral adiposity,[49] and hyperuricemia.[50]

2.2. Dietary Biomarkers

Dietary biomarkers are compounds in biological specimens such as blood, urine, stool, hair, nails, fat tissues, etc. that provide objective estimates of food or nutrient intake.[14] Dietary biomarkers are capable of providing better quantitative estimates of true intake when compared to the estimates obtained from traditional dietary assessment methods, including 24-hour recall, FFQ, etc.[51] Dietary biomarkers could be associated with errors, however, they may be associated with different types of errors than the measurement errors arising from self-reporting dietary assessment methods, such as memory errors in recalling portion sizes and frequency of intake, misreporting and coding errors.[52-54] Dietary biomarkers are promising tools for assessing nutrient intake either alone or in combination with self-report instruments.[55] The development of dietary biomarkers involves a few steps. In order to determine the dose response relationship between the biomarker and diet, investigations in controlled feeding studies with volunteers consuming constant diets of different ranges need to be conducted. This should be followed by evaluating their performance in feeding studies with participants consuming habitual diets since diets vary from day to day in normal life.[14] Although dietary biomarkers are related to issues concerning sample collection, transportation, and storage, and the reliability of laboratory assays, they hold significant potential in diet assessment.[56]

2.2.1. Requirements of Dietary Biomarkers

A dietary biomarker needs to be sensitive to the nutrient or food and respond appropriately in a dose-dependent fashion to intake. As the level of intake increases, the biomarker should show proportional increase. The **sensitivity** of the biomarker to intake highly depends on how well the nutrient is absorbed by the body, i.e., the bioavailability rather than the actual intake level, and homeostatic mechanisms regulating the nutrient concentration in biological tissues. For example, absorption of iron is enhanced by intake of vitamin C in the meal, thereby improving the bioavailability of iron consumed.[57] The sensitivity may also depend on other non-dietary characteristics such as age, genetic, environmental and life style factors, which may affect biomarker level.[55]

Time integration is another important requirement of dietary biomarkers. Biomarkers can be either short-term or long-term. Short-term biomarkers respond rapidly to intake and usually reflect recent exposure in the last several hours or days such as those measured in 24-hour and spot urine, stool, serum, and plasma samples. In this case, the nutrient is absorbed and metabolized rather quickly. On the other hand, long-term biomarkers are reflective of nutrient exposure over the past few months or years, such as biomarkers measured in adipose tissue cells, red blood cells, hair, nails etc., depending on the biomarkers' turnover rate. Long-term biomarkers are capable of measuring long-term nutrient exposures applicable to chronic diseases, which take longer time to manifest and therefore these markers are more useful than short-term markers to uncover diet-disease associations.[58] An example of short and long-term biomarkers are serum folate and red blood cells folate, respectively, to measure recent intake and long-term folate exposure.[59]

A biomarker also needs to be **reliable**, and the reliability depends on its reproducibility and validity. **Reproducibility** is the quality of a biomarker to show similar measurements from biological samples collected at two different time points from the same individual.[51] **Validity** of a biomarker means that it measures what it is supposed to measure. The validity of a marker is determined by how close the observed and true intake levels are close to each other and is often measured using correlation coefficients.[51] High reproducibility and high validity makes a dietary biomarker **reliable.**[51] However, the biomarker reliability also depends on the biological sample, sample collection protocol, as well as the study design.[60] For example, selenium measured in toenails is more reliable than selenium measured in hair due to the possible environmental contamination of hair.[61] Biochemical markers that are sensitive to recent intake could differ largely depending on the time of sample collection during the day and thus early morning fasting samples are highly desirable for valid results. Use of appropriate preservatives and freezing methods for long-term storage of samples such as blood and urine, could considerably improve the validity of the measurements. Reliability also depends on the study design, for example, case-control studies should be designed to ensure samples obtained from cases and controls are handled similarly to reduce systematic errors.[55]

Some dietary biomarkers may reflect intake of more than one dietary substance as they may co-exist and/or be produced as a metabolite of multiple foods and this explains the **specificity** of a dietary biomarker.[55] For example, 24-hour urinary hippuric acid is suggested as a valid biomarker of fruit and vegetable intake in children and adolescents.[62] However, hippuric acid excretion increases with intake of beverages containing polyphenolic compounds such as coffee and tea.[63,64] Thus, urinary excretion of hippuric acid could indicate consumption of fruits and vegetables as well as polyphenol-rich beverages.

2.2.2. Types of Dietary Biomarkers

There are four types of dietary biomarkers, recovery, concentration, replacement, and predictive.[6,14,51]

Recovery biomarkers are biochemical indicators in which a certain proportion of the nutrient is recovered in the biological sample, with high correlation between excretion and levels of nutrient intake, typically >0.8. One well-established biomarker is 24-hour urinary nitrogen, which represents 80% of the nitrogen intake in a 24-hour period, assuming nitrogen balance.[14] Recovery biomarkers can be used to estimate absolute intakes over a set time period. Twenty four-hour urinary potassium,[65] and doubly labelled water[66] as biomarkers of potassium, and energy intake, respectively, are other examples of recovery biomarkers. Recovery biomarkers, however, are available for only a very few dietary factors.[55]

Concentration biomarkers, on the contrary, cannot be used to provide an estimate of absolute intake levels, but the concentration of the nutrient in the biological specimens correlates with the food or nutrient intake.[4] These biomarkers show modest correlations that are much lower than for recovery biomarkers because these biomarkers are impacted by inter-individual variations in absorption and metabolism of nutrients.[55] The strength of the correlation is usually < 0.6 for concentration biomarkers compared to commonly > 0.8 for recovery biomarkers. Some examples for concentration biomarkers include serum ascorbic acid as an indicator of vitamin C, serum 25-hydroxyvitamin D [25(OH)D] as a marker of vitamin D, and serum pyridoxal-5'-phosphate (PLP) as a biomarker of vitamin B-6 status.[4]

Replacement biomarkers are required to measure food or nutrient compounds for which there is limited or no information available in the food composition tables (FCT).[4] An example is the amount of selenium in foods, which is based on the type of soil in which the food was produced and varies across geographical regions.[67] Thus, FCTs cannot be used to determine selenium intakes and replacement biomarkers are needed. Also, assessing the levels of phytoestrogens in foods is suggested to be difficult due to inadequate information on foods containing these compounds in the FCTs. Thus, serum and urinary biomarkers including urinary daidzein, genistein, equol, enterolactone, and kaempferol as a measure of phytoestrogen intake have been developed.[55,56] Replacement biomarkers can be considered as recovery, predictive or concentration biomarkers depending on their inherent characteristics, yet they can be classified as replacement if insufficient data for the nutrient in question in food composition tables exist. For example, urinary selenium can be regarded as a concentration biomarker,[68] however, since the selenium content of foods cannot be accurately determined from FCTs, urinary selenium can be used as a replacement biomarker.

Predictive biomarkers are biochemical markers that show high correlation between intake and excretion, high predictability potential, as well as a dose-response relationship, but their overall recovery is much lower than recovery biomarkers. Most importantly, although predictive biomarkers contain certain level of bias, these biases should not explain high portion of the variability in biomarker, should be stable and can be estimated from a feeding study.[8] 24-hour urinary sucrose and fructose as a biomarker for total sugars intake, is so far the only member of predictive dietary biomarkers.[6] It is classified under predictive biomarkers as a very small fraction of the total sugars intake is recovered in the urine (0.05% of total sugars), but since the correlation between intake and excretion is relatively high (> 0.8) and its biases are known from a feeding study, after being corrected for its biases, it may be used to estimate the absolute intake levels.[6]

2.2.3. Applications of Dietary Biomarkers

The main application of biomarkers is that they can be used as surrogate measures of nutrient intake in epidemiological studies investigating the diet-disease risk associations.[69-71] Dietary biomarkers are prone to lesser biases and errors when compared to the self-report dietary instruments,[51] and is the only available option to estimate nutrient intake for certain nutrients due to the lack of good quality data in FCTs.[72] As an example, urinary isoflavonoids (a major class of phytoestrogens) were used as biochemical indicators to demonstrate that equol, an active metabolite of the isoflavone daidzein, obtained from soy, could have a protective effect against coronary heart disease in Chinese women.[73]

Another application of dietary biomarkers is that they can be used as a true measure of nutrient status when it is obtained both through dietary and non-dietary sources in the body.[55] One example is the biomarker for the carcinogen acrylamide. Acrylamide in the human body can be of dietary source obtained from processed foods such as potato chips as well as of non-dietary origin through exposure to smoking. Furthermore, certain cooking practices such as frying, overcooking and baking of foods generates acrylamide at different levels.[74] Hemoglobin adducts of acrylamide and the metabolite glycidamide are biochemical indicators of the long-term exposure of acrylamide.[75] As the use of self-report dietary questionnaires will fail to accurately estimate the levels of this contaminant, biomarkers are essential. Another example is vitamin D that can be obtained exogenously through diet as well as endogenously through skin exposure to sunlight.[76] The concentration biomarker, serum [25(OH)D] can reflect the “internal dose” of vitamin D, which will be different from the dietary data of vitamin D intake obtained from self-report instruments.[55]

Dietary biomarkers can also be applied as reference instruments in validation studies[8,69,77,78] to assess the magnitude of errors associated with traditional dietary assessment methods and in calibration studies[79,80] to calibrate or correct self-reported intake. Recovery and predictive biomarkers that have high correlation to intake such as 24-hour urinary nitrogen,[81] doubly labelled water[66] and 24-hour urinary sucrose & fructose[6] estimating protein intake, energy intake and total sugars intake, respectively, are useful for these purposes. Dietary biomarkers have also been used as markers of dietary compliance in intervention studies.[82,83]

2.2.4. Discovery of Dietary Biomarkers using metabolomics

The emerging field of metabolomics has been suggested as a promising approach to identify new dietary biomarkers for nutrition research. Metabolomics has the ability to detect metabolites of biological processes with low molecular weights using advanced technologies such as nuclear magnetic resonance (NMR), gas-chromatography mass-spectrometry (GC-MS) and liquid chromatography mass-spectrometry (LC-MS) in biofluids, including blood and urine.[84] Recently, the metabolites anserine, trimethylamine-N-oxide, carnosine, and acylcarnitines were identified in urine as potential biomarkers of meat or fish intake, using a metabolomic approach in a dietary intervention and free-living individuals from the European Prospective Investigation into Cancer (EPIC) cohort study.[85] Using a National food consumption survey and an acute intervention, the metabolites formate, citrulline, taurine, and isocitrate were identified as putative urinary biomarkers of SSB using the ^{1}H NMR technique.[86]

2.3. Biomarker of sugars intake

Urinary sucrose and fructose biomarker for total sugars belongs to the group of predictive biomarkers, and it was first developed in Europe.[6,7,87,88]

One of the earliest studies on this biomarker was conducted by Luceri et al[87] in nine healthy men and women, consuming their “habitual Italian basal” diet for one week, followed by a “low sucrose” diet for 3-days. Spot urine samples were collected on the last day of the dietary period at four different time points, 8 am (fasting), 10 am, 3 pm and 10 pm, to measure the effect of breakfast, lunch, and dinner. The excretion of sucrose and fructose at 10 am, increased from fasting values at 8 am, and remained at a similar level in the subsequent spot samples. The urinary sucrose and fructose excretion significantly increased from 8 am fasting to the 10 am samples with significant difference for fructose excretion ($p<0.01$). The average urinary sucrose in the four-time points during the basal and low sucrose diets, significantly correlated with the sucrose consumed on the collection day ($r=0.7$; $p<0.01$). Similarly, the correlation between sucrose intake and the average fructose excretion in the four-time points during the basal and low sucrose diets was

statistically significant ($r = 0.82$; $p<0.05$).[87] This study suggested that urinary sucrose and fructose could be used as a biomarker of dietary sucrose intake in healthy adults.

In 2005, Tasevska et al[6] conducted two UK-based highly controlled feeding studies with subjects living in a volunteer suite while following their usual daily schedules. The first feeding study was conducted to assess the dose-response of urinary sucrose and fructose to increased total sugars intake. This study recruited 12 healthy men for a randomized 30-day crossover dietary intervention, who were placed on 10-day periods of low, medium and high sugars intakes. Subjects collected 24-h urine on days 4 to 7 of each of the three dietary periods. There was an increase in urinary sucrose and fructose excretion as the sugars intake increased across the three dietary periods. There was also a significant mean difference in urinary sucrose ($P < 0.001$) and urinary fructose ($P < 0.001$) between the three dietary periods.[6]

The second feeding study was done to investigate the performance of the biomarker under usual dietary conditions, as diet does not remain constant from day to day. This was a 30-day study with a total of 13 healthy men and women consuming their habitual diet and collecting 24-h urine samples daily. A strong correlation between the sum of sucrose and fructose in 24-hour urine (24uSF) and total sugars consumption was found ($r = 0.84$, $p < 0.001$). While age, sex, body weight and physical activity explained 10% of variation in urinary sugars, total sugars intake explained an additional 72% of the variation in sugars excretion ($R^2 = 0.82$; $p = 0.002$).[6]

These two feeding studies showed that sugars intake was the main determinant of urinary sucrose and fructose excretion, and the biomarker could be used to estimate total sugars consumption.

In the habitual varying diets study, Tasevska et al[88] further demonstrated the association of extrinsic and intrinsic sugars with sugars excretion. While intrinsic sugars are naturally incorporated into the foods' cellular structure, extrinsic sugars are free in the food[89] and rapidly available for metabolism.[90] Extrinsic sugars' definition is different from the USDA's definition of added sugars, which does not consider fruit juice as a source of added sugars.[89] 24uSF biomarker was found to be strongly associated with extrinsic sugars intake ($r=0.84$, $p<0.001$), and not intrinsic sugars intake ($r=0.43$, $p = 0.144$).[88]

Joosen et al[7] further investigated the characteristics of the 24uSF biomarker using a randomized crossover dietary intervention, which compared biomarker performance between 10 normal-weight and nine obese individuals. Subjects were housed in a volunteer suite for three 4-day dietary periods that provided 13%, 30% and 50% of energy from total sugars, and collected 24-h urine on the last two days of each dietary period. It was demonstrated that there was no significant interaction between BMI and mean urinary sucrose ($p = 0.65$) or fructose excretion ($p = 0.55$) in relation to sugars intake.[7] This study helped to show that BMI is not a determinant of 24uSF and the biomarker can be used to estimate total sugars intake in normal and obese individuals, irrespective of their BMI.

Recently, Moore et al[91] conducted a study to evaluate the validity of urinary sucrose and fructose as biomarkers of added sugars intake in non-obese adolescents in the US. The study was a randomized controlled crossover feeding study with 33 adolescents, 12 – 18 years of age, consuming low added sugars (LAS, 5% of energy intake) and high added sugars (HAS, 25% of energy intake) isocaloric, macronutrient matched diets for 7 days each, with a 4-week wash out period between the diets. Twenty four-hour urine samples were collected on the last 2 days of each diet period. 24uSF were strongly associated with added sugars intake during the HAS diet period ($r = 0.77$, $p < 0.001$), but weakly correlated with added sugars intake during the LAS period ($r = 0.15$, $p = 0.49$). Sucrose and fructose excretion in the LAS period were 0.015 ± 0.01 and 0.199 ± 0.07 mg/day, respectively, whereas in the HAS period, they were 0.028 ± 0.01 and 0.348 ± 0.15 mg/day, respectively.[91] This study concluded that urinary sucrose and fructose can be used as objective indicators of added sugars at higher amounts of sugar intake (25% added sugars) but may not be valid indicators at lower amounts of sugar intake (5% added sugars). In this study, Moore et al observed very low sucrose and fructose excretion levels compared to previous studies,[6,7,88] about 100-fold lower for fructose and 100-to-1000-fold lower for sucrose excretion. The researchers attributed the low excretion to the reduced intestinal permeability for sugars in adolescent population when compared to adults, which may have been the reason for low sucrose but not fructose excretion. In a subsample of 114 prepubertal children, which measured urinary fructose,[92] much higher excretion levels were observed and

similar to previous research,[6,7,88] suggesting that age was not likely the reason for the extremely low excretion levels reported by Moore et al.[91]

Song et al[10] conducted a study to evaluate the relationship between total sugars intake and urinary sucrose & fructose in the US. This study was a subset of the CARB study (Carbohydrates and Related Biomarkers Study), a randomized cross-over feeding study with 53 men and women on two randomly assigned, isocaloric controlled experimental diets of low glycemic load (GL) or high GL, for 28 days each. Participants collected 24-hour urine on the last day of each of the dietary periods. In an unadjusted model, the log 24-hour urinary fructose was significantly associated with last day total fructose intake (defined as sum of fructose and half of sucrose) ($\beta = 0.0110$, 95% CI: 0.0079, 0.0140, $p<0.0005$). In a model adjusted for age, sex and % body fat, sex ($p < 0.0005$) and total percent body fat ($p = 0.004$) were found to be significant predictors of urinary fructose. The log sum of 24-hour urinary sucrose and fructose was also significantly associated with last day of total sugars intake ($\beta = 0.0054$, 95% CI: 0.0034, 0.0074, $p<0.0005$), and after adjustment, gender ($p < 0.0005$) and total percent body fat ($p < 0.0005$) were found to be significant predictors. In an adjusted model controlling for age, gender, and body fat, last day dietary total sugars intake explained 16.3% of variation in sum of urinary sucrose and fructose, and last day dietary fructose explained 24.3% of variation in urinary fructose excretion suggesting that other non-dietary factors could also be determinants of urinary sugars.[10] Nonetheless, these findings are based on one day of measurements on excretion and intake per participant.[6]

2.3.1. Application of Urinary Sucrose and Fructose as a Biomarker of Sugars Intake

Johner et al[92] demonstrated the use of the biomarker as a way of measuring sugars intake in 114 prepubertal boys and girls with a mean age of 9.3 ± 0.8 and 7.9 ± 0.7 years, respectively, in Europe. The study aimed to investigate if fructose biomarker was applicable in free-living children, and whether it relates to added sugars or total sugars intake. The study sample was a subsample of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study. Diet was assessed using 3-day weighted dietary

records, and urinary fructose was measured in a single 24-h urine collection made on the third day of the record. The association of urinary fructose with total sugars intake was better than with added sugars intake (total sugars: $R^2 = 0.181$, $p < 0.001$; added sugars: $R^2 = 0.055$, $p = 0.01$), although still weak, most likely due to a single urine sample and use of self-reported dietary instrument.[92] A preliminary 5-day diet experiment demonstrated lower sucrose than fructose excretion levels, which is why the study focused on urinary fructose alone. The low sucrose excretion levels could be attributed to the fact that the 24-h urine samples were refrigerated without preservatives leading to degradation of urinary sugars. The study concluded that urinary fructose is a potential biomarker for total fructose intake and is applicable for estimation of total sugars intake rather than added sugars intake in children.

Tasevska et al[8,78] applied the sum of 24-h urinary sucrose and fructose as a predictive biomarker of total sugars consumption in two studies that assessed the extent of measurement error in self-reported instruments. The Nutrition and Physical Activity Assessment Study (NPAAS) is a biomarker study that used a subsample of 450 postmenopausal women 60 to 91 years old from the Women's Health Initiative (WHI) Observational Study. FFQ, four-day food records (4DFR) and three 24HDRs were used to measure diet, and attenuation factors (AF) for the dietary instruments were estimated based on the biomarker. AF indicates the measurement error in the instruments that leads to underestimation (attenuation) of the true relative risk estimates of disease occurrence. AF can take values between 0 and 1; values trending towards zero indicate serious attenuation (underestimation) of risk and AF of 1 indicates no attenuation.[93] AF for 24HDR (0.57) was found to be higher than AF for FFQ (0.48) and AF for 4DFR (0.32).[78] Even though all the self-report dietary instruments examined in this study seemed to have measurement error, 24HDR produced the lowest AF and seemed to measure sugars best. The Observing Protein and Energy Nutrition (OPEN) study consisted of 261 men and 223 women in the age range of 40 to 69 years. Two FFQs and two non-consecutive 24HDRs were used to collect dietary data. AF for FFQ was found to be 0.39 for men and 0.33 for women, which was higher than the AF for a single 24 HDR (0.30 for men and 0.24 for women). AF for two 24 HDR was higher, at 0.41 for men and 0.35 for women.[8] Overall, the AFs associated with the

two dietary instruments were greater in men, indicating greater amount of error in self-reported sugars estimates from women, which would lead to greater underestimation of disease risk.

The predictive sugars biomarker has been thoroughly investigated in the feeding studies in the European population.[6,7] However, it is possible that the predictive biomarker will respond differently in different populations, due to inter-ethnic differences between the European and the US populations. The biomarker performance could also vary due to the presence of an ethnically diverse population in the United States with varying rates of absorption and metabolism, and different genetic, environmental and lifestyle determinants of the biomarker.[55] Additionally, the source of the sweeteners used in the two geographical locations could lead to a variation in results. While corn-based HFCS is the common sweetener in the United States, sucrose extracted from sugar beets is the dominant sweetener used in Europe.[9] The glucose-to-fructose ratio of the two sweeteners is different, and also there is no sucrose in HFCS. Owing to all these reasons, it is important to further examine the performance of the biomarker as a valid estimate of the total sugars consumption in the U.S. population.

2.3.2. Gastric diseases leading to variability in sugars biomarker

Increased sucrose permeability was demonstrated to be indicative of gastrointestinal damage.[94,95] Gastrointestinal mucosa, unless damaged, is generally impermeable to large molecules, such as disaccharides, although small amounts pass through the small intestine and gets excreted intact in the urine.[94,96] In a sucrose permeability study, 189 subjects were given a 100 g sucrose drink after fasting, and urine samples collected within the next 10 hours were measured for sucrose. Small amounts of urinary sucrose were observed under normal conditions (controls) and in those with mild symptoms of gastritis and other GI disorders. However, in subjects with severe gastritis and gastric ulcers (confirmed by an endoscopy), high levels of urinary sucrose, approximately in the range 250 – 350 mg/day, were observed ($p < 0.05$) compared to controls.[94] Due to the elevated urinary sucrose in unhealthy gastrointestinal mucosa, studies evaluating the performance of sugars biomarker should ensure exclusion of subjects diagnosed

with gastric diseases, in order to avoid unusually high urinary sucrose levels, that could lead to inaccuracy in predicting sugars consumption.

2.4. Metabolism of sucrose and fructose

2.4.1. Sucrose absorption and metabolism

As sucrose and fructose in urine are the individual components of the biomarker of sugars intake, understanding of the metabolism of these sugars in the human body is critical. Additionally, in order to understand if urinary excretion of sucrose and fructose behaves differently with respect to the sucrose and fructose intake ratio, it is important to understand the differences in their metabolism in humans.

Sucrose is hydrolyzed by the enzyme sucrase to glucose and fructose and is absorbed in the small intestine, specifically, the jejunum.[97] A small fraction of the ingested sucrose escapes hydrolysis and passes through the normal intestinal wall to be excreted in the urine intact as sucrose. Sucrose found in the urine is of dietary source except when endogenous sucrosuria exists.[96]

2.4.2. Fructose absorption and metabolism

After ingestion of fructose either as free fructose from fruits and vegetables, HFCS, or bound in sucrose and released after sucrose hydrolysis, the monosaccharide enters the absorption site, the small intestine, where rapid carrier-mediated energy independent absorption via the glucose transporter protein GLUT5 takes place and is then released into systemic circulation. Different body tissues absorb fructose from the bloodstream, including the liver, which is the primary site of fructose metabolism with an abundance of the glucose transporter protein GLUT2.[98] Free fructose, however, is believed to have a poor absorption capacity.[99] Rumessen et al[99] demonstrated the absorption capacity of fructose in 10 healthy men and women ages 25 to 51 years. After overnight fasting, the subjects were given solutions of 100 g, 75 g, and 50 g of sucrose, 50 g, 37.5 g, 25 g, and 15 g of fructose, and a mixture of 50 g fructose and 50 g, 25 g, or 12.5 g of glucose in a dose dependent manner. Breath hydrogen samples collected for four hours post ingestion revealed high intra-individual variability in the absorption capacity of fructose and fairly

low total absorption capacity. Fructose when ingested in the form of a monosaccharide was found to have a significantly lower absorption capacity when compared to fructose ingested in the form of sucrose ($p<0.01$). Simultaneous ingestion of glucose along with fructose seemed to promote fructose absorption; the facilitating effect of glucose could be due to the disaccharidase related transport system.[100]

Apart from the liver being the main site of fructose metabolism, kidneys were demonstrated to be the secondary site for fructose uptake and clearance. Bjorkman et al[101] conducted a study in 12 healthy men in the age group of 20 to 39 years who were administered 2 mmol/min of fructose intravenously for 45 minutes 60 hours post-fasting. Fructose uptake resulted in glucose production in the kidneys in the presence of enzymes. This renal gluconeogenic effect of fructose explains why blood glucose and muscle glycogen increase after large doses of fructose infusion. An increase in pyruvate and lactate was also evident after infusion of fructose suggesting that some part of the fructose passing through the kidneys plausibly goes through glycolysis as well. Urinary losses of fructose were about 1 to 5% of the infused load suggesting only a small fraction of fructose is excreted in the urine.

2.5. Physical Activity

2.5.1. Physical activity guidelines in the United States

In the year 1995, the Centers for Disease Control and Prevention (CDC) and the American College of Sports Medicine (ACSM) published physical activity recommendations[102] on the amount of physical activity required for promotion of health and disease prevention. CDC and ACSM advised that every US adult should attain 30 or more minutes per day of moderate-intensity activity on at least 5 days, and preferably all days of the week, for a total of at least 150 minutes/week.

The most recent Physical Activity Guidelines for Americans[103] (PAGA) were released in 2008 by the US Department of Health and Human Services (DHHS) with a set of public health recommendations for physical activity for US adults. These newer guidelines are similar to prior recommendations,[102] however, they allow a person to accumulate 150 minutes per week in different ways.

According to PAGA, in order to achieve substantial health benefits, adults are required to obtain at least 150 minutes/week of moderate-intensity aerobic physical activity (MPA), or 75 minutes/week of vigorous-intensity aerobic physical activity (VPA) or 75 minutes/week of a combination of aerobic MPA and VPA (MVPA). Substantial health benefits include lower risks of premature death, coronary heart disease, stroke, hypertension, type 2 diabetes as well as depression.[103] For extensive health benefits, which means even lower risks of heart disease, hypertension or diabetes, adults were advised to increase their aerobic physical activity to 300 minutes/week of MPA or 150 minutes/week of VPA or MVPA.[103]

It is desirable to distribute the aerobic physical activity through the week, for example in bouts of 10 or more minutes at a time. Activities performed on three or more days in a week produce health benefits, rather than activities done for a long period of time in one single day. PAGA also recommends muscle-strengthening activities of moderate or high intensity involving all major muscle groups on two or more days per week for additional health benefits.[103]

2.5.2. Physical activity trends in the United States

The physical activity patterns of the United States population reported here is based on the data collected from 2003 – 2004 through NHANES (n=6329).[104] NHANES 2003-2004 provided the first objective measurement of physical activity data using an accelerometer. For adults ages 18 years or older, moderate intensity activities of 3 METs and vigorous intensity activities of 6 METs were used as threshold criteria. Troiano et al[104] observed that less than 5% of the adults in the US met the physical activity recommendation of 30 or more minutes per day of MPA on five or more days per week put forth by the CDC and the ACSM. The mean time of MPA of adults remained steady up to the age of 40-49 years and decreased with increase in age. When only bouts of activity were counted, adults in the age range of 20 – 59 years attained about 6 – 10 minutes per day of moderate or greater intensity activities, and less than 2 minutes per day of VPA. For older adults over 60 years, mean time in VPA was equal to zero.

Tucker et al[105] evaluated self-reported and objectively measured physical activity in US adults based on data from NHANES 2005-2006 (n=4773) in reference to PAGA. Physical activity was expressed in three different measures; MVPA, M2VPA (Moderate plus two instances of VPA), and METPA (MET-minutes per week). Self-reported physical activity was measured via interviews requiring participants to recall their physical activity, including transportation, household, and leisure activities in the last 30 days. METs were assigned to all activities based on the Compendium of Physical Activities published in the year 2000.[106] Objective physical activity data were obtained using an accelerometer. Physical activity levels estimated by self-reported data were higher compared to PA assessed by accelerometer.[105] Self-reported physical activity data estimated a mean ± SE of 324.5 ± 18.6 minutes/week of MPA, and 73.6 ± 3.9 minutes/week of VPA, whereas the mean ± SE of MPA and VPA through accelerometer was estimated at 45.1 ± 4.6 minutes/week and 18.6 ± 6.6 minutes/week, respectively. Based on self-report, the proportion of US adults meeting the PAGA were 59.6%, 62% and 65.7% using the MVPA, M2VPA, and METPA methods, respectively, whereas using accelerometer, these values were 8.2%, 9.6%, and 44.6% respectively.[105]

According to self-reported data, men showed higher MPA and VPA levels (380.5 and 80.6 minutes/week) than women (272.6 and 67.1 min/week). Self-reported MPA and VPA levels were also higher in non-Hispanic whites (346.9 and 76.1 min/week) than non-Hispanic blacks (282.9 and 71.2 min/week) and Mexican Americans (212.1 and 47.3 min/week). These estimates were also higher in normal weight adults (342.0 and 106.2 min/week) than in overweight (322.1 and 71.5 min/week) and obese class I adults (328.2 and 50.2 min/week). Adults belonging to obese class II reported the lowest MPA and VPA levels at 282.3 and 32.1 min/week, respectively.[105]

Tucker et al[105] also reported that 9.5% of men and 7% of women met the PAGA recommendations based on accelerometry data, which is higher than the report based on NHANES 2003-2004.[104] These differences were partly attributed to increase in physical activity levels between the data collection periods of NHANES 2003-2004 and NHANES 2005-2006 and partly due to the slight changes in guidelines used for the two analyses.[105]

2.5.3. Effects of physical activity on fructose metabolism

A huge body of evidence exists on the effects of fructose consumption on human health. Consumption of fructose-rich foods and beverages promotes body weight gain and visceral adiposity,[107] de novo lipogenesis,[108] fatty liver,[109] hyperglycemia,[110] dyslipidemia,[110,111] insulin resistance,[107] and hypertension,[112,113] affecting both children and adults. As a result of the recent surge in HFCS consumption and its metabolic consequences, the focus of the research community has shifted to ways of mitigating the risks of high sugar consumption, one of them, being physical activity.

Several diet and exercise randomized controlled intervention trials[11,13,114] examined the role of physical activity in reducing the adverse effects of a fructose-rich diet. One trial involving 22 healthy men and women age 18 to 25 years,[11] examined the interaction between a high fructose diet and physical activity levels on postprandial lipidemia and concentration of inflammatory markers; interleukin 6, C-reactive protein and tumor necrosis factor. Subjects were supplemented with a 75 g fructose drink (FR) each day along with their usual diet for two two-week intervention periods, a high physical activity level period (>12,500 steps, FR + Active) and a low physical activity level period (< 4,500 steps, FR + Inactive). Subjects consumed a fructose-rich meal on one study day at the start and end of each intervention period. The results showed an increase in postprandial lipidemia and signs of potential low-grade inflammation in physically inactive subjects, whereas these adverse effects were absent in physically active subjects. Postprandial lipid profiles were measured in terms of total area under curve (tAUC) and ∆peak (the absolute change between baseline and peak concentrations). tAUC for triglyceride (TG) concentrations increased significantly from pre- to post-FR + Inactive intervention ($p = 0.04$), while no changes were observed between pre- and post-FR + Active intervention. An 88% increase in ∆peak for postprandial TG ($p = 0.009$), and 116% increase in ∆peak of IL-6 ($p=0.009$) were also seen in the FR + Inactive intervention, while no changes in these variables were observed in the FR + Active intervention. An 84% and 33% increase in ∆peak for postprandial VLDL was observed in FR + Inactive ($p = 0.002$) and FR + Active interventions ($p = 0.009$), respectively.[11] Thus, physical activity in the form of aerobic exercise seemed to protect against the metabolic adverse effects of a moderate dose of fructose consumption of about 75 g/d over 2 weeks.

In another randomized crossover intervention study, the effects of physical activity on circulating lipids were examined in eight healthy men enduring an aerobic exercise protocol while being fed an energy balance, high fructose diet.[114] The study included three 4-day intervention periods, 1) a low fructose diet - no exercise period (controls), 2) high fructose diet (30% EI) - no exercise period and, 3) high fructose diet (30% EI) - moderate aerobic exercise period. While in the low fructose - no exercise intervention period, fructose intake was associated with a modest but significant increase in total triglycerides (TG) ($p < 0.05$) from baseline, the TG levels after high fructose - no exercise further increased compared to the controls ($p < 0.001$). In the high fructose - moderate aerobic exercise intervention, total TG concentrations were normalized and were not statistically significantly different compared to the controls. These findings further confirm the effects of fructose consumption on the circulating lipid levels suggesting that exercise hindered de novo lipogenesis due to possible metabolic changes.

Similarly, one trial recently investigated the effects of muscle strengthening exercises on fructose metabolism conducted by Wilburn et al.[13] The researchers looked at the effects of strength exercise in a randomized crossover diet-exercise intervention in eight healthy men fed with a high-fructose, high-fat diet. Participants endured a 95-minute bout of strength exercises (four sets of 10 repetitions each with a 90-sec rest period between each set) and consumed a beverage with 0.75 g/kg of body weight (BW) of fructose and 0.5 g/kg BW of fat, after 15 hours of the exercise protocol. Blood samples drawn over the next six hours showed a 20% reduction in triglyceride levels when compared to the controls who were sedentary. This finding demonstrated that strength exercise reduced the post-prandial lipogenic effects of the high-fructose high-fat diet.

Physical activity has been demonstrated as a way to inhibit de novo lipogenesis that results from high fructose consumption. In a meta-analysis reviewing 51 dietary and aerobic exercise intervention trials, Leon and Sanchez[12] looked at the effects of aerobic exercise training on the blood lipid and lipoprotein profiles in adult men and women. It was observed that an aerobic exercise intervention of 12 weeks or more led to a 4.6% raise in high-density lipoprotein (HDL), 5% decrease in low-density lipoprotein (LDL), and a mean reduction of 3.7% in triglyceride levels.[12]

Evidence from these intervention trials demonstrate that physical activity, both in the form of aerobic and resistance training, might have a favorable effect on fructose metabolism. The current study hypothesizes that this in turn may have an effect on fructose excretion, and thus could influence the correlation between dietary and urinary sugars.

2.6. Conclusion

Although several studies as discussed above, strengthened the evidence on the role of physical activity in attenuating the metabolic effects of fructose, there are no studies so far to investigate the effects of physical activity on sucrose and fructose excretion. In this context, it may be important to clarify that the current study is not designed to look at the direct effects of physical activity on the urinary sugars excretion, but to look at the potential effects of physical activity on the association between sugars intake and urinary sugars. This will determine if physical activity is a determinant of the urinary sugar biomarker, which is important in characterizing the biomarker for its future use as an objective tool for accurate and unbiased estimation of total sugars consumption. The study hypothesizes that physical activity does not have a significant effect on the association of urinary sugars and total sugars intake, and is not a significant determinant of sucrose and fructose excretion.

CHAPTER 3

METHODS

3.1 Study Participants

The participants for this study were a subset of an ongoing study investigating biomarkers of sugars intake (Sugars Bio research study). Healthy non-smoking men and women age between 18 and 70 years with BMI < 35 kg/m^2 were recruited from Phoenix Metropolitan area. Those with kidney diseases, bladder incontinence, gastric diseases, type 1 or 2 diabetes, diet restriction due to a medical condition, weight loss in the last 4 months, participation in another diet-related research study in the last 4 months, sunscreen allergy, aminobenzoate potassium (POTABA) or para-amino benzoic acid (PABA) allergy, and, for women, those who were pregnant or lactating, were excluded. Participants were also excluded, if their fasting blood glucose ≥ 100 mg/dl or HbA1C ≥ 5.7%. Study fliers were advertised in and around the campus, at cafes and restaurants, churches, senior centers, etc. Emails were sent to employees in the downtown campus, and through community networks. Participants were also recruited through social media advertisements and word of mouth. Stratified recruitment based on age, gender, and BMI was employed to obtain a heterogenous sample. All participants provided full informed written consent, and the study was approved by Arizona State University Institutional Review Board (See Appendix A and B).

3.2 Study design

This study is a 15-day highly controlled feeding study, wherein the participants consumed their usual diet and collected eight 24-hour urine samples. First, the participants completed a screening questionnaire to determine their eligibility in the study. During the screening visit, participant's anthropometrics were measured, and fasting blood sample was collected and sent to a certified lab to test for fasting blood glucose and HbA1C. The project coordinator described the study, study calendar, biological samples that will be collected and level of involvement to the participant, during the screening visit. If the participant agreed to take part in the study, they signed a consent form. Based on the screening

questionnaire, BMI (< 35 kg/m^2) and laboratory test results (blood glucose <100 mg/dl or HbA1C < 5.7%), if the participants were eligible to take part in the study, they were scheduled for a baseline visit. During this visit, an interviewer-administered baseline questionnaire that included questions on demographics, lifestyle habits and personal medical history was completed. The project coordinator also reviewed the study forms and procedures of the study with participants. Then, participants met with the research chef to go over the procedure of keeping a food diary. The research chef went over the 'Training for 7-day food diary' document with the participants and provided measuring cups, spoons and a food model booklet to help with recording. The food diary assessed the regular diet and eating patterns of the participant, which was used to provide participant's usual diet during the feeding period. See Appendix C for the 7-day food diary, adapted from the EPIC study.[115] The participants then kept two 7-day food diary while at home, in order to assess their usual diet. They recorded everything they ate and drank each day in detail including 'description and preparation' and amounts of each meal. The research chef reviewed each of the 7-day food diary during this period in order to ensure all the necessary details have been recorded. The 15-day feeding period started one week after the food diary period, during which meal plans and preparation were completed. During the 15-day feeding period, participants were provided with all their food. Body weight was measured on all days, except weekends, and BMI computed. Participants were asked to keep a meal checklist and physical activity log daily, and completed eight 24-hour urine collections, one every alternate day. See Appendix D for the study design.

3.3 Feeding protocol

After reviewing participant's food diary, which was used to replicate their usual diet, all the food consumed by participants was prepared in the metabolic research kitchen. The chef prepared two and a half times of the food expected to be consumed by the participants. Half of the food was used to prepare duplicate diets; stored for analysis of sugars and other nutrients of interest in future, and half of the food was given to the participants. Participants consumed breakfast in the kitchen during their daily visit and collected food for the rest of the day, packed in a cooler. On Friday, food for the entire weekend was provided. Participants were allowed to eat as much as they wanted and were instructed to return the leftover

food to the kitchen, the following day, or on Monday morning. Participants were instructed not to consume food other than what was provided to them. All returned food was weighed to the closest gram, to estimate the amount of foods consumed. Feeding data were entered into the NDS-R Software [Version 2016, Nutrition Coordinating Center (NCC), Minneapolis, MN] to estimate energy and nutrient intake during the 15-day feeding study.

3.4 Meal checklist

Participants kept a meal checklist (See Appendix E) daily over the 15-day feeding study period, in order to track the order of food consumption accurately, and to assess compliance to the feeding protocol. Subjects noted down the time of each meal and snack in the Meal Checklist, any beverages not provided by the metabolic kitchen, but allowed for consumption, such as black coffee and tea, beer, wine and hard liquor, along with the amount consumed, accidental consumption of any outside food, any food not consumed from what was provided but not returned to the kitchen, and number of sleep and nap hours. The project coordinator reviewed the meal checklist with participants daily except for weekends. Weekend meal checklists were reviewed on Monday morning.

3.5 24-hour urine collection and storage

Participants collected a total of eight 24-hr urine samples during the 15-day study, on days 1, 3, 5, 7, 9, 11, 13, and 15. Participants were asked to collect urine over an entire 24-h period after discarding the first morning urine on the collection day, and collect all voided urine, starting with the second morning urine of the day until and including the first morning urine the following day. Step by step instructions of urine collection was provided in the directions given to participants. See Appendix F for directions and Appendix G for the 24-hour urine lab log used to record the information regarding sample collection and processing in the laboratory.

Participants were given a 24-hr urine collection kit consisting of a urine collection hat/urinal provided on the first day, two 3.5 L plastic containers filled with 4 g of the preservative boric acid and labeled with subject ID, date of collection and container number. A cooler with frozen ice packs to keep urine cold during collection were given to participants along with the containers, the day before the urine collection day. A temperature logger (HOBOWARE, Bourne, MA) was launched and set up in the inner wall of the cooler, in order to monitor the temperature of the collected urine. Participants were instructed to add ice packs at five different time points during the day to maintain the urine below 22ºC at all times, to prevent sugars in the urine from degradation.

3.5.1 Compliance to the 24-h urine collection protocol

Participants were instructed to complete a 24-hour urine collection log (Appendix H) each day urine was collected. In the log, they recorded the time when the first morning void was discarded, the time of the first collected void (i.e., the second void of the day), and the time each POTABA tablet was taken. If they missed any urine, participants recorded the time and approximate volume of the missed void, as well as the dosage and number of any medications taken. Participants were instructed to avoid taking medications such as sulfonamides, acetaminophen (Tylenol), and furosemide (Lasix) as they are structurally similar to PABA and could interfere with PABA analysis.

Compliance to the 24-hour urine collection protocol was objectively assessed by PABA, a marker of 24-hour urine completeness, which is almost completely recovered in urine after an oral administration.[116] As pharmaceutical-grade PABA is not available in the United States, POTABA, potassium salt of PABA, which has been validated as an alternative to PABA,[117] was used for this study. Participants were instructed to take three 102 mg POTABA tablets on the collection days, first one around breakfast time after the first urine was discarded, and the other two around lunch and dinner time, making sure the tablets are spaced at least five hours apart.

3.5.2 Processing and Storage of urine samples

The morning after the 24-h urine collection day, urine sample coolers were picked up from participant's homes and delivered to the study center by a certified courier, refrigerated and processed within 2 hours. 24-h urine samples were weighed and mixed thoroughly 20 times by rotation and inversion. Urine was then aliquoted into labeled 5 mL and 2 mL vials and frozen at -20°C and -80°C for further analysis.

3.6 Analytical methods

3.6.1 PABA analysis in urine

PABA in urine was measured using high-performance liquid chromatography (HPLC) method, which can differentiate PABA from compounds with similar chemical compositions.[118] The cut-off for 24-hour urine completeness using the HPLC method was determined to be 78%.[119] 24-hour urines were considered to be complete if PABA recovery was greater than or equal to 78% and if participants reported less than one missed void or less than 0.5 oz. missed urine volume in total.

3.6.2 Urine analysis of sucrose and fructose

Urinary sucrose and fructose concentration were measured using a UV method for the determination of sucrose, glucose and fructose using the Sucrose/D-Glucose/D-Fructose kit (R-Biopharm, Germany) on the Beckman DU 730 Life Science UV/Vis spectrophotometer. This method is based on the enzymatic determination of sugars with which very small amounts can be measured. This method has been previously described.[6] Briefly, urine samples were thawed and thoroughly mixed before analysis. The samples were run in duplicates, using standards with concentration of 5, 50, and 100 mg/L sucrose and fructose, and quality controls. The detection limit for sucrose and fructose were 2.4 mg/L and 0.6 mg/L, respectively. The intra-assay and inter-assay CV for the quality controls were 3.8% and 5.5% for sucrose, and 2.8% and 5.2% for fructose, respectively.

3.7 Anthropometric measurements

During screening, participants' body weight (kg), and height (cm) was measured using SECA 284, the digital measuring station for height and weight, and BMI was computed as weight in kg divided by height in meters squared. During the 15-day study period, body weight was measured daily except during weekends. Participants removed their shoes, and any heavy objects, jewelry, or ornaments before measurement. Two measurements were taken each time, and the mean value was recorded.

3.8 Physical activity assessment

3.8.1 Physical Activity Log

To assess physical activity (PA), participants were asked to complete a 15-day PA log inquiring about participants' PA (See **Figure 1** for the PA log). The log asked about 38 different activities organized within six domains (home activities, transportation, occupation, conditioning, sports and leisure activities). Participants circled the type of physical activity (Yes/No), time they began the activity, and time spent for each PA in hours and minutes during a day. Participants were instructed to complete the log at the end of the day and were encouraged to only record the hours and minutes they were actively engaged in each activity. For activities that were done a number of times during the day, for example, "*walking at work*", participants were asked to record the cumulative time spent doing the activity. Participants were instructed to omit logging activities that lasted less than 10 minutes. The log book also allowed participants to enter "other" activities. The project coordinator reviewed the PA log for completeness and accuracy, with participants daily, except during weekends. The logs kept over the weekend was reviewed on Monday. The PA log has been previously described in detail,[120] and validated against an accelerometer in a study investigating the performance of two self-reporting methods for measuring time spent in daily physical activity over a 21-day period among 83 adults. The Spearman correlation coefficients between the accelerometer and the PA log ranged from r = 0.22 to r = 0.36.[120]

3.8.2 PA Data entry into Qualtrics

All data from the completed paper version of the PA log were entered into the web-based Physical Activity logs created in Qualtrics (Qualtrics, Provo, UT). A unique Qualtrics link was generated for each

Day 1: Date ___/___/20___ Day of the Week: ___

Did you do this activity today?	Yes	No	How Long? Hours: Minutes	Time started activity AM or PM
Home Activities				
Sweep, scrub floors, vacuum, washing clothes, etc	Yes	No	___:___	___ AM/PM
Carpentry	Yes	No	___:___	___ AM/PM
Gardening or Yard Work	Yes	No	___:___	___ AM/PM
Transportation				
Walk to work, school, shopping	Yes	No	___:___	___ AM/PM
Bicycle to work, school, shopping	Yes	No	___:___	___ AM/PM
Occupation				
Sitting at work	Yes	No	___:___	___ AM/PM
Standing at work	Yes	No	___:___	___ AM/PM
Walking at work	Yes	No	___:___	___ AM/PM
Lift or carry 10-20 lbs at work	Yes	No	___:___	___ AM/PM
Lift or carry 20+ lbs at work	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Conditioning Activities				
Aerobic Exercise, Aerobic Dance	Yes	No	___:___	___ AM/PM
Bicycling	Yes	No	___:___	___ AM/PM
Calisthenics or gymnastics	Yes	No	___:___	___ AM/PM
Jogging or running	Yes	No	___:___	___ AM/PM
Hiking with pack or in mountains	Yes	No	___:___	___ AM/PM
Martial arts (judo, karate, tai chi)	Yes	No	___:___	___ AM/PM
Rowing a boat, canoeing	Yes	No	___:___	___ AM/PM
Swimming	Yes	No	___:___	___ AM/PM
Walking for exercise	Yes	No	___:___	___ AM/PM
Weight lifting, body building	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Sports Activities				
Baseball or softball	Yes	No	___:___	___ AM/PM
Basketball, European Handball	Yes	No	___:___	___ AM/PM
Surfing	Yes	No	___:___	___ AM/PM
Cross-country skiing	Yes	No	___:___	___ AM/PM
Handball, racquetball, or squash	Yes	No	___:___	___ AM/PM
Ice or roller skating, ice-hockey	Yes	No	___:___	___ AM/PM
Rugby, football	Yes	No	___:___	___ AM/PM
Soccer	Yes	No	___:___	___ AM/PM
Tennis	Yes	No	___:___	___ AM/PM
Volleyball	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Leisure Activities				
Bowling	Yes	No	___:___	___ AM/PM
General Dancing	Yes	No	___:___	___ AM/PM
Golf	Yes	No	___:___	___ AM/PM
Fishing	Yes	No	___:___	___ AM/PM
Table Tennis	Yes	No	___:___	___ AM/PM
Walking for pleasure or social	Yes	No	___:___	___ AM/PM
Yoga	Yes	No	___:___	___ AM/PM
Watching television	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM
Other: ___	Yes	No	___:___	___ AM/PM

End of Day 1

Figure 1. Physical Activity Log

participant, which was used to enter their 15-day data. When participants reported “other” activities in the paper log, the activities were entered into Qualtrics in the appropriate domain regardless of the domain in which the participant might have written. For example, when activities such as *sitting at home*, *standing around house* were reported, they were entered in the leisure domain only, and were not combined with the occupational activities *sitting at work*, *standing at work* respectively. Or when activities such as *circuit*

training, and *skydiving* were reported, they were entered in the conditioning and sports domains respectively. Other leisure related activities were always entered in the leisure domain only, in Qualtrics. Example, *grocery shopping, playing cards, playing with kids, watching movies* and so on.

When "other" activities related to home activities domain were reported such as *home painting*, *installing electricity in the attic* and so on, they were entered as leisure activities in Qualtrics since there was no option to enter "other" activity within the home activities domain in Qualtrics.

For "other" activities, the participants were probed further to provide a brief description of the activity in order to determine the accurate MET score. For example, when a participant reported the activity *loading, unloading of kayaks*, a description of the activity was requested such as how much weight did they lift while loading and unloading. As another example, when *socializing* was reported, a description of the activities involved was requested such as whether the participant was standing, sitting and so on while socializing. For analysis, PA data were downloaded from Qualtrics into an excel file.

3.8.3 Derivation of MET values for the PA log activities

Each activity in the PA log was assigned a Metabolic Equivalent (MET)[1] value generated based on the MET values from the "Compendium of Physical Activities".[121] The Compendium is a widely accepted system for coding of physical activity data. Every activity in the compendium is associated with a 5-digit code and a corresponding MET value, which indicates the energy cost of the activity relative to resting state. METs were derived using an informed judgement, by matching the reported activity in the log to activities listed in the compendium. When the compendium had different METs for a single type of activity with different intensities, a mean MET value was derived (See Appendix J for a list of activities and corresponding MET values). When an "other" activity was reported that was available in the compendium, the MET score was directly assigned based on the compendium. Examples are *Kickball* (15450, 7.0 MET), *circuit training*

[1] Metabolic Equivalent (MET) is the energy expenditure of a body in the state of rest, and it is used as a measure of energy cost of physical activities

(02035, 4.3 MET), *Watching movies* (07025, 1.5 MET), *grocery shopping* (05060, 2.3 MET). When an "other" activity reported was not available in the compendium, for example, the activity *Pickleball,* the description provided by the participant was a combination of racquetball, tennis, and badminton. In this case the MET value for *pickleball* was determined to be 5.8 MET after computing the average of the MET scores for racquetball (7.0 MET), tennis (5.0 MET), and badminton (5.5). As another example, the MET score for the activity *washing dishes/chores -laundry, cleaning etc.* was computed as 2.1 MET by determining the average of washing dishes (05041, 1.8 MET), light cleaning (05011, 2.3 MET), and laundry (05095, 2.3 MET). The most updated Compendium list published in 2011 was used for this purpose.[121,122]

3.8.4 PA Data processing and analysis

For each activity in the PA log, *total time spent in any activity in hours* was calculated as sum of minutes divided by 60. *Total MET hours* per day for each activity was calculated as total time spent in hours multiplied by its MET value. Total MET hours per day were then computed as the sum of total MET hours from all activities. Sedentary activity is defined as "not engaging in any regular pattern of physical activity beyond daily functioning" or "a state of the body in which bodily movement is minimal".[106,123] *Total sedentary MET hours* per day and *total standing MET hours* per day were computed as sum of all MET hours of sedentary activities and sum of all standing MET hours, respectively. For example, sitting at work was considered sedentary under occupation domain, watching television, and "other" activities reported such as reading, homework, socializing etc., were considered sedentary under leisure domain. Standing at work, standing around the house, cooking, getting dressed, and any "other" activity of light intensity (MET ≤ 2.5) involving standing, were considered as standing activities. *Total active MET hours* per day were then computed as total MET hours per day minus total MET hours from sedentary and standing activities. The subjects were stratified by physical activity level into three groups: low, moderate and high physical activity, based on the total active MET hours per week,[17] which was computed by calculating the average of total active MET hours of all the feeding days, multiplied by seven. Subjects with <10 total active MET hours/week (<600 MET minutes/week) were classified as being inactive (low activity), those with 10 – 49.9 MET hours/week (600 – 2999.9 MET-minutes/week) as moderately active (moderate activity), and those

with ≥50 MET-hours/week (≥ 3000 MET-minutes/week) as highly active (high activity). Additionally, the total active energy expenditure/day in kcal was calculated as total active MET hours/day multiplied by the 15-day mean body weight in kg.

We also computed total Walking, Moderate, and Vigorous activity hours for each day. Walking activities with MET ≥ 3.5 from any domain were included in Total Walking category. "Walk to work, school, shopping" with MET = 3.5 under transportation domain, "Walking for exercise" with MET = 4.8 under conditioning, and "Walking for pleasure or social" with MET = 3.5 under leisure domain were used to compute *total walking hours* across domains. "Walking at work", which has a MET value of 2.0 was not included in 'Total walking'. An activity with a MET value of 3 to 5.9 was considered moderate intensity, and an activity with MET ≥ 6 was considered vigorous intensity.[103] *Total moderate hours* per day and *total vigorous hours* per day were computed as the sum of all moderate activity hours and sum of all vigorous activity hours per day, respectively. See Appendix J for the list of moderate and vigorous intensity activities.

3.8.5 PA Data processing rules

A few data processing rules were put in place. Scientific evidence states that a minimum of 10 minutes of activity is required to attain health benefits,[17] thus activity time was re-coded to zero if total activity time for a single activity was less than 10 minutes. This step was done as a way to ensure that activities logged for less than 10 minutes, if any, are not being assessed. Additionally, in order to avoid misclassification into the "high activity" group, if any of the total walking, moderate or vigorous hours/day exceeded 3 hours, the truncation rule was applied.[17] Based on the truncation rule, individual activities contributing to the walking, moderate and vigorous activity times were revisited, and activity times were proportionally reduced to total 3 hours per day for each of the three categories. For example, if the moderate activities *sweep, scrub, vacuum, washing clothes etc.* and *gardening or yard work*, each was reported at 2 hours, each of them was truncated to 1.5 hours. However, if for example, the vigorous activities *jogging/running* was reported as one hour and *skiing* was reported as 6 hours, skiing was truncated to 2 hours, to keep the total vigorous hours/day as 3 hours. However, this was carefully done on a case by case

basis, and care was taken to consider the nature of the participant's profession before this rule was applied. For example, one of the participants was a body builder by profession and reported long hours in strength training, which was not considered as an over reporting.

3.9 Statistical Analyses

Total sugars intake (g/d), added sugars intake (g/d), physical activity expressed as total active MET-hours/day, BMI (kg/m^2), age, gender, ethnicity, total fiber intake (g/d), total fat intake (g/d) and total protein intake (g/d) were treated as independent variables. 24-hour urinary sucrose excretion (mg/d), 24-hour urinary fructose excretion (mg/d) and the sum of the two (24uSF) were treated as dependent variables. Age, gender, and ethnicity were used from the baseline questionnaire. Mean BMI was computed using the mean of participant body weights measured daily except weekends, and baseline height. Dummy variables for gender and ethnicity were created for the linear regression analyses.

Total fructose intake per day for each subject was computed as sum of fructose intake plus sucrose intake divided by two. Naturally-occurring sugars intake was computed by subtracting the sum of added sugars, lactose, and galactose intake from total sugars intake. 15-day mean of total sugars, added sugars, naturally-occurring sugars, dietary sucrose, dietary sucrose plus fructose and dietary total fructose were computed for each subject. Mean 24-hour sucrose excretion, mean 24-hour fructose excretion and mean 24uSF of complete urines were computed for each participant.

All continuous variables were tested for normality. Data were transformed, if they were not normally distributed, in order to apply parametric tests. Total sugars, added sugars, and dietary sucrose were normally distributed. Age, naturally-occurring sugars, and 24-hour fructose excretion were skewed, and were transformed using $\log_{10}$ transformation. 24uSF, walking time, moderate activity time and vigorous activity time in hours/day were transformed using square root transformation. Pre-truncated 15-day mean total MET hours per day was normalized using the "reflect and logarithmic" transformation. In this method, the maximum value of the pre-truncated mean total MET hours was taken, and 1 was added to this value

(max +1). Each value was then subtracted from max+1, and logarithmic 10 transformation of the resulting value was computed.

Normally distributed variables were expressed as means and standard deviations, and skewed variables as medians and interquartile ranges. In order to present the descriptive characteristics, participants were divided into tertiles of 24-hour urinary sucrose and fructose by gender. One-way ANOVA tests were done to compare means across tertiles for men and women. Chi-square test was done to determine the frequency difference across tertiles for categorical variables BMI, ethnicity, education, and marital status. Paired t-tests were performed to compare the means of total and active MET-hours, and total walking, moderate and vigorous activity hours, before and after truncation. Cohen's kappa coefficient (κ) was computed to test if the subjects were classified in concordance according to their physical activity levels, before and after the truncation process.

Pearson correlation coefficient was used to determine the correlation of 8-d mean 24uSF with 15-d mean total sugars, added sugars intake, naturally-occurring sugars and sucrose intake, and between 8-d mean 24-hour fructose excretion and 15-d mean dietary total fructose. The distribution of dietary sucrose plus fructose could not be normalized using $\log_{10}$, square root or inverse transformation methods. Hence, Spearman correlation coefficient was used to determine the correlation between mean dietary sucrose plus fructose and mean 24uSF. Partial Pearson correlation coefficient was used to assess the correlation between dietary and urinary sugars, after controlling for pre-truncated or post-truncated mean active MET hours. Within-subject Spearman correlation coefficients for 15 days of dietary vs. 8 days of urinary sugars were computed for each subject, and the mean within-subject correlation was estimated.

Pearson correlation coefficient was used to determine correlation of 8-day mean 24uSF with 15-day mean total sugars and added sugars by PA level, to check for interactions between physical activity and total sugars and added sugars, in relation to urinary sugars. Significance of mean difference for continuous variables across the moderate and highly active groups were determined using Independent t-test and Mann-Whitney test. Chi-square test was used to determine significant frequency difference for categorical variables. Mean 24uSF was normally distributed in the moderate activity group and skewed in

the high activity group. Hence, Pearson correlation coefficient between dietary sugars and mean sum of sucrose and fructose excretion was computed in the moderate group, and Pearson correlation coefficient between dietary sugars and square root transformed mean sum of sucrose and fructose excretion was computed in the highly active group.

In order to further explore the association between total/added sugars and urinary sugars, simple linear regression models were fitted with total sugars and square root transformed 24uSF and added sugars and square root transformed 24uSF. A multiple linear regression model was fitted by regressing 24uSF on total sugars intake and active MET hours, to test if physical activity was a significant determinant of urinary sucrose and fructose excretion, apart from total sugars intake. Similarly, another multiple linear regression model was fitted with 24uSF as dependent variable and added sugars and active MET hours as independent variables. The covariates age, gender, BMI, ethnicity, fiber intake, fat intake, and protein intake were also added in the two models to be investigated as potential predictors of urinary sugars. The covariates age, gender, BMI, ethnicity, fiber intake, fat intake, and protein intake were also independently added into multiple linear regression models fitted by regressing 24uSF and total sugars intake, and regressing 24uSF and added sugars intake, to see how each of these covariates affect the association between dietary sugars and the biomarker.

IBM SPSS Version 24.0 (SPSS Inc., Chicago, IL) was used for statistical analyses. Association was considered significant if $p < 0.05$.

CHAPTER 4

RESULTS

Of 347 total 24-hour urine collections, 247 collections (71.2%) were complete based on the criteria of PABA recovery and total self-reported missed voids. The collections that were incomplete were excluded from the analysis. Based on the complete urines, the 8-day mean 24-hour excretions of sucrose, fructose and the sum of the two were 26.6 (SD=16.1), 23.1 (20.9) and 51.0 (32.3) mg/day, respectively.

Table 1 shows descriptive characteristics of the study population, presented by tertiles of 24-hour urinary sucrose and fructose by sex. Of the 57 subjects, 21 (37%) were men and 36 (63%) were women. The average age of our population was 35 years with a mean BMI of 26.3 kg/m^2. In men, those in the 2nd tertile were the youngest compared to the first and third tertiles, where as in women, those in the 2nd tertile were the oldest, followed by tertile 1 and 3. However, the differences in age were not statistically significant between tertiles, in neither men nor women. BMI was also not significantly different across tertiles, in both men and women. Neither marital status nor education were found to be associated with 24uSF. In men, the mean total sugars intake was 127.1 g/d, ranging from 47.4 to 203.7 g/d (**Figure 2A**). In women, the mean total sugars intake was 103.5 g/d, ranging from 50.0 to 146.0 g/d (**Figure 2B**). As expected, the 15-d mean total sugars consumption increased across tertile 1 to 3, however this was observed only in men ($p = 0.005$), and not in women ($p = 0.238$). The same trend was observed for added sugars (men: $p = 0.006$; women: $p = 0.234$). The mean added sugars density was 9.6% EI ranging from 3.3 to 19.3% EI in men (**Figure 3A**). In women, the mean added sugars density was 9.9% EI ranging between 1.8 to 18.1% EI (**Figure 3B**). Similarly, sucrose and total fructose consumption increased across tertiles of sucrose and fructose excretion in men, whereas in women, only sucrose intake was associated with 24uSF ($p = 0.017$). None of the PA measures were significantly different across tertiles of sugars excretion (Table 1). See Appendix K for descriptive characteristics of the entire sample (n=57).

Table 1. Descriptive characteristics of the study population from a 15-d highly-controlled feeding study by tertiles of 24-hour urinary sucrose and fructose (24uSF) for men and women (n = 57)

	Men (n = 21)				Women (n = 36)			
	T1	**T2**	**T3**	**p**	**T1**	**T2**	**T3**	**p**
24uSF (mg/d) ‡ **(range)**	32.7 (10.0 – 35.4)	49.6 (37.3 – 54.7)	75.6 (60.5 – 152.1)	-	27.7 (16.3 – 45.7)	54.3 (46.4 – 60.9)	73.3 (61.4 – 120.5)	-
Age (years)‡	34.0 ± 14	30.0 ± 21	34.0 ± 15	0.947	39.5 ± 26	41.0 ± 26	35.5 ± 19	0.906
BMI (kg/m^2) †	25.3 ± 1.7	26.7 ± 4.5	26.8 ± 4.0	0.690	27.7 ± 4.6	25.0 ± 3.6	26.1 ± 4.4	0.320
BMI categories n (%)				0.337				0.553
Normal weight	4 (57.1)	4 (57.1)	2 (28.6)		4 (33.3)	7 (58.3)	5 (41.7)	
Overweight	3 (42.9)	1 (14.3)	4 (57.1)		4 (33.3)	4 (33.3)	5 (41.7)	
Obese	-	2 (28.6)	1 (14.3)		4 (33.3)	1 (8.3)	2 (16.7)	
Ethnicity n (%)				0.660				0.156
White/Non-Hispanic/Caucasian	5 (71.4)	6 (85.7)	5 (71.4)		8 (66.7)	9 (75.0)	12 (100.0)	
Hispanic/Latino	1 (14.3)	1 (14.3)	1 (14.3)		3 (25.0)	-	-	
Other	1 (14.3)	-	1 (14.3)		1 (8.3)	3 (25.0)	-	
Education n (%)				0.340				0.802
Some college or less	2 (28.6)	2 (28.6)	5 (71.4)		1 (8.3)	1 (8.3)	1 (8.3)	
Associate's/Bachelor's Degree	3 (42.8)	3 (42.9)	-		6 (50.0)	5 (41.7)	4 (33.3)	
Master's/Terminal Degree	2 (28.6)	2 (28.6)	2 (28.6)		5 (41.7)	6 (50.0)	7 (58.3)	
Marital status n (%)				0.826				0.644
Single/never married/divorced	3 (42.9)	4 (57.1)	3 (42.9)		8 (66.7)	5 (41.7)	6 (50.0)	
Married/Living with a partner	4 (57.1)	3 (42.9)	4 (57.1)		4 (33.3)	7 (58.3)	6 (50.0)	
15-day mean physical activity measures								
Total MET- hours/d‡	26.3 ± 9.5	27.4 ± 7.3	32.5 ± 6.1	0.065	27.4 ± 8.5	30.1 ± 2.7	30.5 ± 8.8	0.362
Active MET-hours/d†	10.6 ± 4.2	8.5 ± 5.9	14.4 ± 6.2	0.158	13.3 ± 6.2	12.8 ± 3.7	10.6 ± 5.4	0.409
Walking time (hours/d) ‡	1.09 ± 1.0	0.42 ± 0.5	0.52 ± 0.8	0.251	0.50 ± 0.5	0.64 ± 0.9	0.16 ± 0.8	0.093

Moderate activity time (hours/d) ‡	1.60 ± 1.8	0.60 ± 0.9	1.01 ± 2.3	0.159	1.34 ± 1.9	2.03 ± 1.6	0.52 ± 0.5	0.059
Vigorous activity time (hours/d) ‡	0.13 ± 0.3	0.13 ± 0.7	0.57 ± 1.1	0.130	0.33 ± 0.6	0.20 ± 0.2	0.53 ± 0.9	0.545
15-day mean dietary intake								
Total energy (kcal/d) †	3130.3 ± 923.8	3250.9 ± 615.8	3240.9 ± 452.7	0.844	2346.0 ± 442.8	2462.7 ± 277.2	2370.8 ± 351.3	0.712
Total sugars (g/d) †	95.6 ± 29.7	127.0 ± 28.2	158.5 ± 35.2	0.005	93.3 ± 30.7	109.1 ± 24.4	108.3 ± 19.1	0.238
Added sugars (g/d) †	51.6 ± 15.9	69.4 ± 22.6	107.3 ± 40.6	0.006	50.7 ± 27.3	63.8 ± 23.8	65.0 ± 13.2	0.234
Added sugars density (% EI) †	6.8 ± 2.2	8.8 ± 3.4	13.2 ± 4.7	0.011	8.3 ± 3.7	10.2 ± 3.5	11.1 ± 2.6	0.107
Naturally-occurring sugars (g/d) ‡	31.0 ± 39.2	45.9 ± 46.3	31.7 ± 43.7	0.817	31.5 ± 14.6	35.2 ± 21.4	23.4 ± 18.7	0.609
Sucrose (g/d) †	44.2 ± 17.1	70.1 ± 20.6	78.8 ± 17.6	0.007	44.2 ± 23.8	59.7 ± 19.3	61.9 ± 12.1	0.017
Fructose (g/d) ‡	23.3 ± 15.4	20.0 ± 14.0	33.1 ± 23.2	0.070	16.4 ± 7.8	16.9 ± 9.3	14.0 ± 12.0	0.566
Total fructose (g/d) †	43.3 ± 13.9	55.0 ± 14.3	72.0 ± 18.7	0.011	39.7 ± 14.1	48.0 ± 10.6	47.2 ± 9.9	0.179
Carbohydrate (g/d) ‡	322.5 ± 185.5	328.5 ± 164.6	361.2 ± 96.5	0.424	249.7 ± 66.4	271.6 ± 54.3	251.9 ± 80.2	0.467
Total fiber (g/d) ‡	26.2 ± 54.9	35.8 ± 31.6	25.5 ± 21.4	0.669	27.9 ± 12.1	32.1 ± 7.4	25.7 ± 14.9	0.675
Protein (g/d) ‡	106.5 ± 39.8	132.5 ± 55.6	116.1 ± 59.9	0.845	99.9 ± 50.4	102.3 ± 14.2	88.0 ± 23.9	0.299
Fat (g/d) ‡	106.6 ± 48.9	141.1 ± 82.8	133.0 ± 11.6	0.657	102.6 ± 40.1	112.1 ± 21.1	107.3 ± 24.3	0.705

† Mean and SD

‡ Median and IQR

[a] One-way ANOVA test to compare mean difference for continuous variables and Chi-square test to compare frequency difference for categorical variables

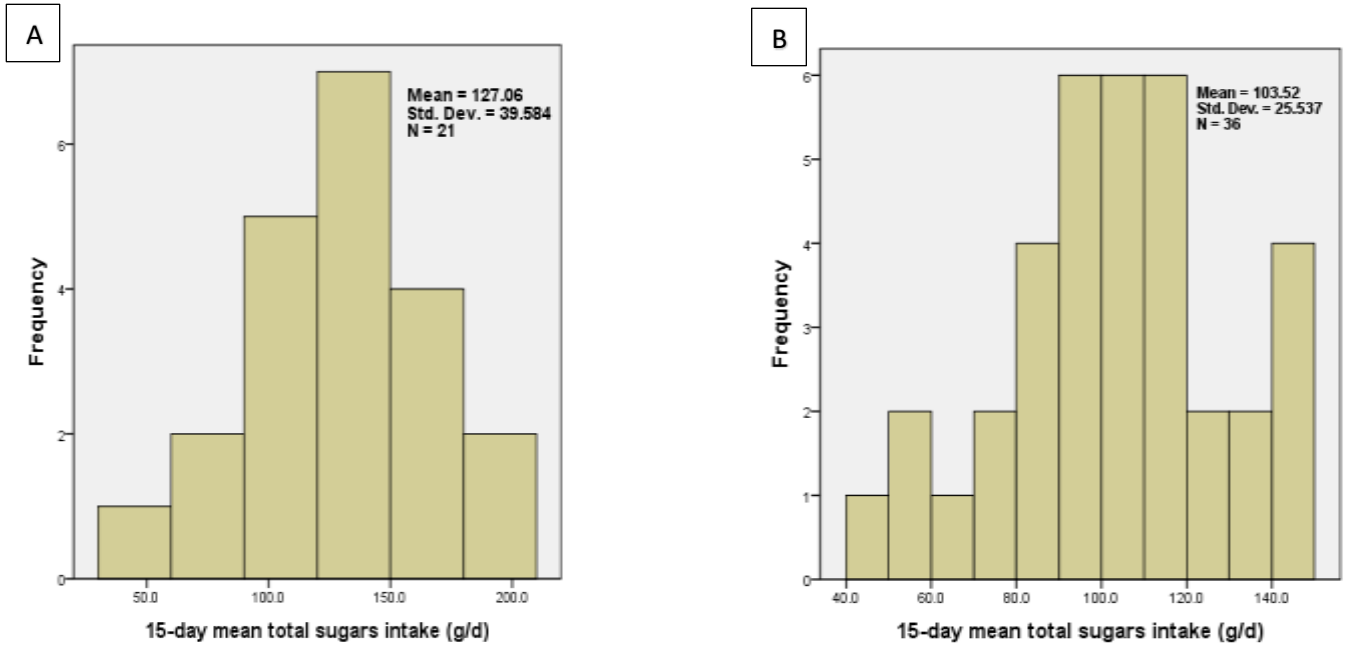

Figure 2. Histograms showing the distribution of total sugars in men (n=21) and women (n=36). (**A**) total sugars (g/d) in men; (**B**) total sugars (g/d) in women.

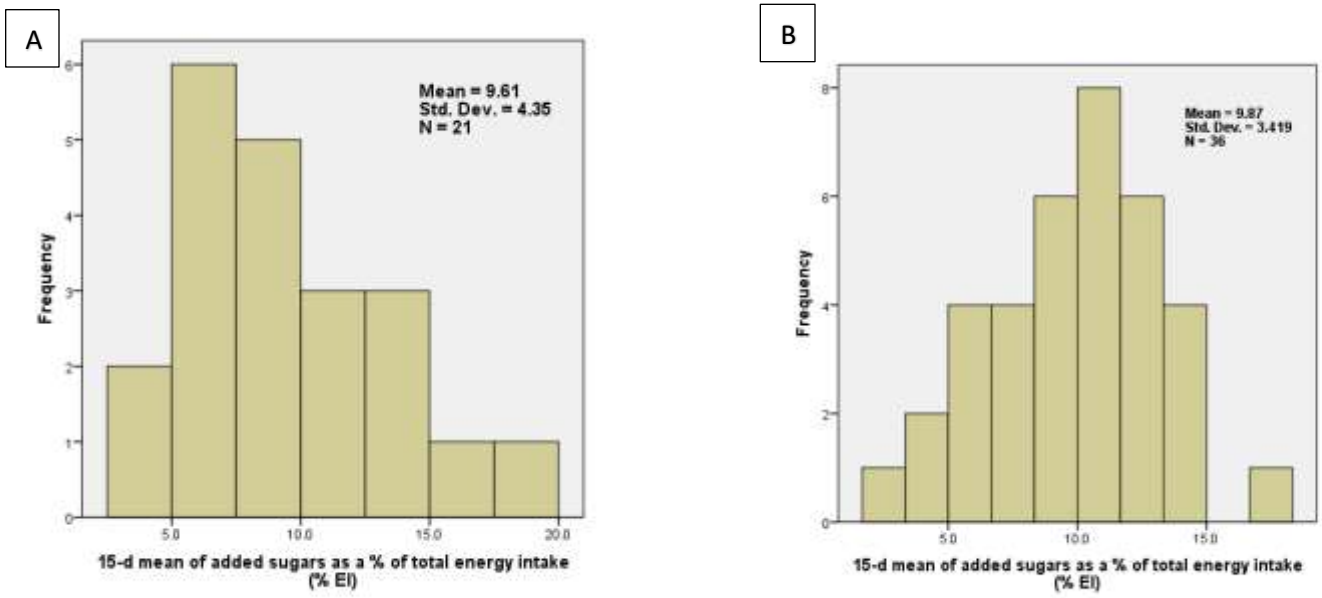

Figure 3. Histograms showing the distribution of added sugars in men (n=21) and women (n=36). (**A**) added sugars density (%EI) in men; (**B**) added sugars density (%EI) in women.

Total MET-hours and active MET-hours for the study population were 28.7 ± 7.5 and 11.9 ± 5.4 per day, respectively (**Table 2**). After applying the PA truncation procedure, which was done to normalize the distribution of PA levels, and avoid misclassification of subjects into high activity level category, the post-truncated 15-day total MET-hours and active MET-hours decreased to 26.7 ± 4.7 and 10.9 ± 5.0 per day, respectively, and were statistically significantly lower compared to pre-truncated estimates (p-values for all PA measures were < 0.05; Table 2).

Table 2. Physical activity measures of the study population before and after applying the truncation rule (n = 57)

15-day mean physical activity measures	Pre-truncation	Post-truncation	Paired t-test p-value
Total MET- hours/d	28.7 ± 7.5‡	26.7 ± 4.7†	<0.001
Active MET-hours/d	11.9 ± 5.4†	10.9 ± 5.0†	<0.001
Walking time[a] (hours/d)	0.47 ± 0.7‡	0.47 ± 0.6‡	0.003
Moderate activity time[b] (hours/d)	0.93 ± 1.6‡	0.78 ± 1.1‡	<0.001
Vigorous activity time[c] (hours/d)	0.28 ± 0.6‡	0.28 ± 0.5‡	<0.001

† Mean and SD

‡ Median and IQR

[a] Walking = Walking activities with MET≥3.5

[b] Moderate activities = activities other than walking with MET 3.0-5.9

[c] Vigorous activities = activities with MET ≥6

The 15-day mean total sugars and 15-day mean added sugars, each, were moderately positively correlated with 8-day mean 24uSF (r = 0.56, p < 0.001) (**Figures 4** and **5**). There was a statistically significant moderate positive correlation between mean dietary sucrose and mean 24uSF as well (r = 0.55, p < 0.001) (**Table 3).** We found no correlation between 15-d mean naturally-occurring sugars and 8-d mean 24uSF (r = 0.070, p < 0.001). All other correlations between dietary sugars and urinary sugars were statistically significant at p < 0.001. Controlling for 15-day mean pre-truncated or post-truncated active MET hours did not change any of the correlation estimates (data not presented).

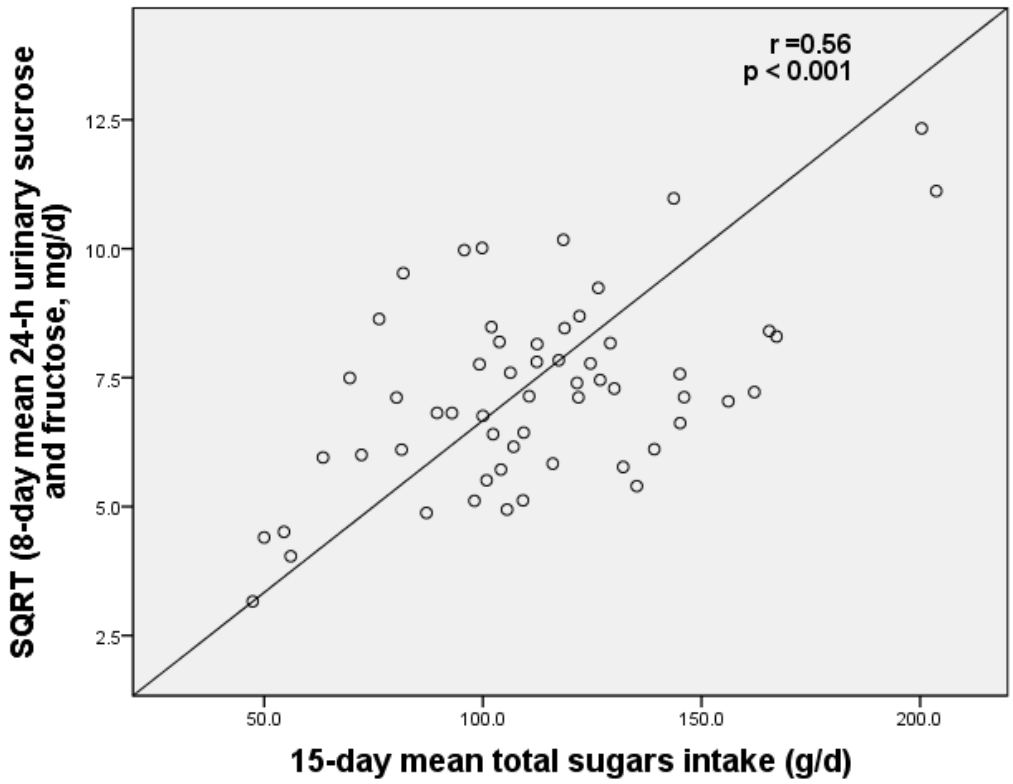

Figure 4. Scatterplot showing the association between 15-d mean total sugars (g/d) and SQRT (24-h urinary sucrose and fructose, mg/d) for the study population (n = 57)

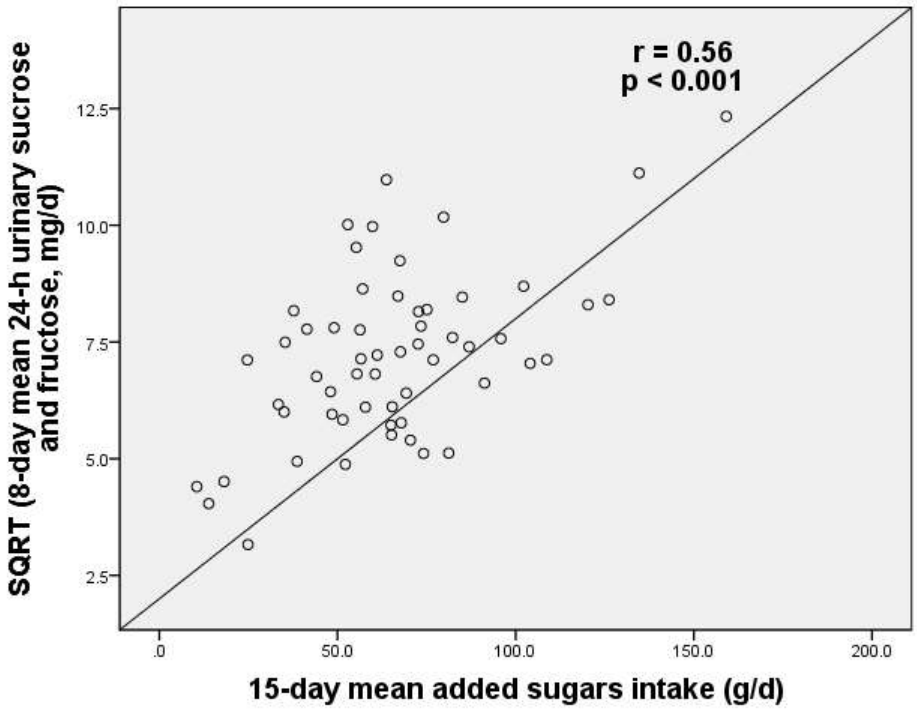

Figure 5. Scatterplot showing the association between added sugars (g/d) with SQRT (24-h urinary sucrose and fructose, mg/d) for the study population (n = 57)

Table 3. Pearson correlation coefficients (r) between 15-d mean dietary sugars and 8-d mean urinary sugars (n = 57)

	SQRT (Urinary sucrose and fructose excretion, mg/d)	**Log (Urinary fructose excretion, mg/d)**
	r	**r**
Total sugars (g/d)	0.56	
Added sugars (g/d)	0.56	
Dietary sucrose (g/d)	0.55	
Dietary sucrose+fructose (g/d)	0.46†	
Log (Dietary total fructose, g/d)	**-**	0.51
Log (Naturally-occurring sugars, g/d)	0.070	

All correlations are significant at $p < 0.001$

† Spearman correlation coefficient

In men, there was a strong significant correlation between 15-day mean total sugars and 8-day mean 24uSF ($r = 0.82$, $p < 0.001$) and 15-day mean added sugars and 8-day mean 24uSF ($r = 0.83$, $p < 0.001$). In women, though statistically significant, the correlation was much weaker for both total sugars ($r = 0.39$, $p = 0.020$) and added sugars ($r = 0.34$, $p = 0.046$). Nonetheless, the mean within-subject correlation between daily estimates of total sugars and 24-uSF was greater in women (0.72) than in men (0.57).

After stratifying the population based on active MET-minutes per week as shown in **Table 4**, there were no participants in the low activity level group, 19% of the population were in the moderate activity group (600-2999.9 MET-minutes/week), and 81% were in the high activity group (≥ 3000 MET-minutes/week). Cohen's kappa coefficient (κ) used to test the agreement in ranking subjects into moderate, and high activity groups, before and after truncation, was 0.95, indicating that the agreement between the rankings was almost perfect.

Table 4. Stratification of study population based on level of physical activity and Cohen's kappa coefficient (κ) of agreement in ranking subjects pre- and post-truncation (n=57)

		Post-truncation			**κ**
	Activity level	**Low[a]**	**Moderate[b]**	**High[c]**	0.95
Pre-truncation	**Low[a]**	0			
	Moderate[b]		11		
	High[c]		1	45	

[a] Low - < 600 MET-minutes/week,

[b] Moderate - 600-2999.9 MET-minutes/week

[c] High - ≥ 3000 MET-minutes/week

Table 5 shows the descriptive characteristics of the population by physical activity level. As expected, the 15-day mean total active MET-hours of the highly active group was significantly different from the moderately active group ($p<0.001$) with the highly active group being about three times more active, 12.9 ± 7.5 vs. 4.6 ± 2.1 MET-hours/day. No significant difference in total sugars, added sugars intake or 24uSF excretion was found between moderately and highly active group. However, the range of total sugars

and added sugars intake were much wider in the highly active group when compared to the range in the moderately active group.

Table 5. Descriptive characteristics of the study population based on physical activity level

	Moderate activity level (n=11)	**High activity level (n=46)**	**p-value [a]**
Age (years)	42 ± 16.5† (22 – 68)	34.5 ± 20 ‡ (20 – 63)	0.498
Gender n (%)			0.510
Male	5 (45.5)	16 (34.8)	
Female	6 (54.5)	30 (65.2)	
BMI (kg/m^2)	26.8 ± 4.3† (18.7 – 32.5)	26.2 ± 3.9† (19.8 – 35.2)	0.641
Ethnicity n (%)			0.734
White/Non-Hispanic/Caucasian	10 (90.9)	35 (76.1)	
Hispanic/Latino	-	6 (13.0)	
Other	1 (9.1)	5 (10.9)	
15-day mean physical activity measures			
Total MET-hours/d	21.4 ± 3.5† (14.7 – 26.3)	30.0 ± 4.7‡ (17.6 – 37.1)	< 0.001
Active MET-hours/d	4.6 ± 2.1† (1.5 – 7.0)	12.9 ± 7.5‡ (7.6 – 24.9)	< 0.001
Walking time (hours/d)	0.36 ± 0.3† (0.0 – 1.1)	0.51 ± 0.8‡ (0.0 – 1.7)	0.078
Moderate activity time (hours/d)	0.41 ± 0.3† (0.0 – 0.9)	1.27 ± 1.7‡ (0.0 – 5.1)	0.001
Vigorous activity time (hours/d)	0.02 ± 0.1‡ (0.0 – 0.3)	0.32 ± 0.5‡ (0.0 – 2.0)	0.001
15-day mean dietary sugars			
Total sugars (g/d)	112.8 ± 16.6† (81.8 – 139.2)	112.0 ± 36.1† (47.4 – 203.7)	0.908
Added sugars by total sugars (g/d)	69.4 ± 11.4† (49.0 – 86.9)	65.0 ± 31.9† (10.5 – 159.1)	0.449
Added sugars density (% EI)	11.0 ± 2.1† (8.2 – 14.6)	9.5 ± 4.0† (1.8 – 19.3)	0.083
8-day mean urinary sugars			
24-hour urinary sucrose plus fructose (mg/d)	51.5 ± 19.7† (29.1 – 90.7)	51.6 ± 33.4‡ (10.0 – 152.1)	0.762

† Mean and SD (range)

‡ Median and IQR (range)

[a] Independent t-test for normally distributed continuous variables, Mann-Whitney test for continuous variables with skewed distribution to compare mean differences, Chi-square test to test frequency difference for categorical variables

In the moderately active group (n = 11), the 8-day mean 24uSF was inversely correlated with 15-day mean total sugars (r = – 0.479, p = 0.136) and added sugars intake (r = -0.038, p = 0.912), however they were not significant. In the highly active group (n = 46), 8-day mean 24uSF was positively correlated with the 15-day mean total sugars (r = 0.633, p < 0.001) and added sugars intake (r = 0.603, p < 0.001) (**Table 6**). Correlation estimates remained the same after adjusting for pre- and post-truncated 15-d mean active MET hours (data not shown).

Table 6. Pearson correlation coefficients (r) between 15-d mean dietary sugars (g/d) and 8-d mean 24-h urinary sucrose and fructose (24uSF) (mg/d) in the moderate and highly active group

	Moderate activity level (n=11)	**High activity level (n=46)**
Total sugars intake vs. 24uSF	-0.479	0.633[a]
Added sugars intake vs. 24uSF	-0.038	0.603[a]

[a] p-value < 0.001

Table 7 summarizes the multiple linear regression models fitted to investigate the association between total sugars and urinary sugars and to predict 24uSF from total sugars and other covariates. In a simple linear regression model, total sugars intake explained 30% of the variation in sucrose and fructose excretion (Adjusted R^2 = 0.297, p < 0.001), whereas adding sex and protein intake along with total sugars explained an additional 7.6% in variability in excretion (Adjusted R^2 = 0.373, p < 0.001). Pre-truncated and post-truncated active MET-hours did not explain any fraction of the variation in urinary sugars when added with total sugars (Adjusted R^2 = 0.286, p < 0.001; and 0.285, < 0.001; respectively), showing that physical activity is not a significant determinant of urinary sugars. Additionally, adding age, BMI, ethnicity, fat, and total fiber intake individually with total sugars, did not explain any portion of the variability in urinary sugars.

Table 7. Summary of multiple linear regression models with SQRT (24-hour urinary sucrose and fructose, mg/d) as dependent variable and total sugars and other covariates as independent variables

Variables in the model	R^2 change	p-value	β (SE)	p-value
Exploratory models				
Total sugars (g/d)	0.310	<0.001	0.031 (0.006)	< 0.001
Model adjusted R^2	**0.297**	<0.001		
Total sugars (g/d)	0.310	<0.001	0.031 (0.006)	< 0.001
Pre-truncated active MET-hours/d	0.002	0.694	-0.015 (0.038)	0.694
Model adjusted R^2	**0.286**	<0.001		
Total sugars (g/d)	0.310	<0.001	0.031 (0.006)	< 0.001
Post-truncated active MET- hours/d	0.001	0.737	-0.014 (0.042)	0.737
Model adjusted R^2	**0.285**	<0.001		
Total sugars (g/d)	0.310	< 0.001	0.031 (0.006)	< 0.001
Log (Age, years)	0.000	0.991	0.016 (1.463)	0.991
Model adjusted R^2	**0.284**	<0.001		
Total sugars (g/d)	0.310	< 0.001	0.031 (0.006)	< 0.001
BMI (kg/m^2)	0.003	0.636	-0.025 (0.052)	0.636
Model adjusted R^2	**0.287**	<0.001		
Total sugars (g/d)	0.310	<0.001	0.030 (0.006)	< 0.001
Ethnicity	0.012	0.618		
Hispanics			-0.658 (0.671)	0.331
Others			-0.145 (0.679)	0.831
Model adjusted R^2	**0.284**	<0.001		
Total sugars (g/d)	0.310	< 0.001	0.036 (0.006)	< 0.001
Sex (Males)	0.061	0.026	-0.985 (0.431)	0.026
Model adjusted R^2	**0.347**	<0.001		
Total sugars (g/d)	0.310	<0.001	0.032 (0.007)	<0.001
Log (Fat intake, g/d)	0.002	0.713	-0.725 (1.956)	0.713
Model adjusted R^2	**0.286**	<0.001		
Total sugars (g/d)	0.310	<0.001	0.034 (0.007)	<0.001
Log (Fiber intake, g/d)	0.026	0.152	-1.820 (1.254)	0.152
Model adjusted R^2	**0.311**	<0.001		
Total sugars (g/d)	0.310	< 0.001	0.036 (0.006)	< 0.001
Log (Protein intake, g/d)	0.075	0.013	-4.438 (1.733)	0.013
Model adjusted R^2	**0.361**	<0.001		
Final model				
Total sugars (g/d)	0.310	<0.001	0.038 (0.006)	< 0.001
Sex (Males)	0.061	0.026	-0.654 (0.460)	0.161
Log (Protein intake, g/d)	0.037	0.076	-3.381 (1.871)	0.076
Model adjusted R^2	**0.373**	<0.001		

Additionally, multiple linear regression models were fitted to predict 24uSF from added sugars and other covariates (see **Table 8**). Similar to total sugars, added sugars intake explained 30% of variation in sucrose and fructose excretion (Adjusted $R^2 = 0.301$, $p < 0.001$), whereas adding sex and BMI explained an additional 6.3% in variability in excretion (Adjusted $R^2 = 0.364$, $p < 0.001$). Active MET-hours did not explain any proportion of the variation in urinary sugars (Adjusted $R^2 = 0.289$, $p < 0.001$ for the model with pre-truncated; and 0.290, $p < 0.001$ with post-truncated active MET-hours). Additionally, when age,

ethnicity, fat, total fiber, and protein intake were included with added sugars, they did not show to have any effect on urinary sugars.

Table 8. Summary of multiple linear regression model with SQRT (24-hour urinary sucrose and fructose, mg/d) as dependent variable and added sugars and other covariates as independent variables

Variables in the model	R^2 change	p-value	β (SE)	p-value
Exploratory models				
Added sugars (g/d)	0.314	<0.001	0.035 (0.007)	<0.001
Model adjusted R^2	**0.301**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.035 (0.007)	<0.001
Pre-truncated active MET-hours/d	0.001	0.816	0.009 (0.038)	0.816
Model adjusted R^2	**0.289**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.036 (0.007)	< 0.001
Post-truncated active MET-hours/d	0.002	0.672	0.018 (0.042)	0.672
Model adjusted R^2	**0.290**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.035 (0.007)	<0.001
Log (Age, years)	0.003	0.620	-0.724 (1.449)	0.620
Model adjusted R^2	**0.291**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.039 (0.007)	<0.001
BMI (kg/m^2)	0.038	0.083	-0.093 (0.052)	0.083
Model adjusted R^2	**0.327**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.039 (0.007)	<0.001
Sex (Males)	0.040	0.073	-0.778 (0.426)	0.073
Model adjusted R^2	**0.329**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.035 (0.007)	<0.001
Ethnicity	0.027	0.346		
Hispanics			-0.864 (0.662)	0.198
Others			-0.547 (0.662)	0.412
Model adjusted R^2	**0.303**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.037 (0.008)	<0.001
Log (Fat intake, g/d)	0.003	0.647	-0.903 (1.962)	0.647
Model adjusted R^2	**0.291**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.037 (0.007)	<0.001
Log (Protein intake, g/d)	0.028	0.133	-2.614 (1.715)	0.133
Model adjusted R^2	**0.317**	<0.001		
Added sugars (g/d)	0.314	<0.001	0.036 (0.007)	<0.001
Log (Fiber intake, g/d)	0.011	0.351	1.114 (1.184)	0.351
Model adjusted R^2	**0.300**	<0.001		
Final model				
Added sugars (g/d)	0.314	<0.001	0.043 (0.007)	< 0.001
Sex (Males)	0.040	0.073	-0.848 (0.417)	0.047
BMI (kg/m^2)	0.045	0.051	-0.102 (0.051)	0.052
Model adjusted R^2	**0.364**	<0.001		

CHAPTER 5

DISCUSSION

In our 15-d controlled feeding study including healthy participants consuming their habitual diet, both total sugars and added sugars were moderately positively correlated with 24-hour urinary sucrose and fructose ($r = 0.56$, $p < 0.001$). We found no effect of physical activity on the association between dietary and urinary sugars, indicating that physical activity is not a significant determinant of the sugars biomarker. Physical activity was shown to interfere with the hepatic metabolism of fructose[11-13] and possibly alter fructose excretion in the urine. However, our results suggest that physical activity level does not interact with dietary sugars in relation to sucrose and fructose excretion in urine. We found strong significant association between the sugars biomarker and dietary sugars in men, whereas the association was weaker in women. We also found sex to be a significant predictor of urinary sucrose and fructose excretion when included with total sugars and added sugars. More detailed investigation of the association between diet and urinary sugars in a large sample size with a wider range of sugars intake is needed to better investigate the characteristics of the biomarkers and its uses in the US.

5.1 Dietary Sugars Distribution

On average, total and added sugars intake of our sample population was 112.2 ± 33.1 and 65.8± 29.0 g/day, respectively. The total sugars intake of our sample was lower than that of the nationally representative adult population of 130 grams/day evaluated in the period 1999 – 2006.[19] Approximately 10% of the total energy intake in our participants came from added sugars, which is in compliance with the 2015 - 2020 Dietary Guidelines for Americans,[21] but lower than the national average intake of 14% based on NHANES 2011-2012 survey data for US adults.[24] Mean total fructose intake of our sample population was 49.3 ± 15.9 grams/day, which is slightly higher than 48 grams/day based on the NHANES 1999-2006.[19] The mean sucrose intake of our sample of 59 grams/day was also higher than the mean consumption of sucrose among US adults of 54 grams/day according to NHANES III 1988 - 1994.[18] While we expected our

sample to have a higher free fructose intake than sucrose intake, we observed the reverse, the mean sucrose intake was 58.6 ± 21.5 grams/day and mean free fructose intake was 17.0 ± 12.9 grams/day. This could possibly be attributed to the lower consumption of processed foods high in HFCS in our participants. The overall low sugars intake (except sucrose) could be due to a highly educated and health-conscious study population. Participants may have also altered their sugar consumption during the feeding period as they knew they are being observed and have all their intake measured.

We found no association between age, ethnicity, education and marital status and sugars excretion. Assuming that sugars excretion is an objective indicator of sugars intake, this does not agree with the findings reported by NHANES and National Health Interview Survey (NHIS) in the general US adult population that show that adults with higher levels of added sugars intake are younger.[23,25] Based on the findings of the national population, men and women with the highest level of added sugars intake were non-Hispanic blacks,[23] less educated and not in a domestic relationship.[25] The lack of consistency in findings could possibly be attributed to our sample being 80% whites, predominantly young, with a small number of subjects under each of these sociodemographic groups of comparison.

5.2 Distribution of Physical Activity

Our sample population was in compliance with the 2008 PAGA[103] of 300 minutes or 150 minutes per week of moderate or vigorous physical activity for extensive health benefits put forth by the US DHHS. On average, our population spent about 390 minutes per week (0.93 hours/day) in moderate-intensity physical activity and about 120 minutes per week (0.28 hours/day) in vigorous-intensity physical activity that included both aerobic and muscle-strengthening activities.

In comparison to the physical activity estimates of the US adult population based on NHANES 2005-2006 measured using a self-reporting questionnaire in a household interview,[105] our sample population spent longer hours in moderate and vigorous intensity physical activity. Based on this report, US adults spent 324 ± 18.6 and 73.6 ± 3.9 minutes per week in moderate and vigorous physical activity, respectively,[105] which is lower than the PA reported by our sample population. The higher estimates of our

sample could be due to the differences between our methods of measuring physical activity and NHANES methods. Our study collected 15 days of physical activity data daily using the physical activity log, with participants self-reporting hours and minutes actively spent in 38 activities across six domains including home activities, transportation, occupation, conditioning, sports, and leisure activities. Our participants could also add other activities that were not listed in the log. The NHANES study collected the average frequency and duration of time spent in about 30 physical activities performed in transportation, occupation, household and yard activities, exercises, sports and other physical activities done in leisure time over the past 30 days. The higher estimates of moderate and vigorous activity of our study could also be attributed to the relatively younger and more educated sample compared to the nationally representative NHANES sample population, and to the possible overestimation of physical activity while keeping the physical activity log and accounting for every hour of the day.

In our sample population, those in the high physical activity group were younger, slightly leaner, and consumed slightly less added sugars, when compared to those in the moderate activity group, although none of these variables were statistically significantly different between the two groups. This pattern is comparable to the trends observed in the general US adult population.[104] In a representative sample of the US population, physical activity reported by non-Hispanic whites was the highest followed by PA reported by non-Hispanic blacks and Mexican-Americans.[105] However, our study sample included very few subjects of ethnicities other than whites and hence these results cannot be truly compared with the results from the general population. Our findings also compare well with the results from the general population where those with highest levels of added sugars intake were less physically active,[25] however the difference in added sugars intake between moderately and highly active participants was not statistically significant.

5.3 Performance of the sugars biomarker

In our study, we found diet to be the most significant predictor of urinary sucrose and fructose demonstrating that the biomarker is sensitive to dietary sugars. Total sugars and added sugars both

explained 30% of the variation in excretion of urinary sugars. We collected 24-hour urine samples to reflect intake throughout the day and found positive moderate correlation between 8-day mean urinary sugars and 15-day mean total sugars ($r = 0.56$, $p < 0.001$). This indicates that urinary sucrose and fructose is a valid short-term biomarker that responds to recent intake of total and added sugars, thus fulfilling the criterion of time integration. Reproducibility is a function of between-subject variability and within-subject variability in excretion in repeated number of urinary samples. Assessing reproducibility of the sugars biomarker was not within the scope of our study. Based on previous data on the reproducibility of the biomarker, we used the mean of eight urinary measurements, which has been shown to be an acceptable number for measuring correlations between dietary and urinary sugars.[6] We found strong significant positive correlation between total sugars and urinary sugars ($r = 0.82$, $p < 0.001$) and added sugars and urinary sugars ($r = 0.83$, $p < 0.001$) in men. However, the correlation was much weaker in women ($r = 0.39$, $p = 0.020$ and $r = 0.34$, $p = 0.046$). This could partly be explained by the smaller range in total sugars intake in women (50.0 to 146.0 grams/day) compared to the range in men (47.4 to 203.7 grams/day). Similarly, the range of added sugars intake was wider in men (24.9 - 159.1 grams/day) compared to women (10.5 - 108.7 grams/day). However, when we correlated daily dietary and urinary sugars by subject, the mean within-subject correlation was greater in women than in men suggesting that the lack of compliance among women was unlikely reason for the lower between subject correlation. In addition, mechanisms such as hormonal differences between men and women cannot be ruled out in having a role in the difference in association between dietary and urinary sugars between men and women. Some women in our study were also on oral contraceptives.

Our findings have been consistent with previous biomarker studies, however some investigations showed stronger associations[6,10,88,91] and one showed weaker association[92] between dietary sugars and urinary sugars than what we found. In a controlled feeding study with 13 participants consuming their habitual diet under controlled conditions and collecting 30 daily 24-hour urine samples, significant strong correlation was found between total sugars and 24-hour urinary sucrose and fructose ($r = 0.841$; $p < 0.001$). Total sugars explained 72% of variation in biomarker excretion. The differences in study methods and study population could have contributed to difference in findings. We have US-based population whereas

Tasevska et al[6] used UK population. Our subjects had a lower and smaller range of total sugars intake between 47 and 204 grams/day with a majority of them having intake lower than 150 grams/day and a few outliers skewing the distribution, while the UK feeding study had a range of intake between 95 and 323 grams/day. While their sample size was smaller (n=13), their participants collected 30 daily 24-hour urine samples and had 30 days of dietary data. However, 15 days of diet and eight urinary measurements have been shown to be sufficient for estimating correlations for the sugars biomarker[6] .

We found no correlation between naturally-occurring sugars and urinary sucrose and fructose ($r = 0.070$, $p < 0.001$), and moderate correlation with added sugars ($r = 0.56$, $p < 0.001$). In their habitual varying diet study, Tasevska et al[88] found urinary sucrose and fructose to be strongly correlated with extrinsic sugars ($r = 0.84$, $p < 0.001$), while they observed no significant correlation between 24uSF and intrinsic sugars ($r = 0.43$, $p = 0.144$).[88] The higher correlations with intrinsic sugars observed in their study could partly be due to the two-fold higher consumption of intrinsic sugars (68 ± 23 grams/day) by their study subjects compared to the naturally-occurring sugars intake of our subjects (31.2 ± 28.4 grams/day).

In a randomized cross-over feeding study with 53 participants consuming isocaloric controlled experimental high and low GL diets for 28 days, Song et al[10] found 41.7% of the variation in excretion of sucrose and fructose explained by total sugars along with age, gender, and percent body fat. We found 40.7% (R^2) of the variation in sucrose and fructose excretion explained by total sugars, sex, and protein, which is comparable to their results. However, in our study, total sugars explain 31% of the variation in sucrose and fructose excretion ($R^2 = 0.310$, adjusted $R^2 = 0.297$), while only 10% was explained by sex and protein, whereas in Song et al,[10] total sugars explained 16.3% of the variation in the biomarker. To our knowledge, this is the only other study investigating the association of urinary sugars and dietary sugars in US adult population. Subjects consumed constant but extreme levels of experimental diets (high and low GL), while ours were on a diet that differed from day to day and mimicked their usual diet. Furthermore, their study investigated the association between dietary and urinary sugars based on a single day's diet and one 24-hour urine sample which could introduce random error in the biomarker.[1] Moreover, they used no preservative during urine collection, which may have led to degradation of urinary sugars. Boric acid has

been recommended as urine preservative for measuring 24-hour urinary dietary biomarkers.[6,7] In reality, there is a high day-to-day variability in dietary intake, and total sugars require a minimum of three days to precisely rank individuals by intakes.[124] Additionally, repeated dietary measurements are needed to provide a good estimate of the individual's usual intake in order to reduce random error.[124] Song et al[10] found sex ($p < 0.0005$) and percent body fat ($p < 0.0005$) to be significant predictors of 24uSF other than total sugars. We fit several factors, including physical activity, age, BMI, sex, ethnicity, and dietary factors, such as fiber, fat, and protein intake into our regression models. None of these factors showed any potential in explaining the variation in urinary sucrose and fructose excretion when included with total and added sugars, except for sex, BMI, and protein intake. Sex and dietary protein explained only a small portion of variation in total sugars excretion (7.3%) in addition to 30% explained by total sugars, while sex and BMI additionally explained only 6.4% of variation in urinary sugars in addition to 30% explained by added sugars. Although BMI alone was not a significant predictor of variation of urinary sugars when added in the model with added sugars, it improved the predictability of urinary sugars by 6.4% along with sex. BMI is a ratio of body weight to square of height, and correlates to body fat measurement. Earlier studies have shown body weight[6] and body fat[10] to explain certain percent variation in urinary sucrose and fructose. In a randomized crossover intervention conducted in the UK which included 10 normal weight and 9 obese individuals consuming low sugar (13% EI), medium sugar (30% EI), and high sugar (50% EI) diets for 4 days each,[7] no significant interaction effect of BMI on urinary sucrose ($p = 0.65$) or urinary fructose ($p = 0.55$) with different sugar intakes was found.[7] As sex seemed to explain a proportion of variation in sugars excretion along with total sugars and added sugars in both models, future research could focus on investigating the performance of the sugars biomarker by sex and the sources of biomarker differences such as hormonal variations between men and women.

Dietary protein could help with the synthesis of glucose transporter proteins, which facilitates fructose absorption in the small intestine[98] and subsequent reabsorption in the kidneys. This could be a plausible mechanism behind the role of protein in improving the predictability of urinary sucrose and fructose, along with total sugars.

A recent randomized controlled crossover feeding study evaluated the validity of urinary sucrose and fructose as a biomarker of added sugars in 33 non-obese adolescent US population consuming isocaloric low added sugar (5% EI, LAS) and high added sugar (25% EI, HAS) diets for 7 days each with a 4-week wash out period while collecting two 24-hour urine samples. Moore et al[91] found urinary sugars to be strongly correlated with added sugars ($r = 0.77$; $p < 0.001$) in a diet with 25% of energy intake from added sugars, and weakly correlated with added sugars in the 5% EI dietary period ($r = 0.15$; $p = 0.49$). Added sugars was shown to be a better predictor of variation in urinary sugars, explaining 36% of variation in urinary sucrose and fructose ($R^2 = 0.36$) than total sugars which explained 28% of variation in excretion ($R^2 = 0.28$).[91] Though our study findings were comparable, the mean excretion of sucrose and fructose they measured was 0.028 ± 0.01 and 0.348 ± 0.15 mg/d in their HAS period respectively, compared to sucrose and fructose excretion levels measured in our study (26.6 ± 16.1 and 23.1 ± 20.9 mg/d, respectively). It is concerning that they observed sugars excretion levels 100-1000 times lower compared to what has been reported on the biomarker in any other prior study in both adults[6-8,10,78,87] and children.[92]

In an investigation of 24-hour urinary fructose as a biomarker of dietary sugars in a subsample of 114 free-living pre-pubertal children from the DONALD study, variation in urinary fructose was better explained by total sugars intake ($R^2 = 0.181$, $p < 0.001$) than by added sugars ($R^2 = 0.055$, $p = 0.01$).[92] In this study, diet was measured using 3-day weighted dietary records and one 24-hour urine sample on the 3rd dietary record day was collected. Lower R^2 seen in this study may be due to measurement error in self-reported sugars, availability of single 24-h urine and a preservative free urine sample.

The reason for the slightly different findings of our study compared to the other biomarker studies may partly be explained by the methodological differences such as study populations,[6,7,91,92] study design,[10,91,92] number of urinary measurements,[6,10,91] use of preservative,[10,91,92] and criteria for determination of urine completeness.[6,10,91,92]

Only one feeding study so far[6] examined physical activity as a potential determinant of the sugars biomarker. Along with other factors such as age, sex, and body weight, PA was found to explain 10% of variability in urinary sugars, whereas 72% of the variation was explained by total sugars intake alone.[6]

When we included physical activity in the regression models along with total sugars and added sugars, PA was not found to be a significant predictor of the biomarker nor it increased the predictability of the model. Our study collected time engaged in physical activities daily using the log which had 38 different activities under six different domains while also allowing participants to log other activities not listed in the log. We then computed the physical activity expressed as active MET-hours/day for statistical analyses. The UK-based feeding study collected much less detail on participants' PA. Participants recorded time spent in different types of exercises on a daily basis which were used to generate a four-level score (i.e., inactive, moderately inactive, moderately active, and active) by combining occupational activities with higher-intensity activities such as cycling, aerobics, swimming, jogging etc. In spite of the methodical differences, our study replicates their findings that physical activity has no effect on the association between the sugars biomarker and dietary sugars.

One of the features of predictive biomarkers is that they should not have too many non-dietary determinants. Diet only explained a third of the variability in urinary sugars and physical activity did not explain any proportion of the variation in our study. We found sex, protein intake, and BMI to be additional predictors of urinary sugars, although these factors explained only a small proportion of variability apart from dietary sugars. We also investigated age, ethnicity, and dietary fat and fiber, but we could possibly not be capturing other non-dietary determinants of the biomarker. Thus, future research in this area could consider investigating other factors that were not investigated extensively in this study such as body fat and life style factors such as alcohol use. In general, diet reflected lower predictability potential compared to previous research,[6,10] and we have no definite explanation on why this could have occurred. We think variation in aspects such as sources of sugar, genetics, environment, life-style factors such as alcohol use, and drug-nutrient interactions could have all played a role in this difference. Corn based sweetener HFCS is different from sucrose extracted using beet sugar in the UK, leading to possible variations in the way sugars are metabolized.[9] Some of our subjects were on medications and this might have interfered with the metabolism pathways of sucrose and fructose resulting in lower diet to excretion association.

5.4 Strengths and Limitations

The study had several strengths, most notably, the controlled feeding study design with free-living subjects, consuming their habitual diet, and following their normal life style. We had information on participants' true intake. Our study collected eight urine samples, which was found to be sufficient for assessing correlation with true intake,[6] and measured urine completeness prior to analyzing the samples. We also stratified recruitment by age, gender, and BMI in an attempt to obtain a heterogenous sample. We used a validated physical activity log[120] which allowed collection of detailed information on PA daily level across six domains. However, the instrument still relies on self-report and may be associated with measurement errors due to reporting and social desirability bias.[125] The intensity of the current study protocol and concerns over participants' burden prevented us from using an objective instrument for PA assessment such as an accelerometer that measures the intensity of movement throughout the day. We used a convenient healthy sample who were somewhat highly educated, had a healthy diet and were physically very active. This could have led to low generalizability of results.

5.5 Conclusions

In conclusion, our study further strengthened the existing evidence that urinary sucrose and fructose can be used as a biomarker for total sugars consumption. Additionally, we found some gender differences in the association between the sugars biomarker and dietary sugars. Future feeding studies should include subjects with wider range of total and added sugars intake and of different ethnic/racial backgrounds. In lieu of our contrasting findings of the association between dietary sugars and the sugars biomarker in men and women, future studies should focus on investigating this further to evaluate biomarker gender differences. Finally, we found no effect of physical activity on the association between sugars biomarker and dietary sugars in the US adult population.

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APPENDIX A

CONSENT FORM

What is the purpose of this form?

The purpose of this form is to provide you (as a prospective research study participant) with information that may affect your decision as to whether or not you would want to participate in this study and to record your consent that you agree to take part in the study.

Who are the researchers?

Dr. Natasha Tasevska, an Assistant Professor at the Arizona State University (ASU) School of Nutrition and Health Promotion, is inviting you to participate in a research study that will be conducted over 11 weeks.

Why am I being invited to take part in a research study?

We are asking you to take part in this research study because we wish to recruit healthy, non-smoking volunteers 18-70 years, like yourself.

Why is this research being done?

Sugars are thought to play very important role in developing many diseases such as diabetes, cancer and cardiovascular disease. To see if this is true we need to measure the food people eat accurately and see if type of food people eat relates to the sort of diseases they develop. This study will test how accurately urine and blood biomarkers can predict the usual consumption of sugars.

How long will the research last?

While the study will run over 11 weeks, individuals will spend one month actively participating in the proposed activities.

How many people will be studied?

We plan to recruit 107 people in this research study.

What happens if I say yes, I want to be in this research?

It is up to you to decide whether or not to participate. If you decide to take part in the study:

- A **screening fasting blood sample** will be taken to check your plasma glucose and HbA1c levels. If your fasting blood glucose <100 mg/dl and HbA1c <5.7%, you will be scheduled for a baseline visit.
- **Your body weight and height will be measured.**
- **You will complete a questionnaire** with questions on your demographics, lifestyle habits, and personal medical history.
- **You will record all the foods and drinks you consume over two weeks**. For that purpose, you will be given a food diary in which you will find set of instructions to help you record your diet, and measuring cups, spoons, and a food model booklet to help you record quantities. Following each

week, you will be invited to meet with our Research Kitchen Coordinator and Chef to discuss what you have recorded in your food diary and help us gather more information.

- A week after you have completed the 2-wk food diary, **you will participate in a 15-day feeding study**. During the feeding period, you will be provided with all your food on a daily basis. This is the food that you would usually eat, which we have purchased and prepared for you based on the food diaries you kept over the previous 2 weeks. You will come to our kitchen daily Monday-Friday where you will eat your breakfast or lunch and then collect your dinner, snacks and breakfast or lunch for the following day. On Fridays, you will collect your food for the entire weekend. We will provide you with cooler bags on wheels to ease the transport of meals to your home. You will be free to eat as much as you like from the food prepared for you, and you will NOT be allowed to consume any foods or drinks prepared outside of our kitchen, besides water, alcohol, and black coffee and tea (no added sugar, sweetener, milk, creamer, etc.). If you drink alcohol, you will record the type and amount consumed; you are allowed to drink wine, beer or spirits (i.e., hard liquor, such as whisky, vodka, tequila, gin, etc.) ONLY. Please note that any alcohol beverages that contain added sugars, fruits, cream, spices, herbs, flowers or nuts, such as liqueurs (e.g., Grand Marnier, schnapps) or cocktails are not allowed. We ask you to keep your intake of coffee and tea consistent throughout the 15-day feeding study. You will keep the unconsumed food/drinks in the respective container/bottle and return them to the metabolic kitchen on your next visit. Please note that no one else is allowed to eat the leftovers, and you have to return all leftovers to the metabolic kitchen, so we can calculate exactly how much food you have consumed.
- You will **collect nine breath samples during the 15-day feeding study** (three samples per day on three randomly selected days; on the breath collections days, you will collect one breath sample before breakfast, and two samples at randomly selected time points during the day).
- **We will collect 3 blood samples from you:** before and at the end of the 15-day feeding study and 5 weeks later.
- **You will collect 24-hour urine every other day during the 15-day feeding study (8 in total).** On two urine collection days, you will collect each of your urine voids in a separate container. We will give you a trolley bag for carrying urine bottles when away from home. To alleviate your burden, we will organize a pick-up service to collect the 24-h urine from your home the morning after the urine collection day (including weekend and holiday). In order to determine whether the collections are complete, you will be requested to take a capsule of aminobenzoate potassium (POTABA) with your breakfast, lunch and dinner (three capsules per day) on the urine collection days. POTABA is commonly used as a marker for ***urine completeness in research studies***, as it is nearly completely excreted in the urine soon after taking a tablet of POTABA.
- **You will keep study logs during the 15-day feeding study**: a brief physical activity log (<5 minutes to complete), and a meal checklist daily, and a urine collection log on the urine collection days.
- **You will be asked to refrain from taking any dietary supplements** during the feeding study and until collection of the final blood sample.

Samples will be stored and may be used at a later date to see if we can find other dietary biomarkers.

Participant Timeline:

Visit Timeline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 12
Screening Visit	Day 1							
Baseline Visit	Day 4							
Food Diary		All Days	All Days					
Meeting with Chef	Day 4		Day1	Day 1				
Feeding Study					All Days	All Days	Day 1	
24-hour Urine Collection					Day 1, 3, 5, 7	Day 2, 4, 6	Day 1	
Blood Draw[†]	Day 1				Day 1[‡]		Day 2[‡]	Day 1[‡]
Breath Sample					Randomly selected day (3x)	Randomly selected day (3x)		

[†] 6 ml blood.

[‡] ***24 ml per blood draw (3x).***

What happens if I say yes, but I change my mind later?

Even if you say "yes" now, you are free to say "no" later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled. If you decide to leave the research, you should contact the investigator so that the investigator can notate your departure in our database. If you stop being in the research, already collected data may not be removed from the study database. If it becomes evident that you are not complying with the feeding, urine collection or blood collection protocol, the research staff may remove you from the study without your consent. If this occurs, you will only be compensated for the portions of the protocol you completed.

Is there any way being in this study could be bad for me?

There are no risks associated with the feeding portion of the study. All food safety precautionary measures will be taken to ensure safe food handling and prevention of food borne illnesses. You may experience slight pain from the blood draws (4 in total, including the blood draw at screening). Although unlikely, some bruising and/or infection can occur from the blood draws. You may be inconvenienced by

collecting 24-h urines (8 in total) and by not being able to eat or drink anything prepared outside of our kitchen (except for water, alcohol, coffee and tea) during the 15-day feeding study. On the urine collection days, you will be asked to take three 102 mg capsules of POTABA, one with each main meal, as a marker for 24-h urine completeness. Only few instances of side effects, such as upset stomach, nausea, loss of appetite, fever and skin allergy (rash), have been reported following intake of POTABA, and in doses much larger than the dose in this study. If you experience these symptoms, please notify the research staff, and taking of the capsules will be discontinued. An allergic reaction to sunscreen may indicate that side effects from POTABA can occur. At screening, you have informed us that you have never experienced an allergic reaction to sunscreens.

Will I be able to obtain any of the results from the samples I provide?

Participants can electively choose to receive their data from the screening blood collection, which includes fasting blood glucose and HbA1c levels. To receive these data:

a. Participation in the study must be complete (i.e., based on the screening blood results you are not eligible to participate, you voluntarily withdraw or are removed from the study, or you complete the entire study); and

b. You must sign a Research Results Acknowledgment Statement form that states that this information does not constitute medical advice or diagnosis, and that you take responsibility for sharing this information with your physician or health care provider.

Research Results Acknowledgement Statement forms available upon request.

Will being in this study help me in any way?

If you chose to sign the Research Results Acknowledgment Statement, you will be given the results on your fasting blood glucose and HbA1c level from your screening blood collection. We cannot promise any benefits from taking part in this research to you directly. However, the potential benefit to others is large, due to long-term public health impact of this project. This study will help in determining the role of sugars in risk of obesity, cardiovascular disease, cancer, type 2 diabetes, and other chronic diseases.

What happens to the information collected for the research?

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. In order to maintain confidentiality of your records, we will assign you a participant number at study entry, which will be used on all forms, meals and specimens. Your name will not appear anywhere aside from this consent form. This form will be kept in a locked cabinet in Dr. Natasha Tasevska's office to maintain your confidentiality.

What else do I need to know?

This research is being funded by the National Institutes of Health (NIH).

If you agree to take part in this research study, we will pay you up to $599: $10/day for keeping food diary for 2 weeks, $20/day during the 15-day dietary study and an additional $159 as an incentive for completing the entire study protocol. If you agree to participate in the study, this consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

At the end of this research project, we will be happy to explain individual results.
Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, please contact ***Natasha Tasevska, at*** Natasha.Tasevska@asu.edu or 602 827-2485 ***or Cassandra Kettenhoven, Project Coordinator, at*** Cassandra.Kettenhoven@asu.edu ***or 602-827-2545.***

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788 or research.integrity@asu.edu.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent

conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of investigator	Date

Printed name of investigator

APPENDIX B

ASU IRB

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Natasha Tasevska
SNHP - Nutrition
602/827-2485
Natasha.Tasevska@asu.edu

Dear Natasha Tasevska:

On 5/22/2015 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Investigation of Biomarkers for Sugars Intake – A Controlled Feeding Study
Investigator:	Natasha Tasevska
IRB ID:	STUDY00002695
Category of review:	(3) Noninvasive biological specimens, (2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(b) Social science methods, (7)(a) Behavioral research
Funding:	Name: NCI: National Cancer Institute, Grant Office ID: FP00001446 , Funding Source ID: 1R01CA197902-01
Grant Title:	FP00001446 ;
Grant ID:	FP00001446 ;
Documents Reviewed:	• Appendix 7, Category: Participant materials (specific directions for them); • Appendix 12 - Consent Form, Category: Consent Form; • Appendix 3 - Screening Q, Category: Recruitment Materials; • Appendix 6, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Appendix 7a, Category: Participant materials (specific directions for them); • Appendix 11, Category: Measures (Survey questions/Interview questions /interview guides/focus

	group questions); • Appendix 10, Category: Other (to reflect anything not captured above); • Appendix 4, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Appendix 2 - Information Sheet, Category: Recruitment Materials; • FP 1446 Tasevska FP.pdf, Category: Sponsor Attachment; • Sugars Biomarkers Protocol, Category: IRB Protocol; • Appendix 1 - Study Advertisement, Category: Recruitment Materials; • Appendix 9, Category: Other (to reflect anything not captured above); • Appendix 8a, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Appendix 5, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • IRB Exemption letter.pdf, Category: Off-site authorizations (school permission, other IRB approvals, Tribal permission etc); • IRB clarification requested May12.pdf, Category: Other (to reflect anything not captured above); • Appendix 8, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

The IRB approved the protocol from 5/22/2015 to 5/21/2016 inclusive. Three weeks before 5/21/2016 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 5/21/2016 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

Carol Johnston
Kate Zemek

APPENDIX C

7-DAY FOOD DIARY

FOOD DIARY

ARIZONA STATE UNIVERSITY
Investigation of Biomarkers for Sugars Intake

Instructions

We would like you to keep a record of everything you eat and drink over the next 7 days.

It's extremely important that you don't make changes from what you'd normally eat or drink when completing this.

As you will see, each day is marked in sections, beginning with the first thing you eat when you get up in the morning, and ending with an evening snack before bedtime. For each part of the day write down all food and drink consumed, the amounts and a description if necessary. If nothing is eaten or drunk during a part of the day, draw a line through that section or include N/A. It is important that you record everything immediately after or at the time of eating, and not from memory at the end of the day.

On the next 4 pages is a list of popular foods and drinks. Next to each item is the information we need to know so we can tell what the food item is made of and how much you had. This list cannot cover all foods and drinks, so try to relate to a similar item if any items that you have eaten are missing. Please give as much detail as you can.

Use provided measuring cups and spoons to help you estimate amounts. For some foods you may find it easier to describe how much you had by using the pictures in the food models booklet you received. Use one or the other approach to estimate amount.

Drinks and many packaged foods have weights printed on them, so please use these to show how much you consumed.

At the end of each day there is a list of snacks and drinks that can easily be forgotten. Please write down any extra items in here, if you have not already included them in some other part of the day.

FOR EACH ITEM THAT YOU EAT OR DRINK PLEASE READ THE FOLLOWING FOR DETAILS WE NEED:

Eat as you normally would.

Always state what sort of **oil** or **fat** was used for baking, frying, etc.

Give **brand and full name** of products where possible.

For **meals/snacks eaten away from home**, please note where these items were eaten, giving name and/or type of restaurant, café, bar, etc. where appropriate.

Record **serving sizes of meats in ounces** or by the piece (1 thigh and 1 breast of chicken).

Measure or estimate the volume of fluid in your glass or cup (4 fl oz, 8 fl oz). It's okay to estimate serving sizes for all foods.

Don't forget to record all the little "extras" like sugar in your coffee or on your cereal, fruit on your cereal, butter on your vegetables or bread, honey in tea, mustard, mayo or ketchup on sandwiches, cream in coffee and so forth.

Fully describe the food e.g., 2% milk, water-packed tuna, low-fat cottage cheese, low-salt Wheat Thins, tomato soup made with whole milk, sugar-free Jell-O, canned peaches in heavy syrup, breast of chicken with or without skin.

Check food labels for weights, etc., such as candy bars, individually wrapped cheeses, cookies and juices – write it down and/or take photos.

Please remember to provide us with as much detail as you possibly can

WHERE POSSIBLE, ALWAYS STATE WHAT SORT OF OIL OR FAT WAS USED FOR BAKING, FRYING, ETC.

Food/Drink	Description & Preparation	Amount	USDA Food Models
Home-made dishes	Please say what the dish is called and give recipe or ingredients, including amounts	Ounces	Mounds, grid, blocks, wedges
Ready-made meals	What type; e.g., pizzas, microwave dishes, brand name. Please give main ingredients and nutritional information on packet, and enclose label and bar code if possible.	Weight from package	
Meals eaten away from home	What type; e.g., pizzas, Chinese, Indian, fish and chips, hamburgers, hot dogs. Please say what the dish is called and give ingredients where possible. Give the name of the restaurant.	Ounces	Mounds, grid, blocks, wedges
Cider	Sweet, dry, vintage, low alcohol, % alcohol	Ounces; number of cans/bottles, including size	Cups, mugs, glasses
Coffee	With/without milk, what sort; half and half; 1%, 2%, whole milk; ground, instant, decaffeinated/caffeinated; strong, average or weak, sweetened or unsweetened	Ounces	Cups, mugs
Spices	Pepper, salt, or other	½ or ¼ teaspoon, pinch, etc.	
Cooking oil	Type, brand name	Teaspoons	
Cream	Half, single, sour, whipping, double, clotted, low fat; fresh or substitute; sweetened or unsweetened	Tablespoons	
Chips	Brand name; baked or low-fat; low salt	Packet weight or number of chips	
Egg	How was it cooked: boiled, fried, scrambled, poached, omelette, etc.; full egg, egg white only, yolk only, etc; any oils or fat used to cook it	Number	

Food/Drink	Description & Preparation	Amount	USDA Tools
Fish	What sort, fried, boiled, grilled, poached, microwaved, pickled, smoked or salted; with batter or breadcrumbs; tinned with oil or tomato sauce; size; give brand name where applicable	Ounces	Blocks, grid
Fish sticks	What sort; large, medium or small size; fried or grilled; give brand name if possible	Number	Circles, blocks, inches
Fruit – fresh	What type and variety e.g. Granny Smith apple; cored, with or without skin, size	Number or cups	
Fruit – frozen or canned	What type and variety; with or without sugar; in fruit juice or syrup	Size, cups or can size	
Fruit – juice	What type; sweetened or unsweetened	Ounces	Glasses, cups
Gravy	Thick or thin, instant or packet, made with or without dripping, meat juices, etc.	Tablespoons	
Herbs	Type, fresh or dried	Teaspoons	
Honey, jam	Type, specify if low sugar	Teaspoons	
Ice-cream	Dairy or non-dairy, flavor or variety, brand name if possible	Cups	
Liver, Kidney	Pig, lamb, ox; fried or stewed	Picture	
Margarine	Hard, soft, polyunsaturated, low fat, very low fat; give name and brand, if possible	Tablespoons	Mounds
Mayonnaise	Give name and brand; state if low fat	Teaspoons	
Meat pie, pasty, pastry	What type; individual or helping; size; fat used for pastry; give brand name, where possible	Number	Mounds, circles
Meats	What type; lean or fatty; fried, microwaved, grilled, roast, barbequed; well done, rare, etc.; with or without gravy, cut used; pickled, smoked, breaded, salted, etc.	Ounces, slices, helping	Grid, blocks

Food/Drink	Description & Preparation	Amount	USDA Tools
Milk – for drinking on its own or for cereals	Whole milk, 1%, 2%, raw, pasteurized, flavored, soy, or nut, sweetened, unsweetened, light	Ounces	Glasses, cups
Peanuts	Dry roasted or regular	Number or packet weight	
Potatoes	Baked, boiled; with or without skin, mashed, creamed, fried/fries, instant, roasted; with butter, margarine, oil, etc.; variety e.g., sweet potato, Idaho	Ounces	Circles, mounds
Pudding	What type and brand: e.g. Jello brand chocolate pudding; with fruit; pie (what type); jelly; mousse, instant desserts; milk puddings, give recipe/ingredients	Tablespoons	Bowls, circles, mounds
Rice	Brown or white; boiled, steamed or fried; rice pudding	Cups	Bowls, circles, mounds
Salad	Describe ingredients, with dressing (type – e.g., oil and vinegar, ranch)	Cups, tablespoons	Bowls, circles,
Sandwiches	Type of bread (e.g. white, whole wheat) type of filling; butter, margarine, mayonnaise, etc.; large or small loaf; thick medium or thin slices	Slices of bread, ounces	Blocks, grid
Sauce – hot	(for vegetables, meat or fish; puddings) what type; savory or sweet; thick or thin, give recipe or brand, if possible	Tablespoons	Circles
Sauce – cold	What type: e.g., tomato ketchup, mustard, soy sauce, salad dressing, sweet or savory	Tablespoons	Circles
Sausages	What type: e.g., pork, beef, pork and beef; low fat; large or small, how cooked	Number and ounces	
Soups	What type: e.g. minestrone, recipe or brand and ingredients	Serving, cups	Bowls

Day 1: Date ___/___/20___ Day of the Week: _____		
Before Breakfast		
Food/Drink	Description and Preparation	Amount
Breakfast		
Food/Drink	Description and Preparation	Amount
Morning Snack		
Food/Drink	Description and Preparation	Amount

Lunch		
Food/Drink	Description and Preparation	Amount
Afternoon Snack		
Food/Drink	Description and Preparation	Amount

Dinner		
Food/Drink	Description and Preparation	Amount

Evening Snack		
Food/Drink	Description and Preparation	Amount

Between Meals, Snacks and Drinks if not already written in before		
Food/Drink	**Description and Preparation**	**Amount**
Chocolate		
Sweets		
Chips		
Peanuts		
Other snacks		
Beer, wine		
Spirits, liquor		
Soda, pop		
Energy drinks		
Other cold drinks		
Tea, coffee		
Other hot drinks		
Ice cream		
Anything else?		

Space to write in the recipe or ingredients of any homemade dishes, take-out meals, etc. that you have mentioned but not described previously. Where applicable, please list amounts of ingredients and brand names. **For recipes, takeout meals, etc. please indicate amount/proportion actually consumed by yourself.**

(continue)

Did you consume any dietary supplements today? ☐ **Yes** ☐ **No**
If yes, please record type, brand name and dose.

	Type	Brand	Dose
1)			
2)			
3)			
4)			
5)			
6)			
7)			
8)			

End of Day 1

Sample Food Diary

Sample Food Diary Day 1

Day 1: Date: 2 / 17 /2016 Day of the Week: Monday

Before Breakfast

Food/Drink	Description and Preparation	Amount
N/A	N/A	N/A

Breakfast

Food/Drink	Description and Preparation	Amount
Coffee	Starbucks, home-brewed	8 fl oz
Creamer	Int'l House Caramel Macchiato (regular not fat free)	2 Tablespoons
Bagel	Trader Joe's Sprouted Wheat, toasted dark (4 1/2" bagel, large)	1/2 (bottom only)
Cream cheese	Trader Joe's, whipped, regular fat	2 teaspoons
Banana	Large (8"), Chiquita	½ (4 inches)

Morning Snack

Food/Drink	Description and Preparation	Amount
Greek yogurt	Oikos, vanilla, 0% fat	6 oz.
Fresh strawberry	large HUGE	1

Lunch

Food/Drink	Description and Preparation	Amount
Spinach	Fresh, Trader Joe's baby spinach (not organic)	1 packed cup
Grape tomatoes	Trader Joe's	5 ea
Egg	Hard boiled, Hickman's large	1 ea
Honey Dijon Vinaigrette	Homemade (recipe attached)	2 Tablespoons
Smoked almonds	Salted, roasted, smoked	8 ea

Afternoon Snack

Food/Drink	Description and Preparation	Amount
N/A	N/A	N/A

Dinner

Food/Drink	Description and Preparation	Amount
Hummus	Herbed hummus (The Greene House in Scottsdale) (pic)	2 Tablespoons

Grilled Flatbread	White, no pocket (The Greene House in Scottsdale) (pic)	1 wedge [4 ½ D]
Tomato, Onion & Feta Salad	Tossed in light vinaigrette (The Greene House in Scottsdale) (pic)	¼ cup
Asian Chicken Salad	Romaine, endive, shredded carrot, crunchy flat noodles, Sesame Seed, Ginger Miso Dressing (The Greene House in Scottsdale) (pic)	2 ¼ cups (1/2 of the plate)
Grilled Mahi Tacos	Mahi mahi, lime juice from wedge, grilled corn tortilla Pico de Gallo, Salsa Verde, and cotija cheese (The Greene House in Scottsdale) (pic)	4 oz., 1 wedge of lime, 1 tortilla, 1 1/2 Tablespoons pico, 1 Tablespoon salsa verde, 1 Tablespoon cheese
Avocado & White Bean	Avocado with white beans, pico de gallo and cotija cheese (pic)	2 Tablespoons, ½ cup, 1 Tablespoon pico, 1 teaspoon cheese
White wine	(Sauvignon Blanc) North Coast, CA	1 glass [G8-B]
Chocolate Mocha Bars	Dark Chocolate Praline Bar with Choc Mousse, Vanilla Bean Gelato, Raspberries(The Greene House in Scottsdale) (pic)	¾ 1 bar, ¼ cup gelato, 2 raspberries
Fresh Parmesan Ciabatta with Bacon Scallion Butter	Cake-like ciabatta, fresh parmesan, pork bacon butter	1 partial piece (consumed 2.5" x 2.5 x 2""), 1 large flake fresh parm
	(The Greene House in Scottsdale) (pic)	1 teaspoon bacon butter

Evening Snack		
Food/Drink	Description and Preparation	Amount
Red wine	Cabernet, Charles Shaw	6 oz. [G8-B]

Between Meals, Snacks and Drinks if not already written in before		
Food/Drink	**Description and Preparation**	**Amount**
Chocolate	dark chocolate PB cups from Trader Joe's	4 small
Sweets		
Chips		
Peanuts		
Other snacks		
Beer, wine		
Spirits, liquor		
Soda, pop		
Energy drinks		
Other cold drinks		
Tea, coffee	Arizona Peach Tea	1 drink, 2 T
Other hot drinks		
Ice cream		
Anything else?	Green olives, Queen Creek Olive Mill	5 ea.

Space to write in the recipe or ingredients of any homemade dishes, take-out meals, etc. that you have mentioned but not described previously. Where applicable, please list amounts of ingredients and brand names. For recipes, takeout meals, etc. please indicate amount/proportion actually consumed by yourself.

Vinaigrette for Green Salad

Ingredients:
1 t. Dijon mustard
1 t. Minced garlic
3 T champagne vinegar
Kosher salt to taste
Black pepper to taste
½ cup olive oil

Directions:
In a small bowl whisk together the mustard, garlic, vinegar, 1 teaspoon salt and ½ t. pepper. After well combined, slowly pour in olive oil while whisking until completely emulsified.

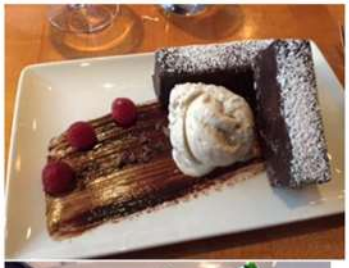
Toss over a salad of choice immediately and enjoy.

(Consumed 1 serving)

Did you consume any VITAMINS or SUPPLEMENTS today? ☒ Yes ☐ No
If YES, please list below, including type, brand name, dosage and time of day.
Please note: No supplements are to be consumed 7 days prior to the 1st day of the feeding study.

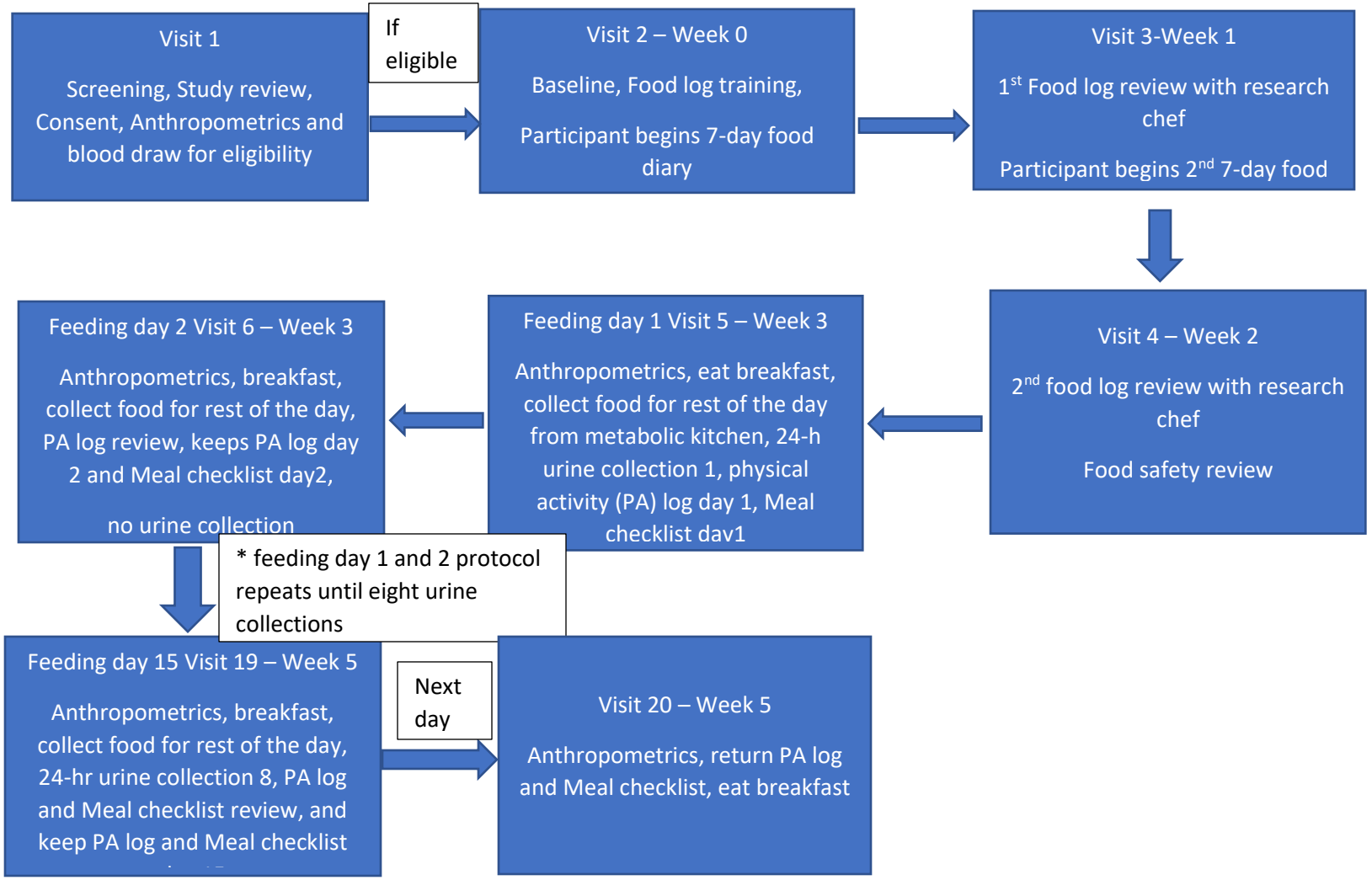
Vitamin B-12, 6am, 1 Tablet, 500 mcg, 6 am

End of Day 1



APPENDIX D

STUDY DESIGN


Visit 1
Screening, Study review, Consent, Anthropometrics and blood draw for eligibility
If eligible
Visit 2 – Week 0
Baseline, Food log training,
Participant begins 7-day food diary
Visit 3-Week 1
1st Food log review with research chef
Participant begins 2nd 7-day food
Visit 4 – Week 2
2nd food log review with research chef
Food safety review
Feeding day 1 Visit 5 – Week 3
Anthropometrics, eat breakfast, collect food for rest of the day from metabolic kitchen, 24-h urine collection 1, physical activity (PA) log day 1, Meal checklist dav1
Feeding day 2 Visit 6 – Week 3
Anthropometrics, breakfast, collect food for rest of the day, PA log review, keeps PA log day 2 and Meal checklist day2,
no urine collection
* feeding day 1 and 2 protocol repeats until eight urine collections
Feeding day 15 Visit 19 – Week 5
Anthropometrics, breakfast, collect food for rest of the day, 24-hr urine collection 8, PA log and Meal checklist review, and keep PA log and Meal checklist
Next day
Visit 20 – Week 5
Anthropometrics, return PA log and Meal checklist, eat breakfast

APPENDIX E

MEAL CHECKLIST

School of Nutrition & Health Promotion

Sugars Biomarkers Study

Study ID: _____

Starting with _____, please track all of the meals and snacks that you eat. **Please do not eat anything not provided to you by the metabolic kitchen.** However, if you did eat something outside of the food provided to you, please record it on this checklist. Make sure to check the meals off as you eat them and not wait until the end of the day.

- You will need to consume **1 meal per day (breakfast or lunch) Monday-Friday in the metabolic kitchen.**
  - During this visit, you will pick up any remaining meals or snacks for the day and the next day's meal(s) to be consumed prior to your next visit.
- On Fridays, you will collect all of your meals and snacks for the weekend and the meals and snacks to be consumed prior to Monday's visit.
  - You will be provided with a cooler bag on wheels to ease the transportation of the meals to your home.
- You are free to eat as much as you want from the foods provided for you. **Please keep any uneaten portions in the respective container and return them to the metabolic kitchen on your next visit.**
- All meals are categorized on your Menu Plan. **Use the Menu Plan to identify which "meal" you are consuming**. Mark the correct time for each meal for example:
  - Grilled Chicken Salad is listed as "Lunch" on the menu plan, but you eat it for dinner at 7:30pm. Mark 7:30 pm next to "Lunch" on your meal checklist.
  - Pita with Hummus is listed as "afternoon snack" on the menu plan, but you eat it for your morning snack at 10am. Mark 10am next to "afternoon snack" on the meal checklist.
- If you consume one component of a meal or snack with another meal or snack please indicate that **in the notes section**. For example:
  - Chips and a Coke are listed as your afternoon snack, and you have the Coke with lunch at 12:00pm. Write in the notes section next to "Lunch" had Coke from afternoon snack.
  - Fish with rice, black beans, and a salad is listed as your dinner, and you have the rice (or some amount of rice) for afternoon snack at 3pm. Write in the notes section next to "afternoon snack" had rice from dinner (note estimated amount if different from the total amount given to you).
- Check Yes, No, and N/A according to your Menu Plan
  - No means meal was provided on Menu Plan but was not eaten
  - N/A meals meal was not provided on Menu Plan
- **In the notes section**, please specify type and amount of any **unconsumed food that you did not return to us** for any given reason:
  - Forgot to eat a meal,
  - Threw any of it away,
  - Failed to return some of the food for any given reason, or
  - Someone else consumed it.
- Please record your **alcohol consumption** throughout the day. Indicate type and amount of alcohol consumed. You are allowed to drink wine, beer or spirits (i.e., hard liquor, such as whisky, vodka, tequila, gin, etc.), only. Please note that any alcohol beverages that contain added sugars, fruits, cream, spices, herbs, flowers or nuts, such as liqueurs (e.g., Grand Marnier, schnapps) or cocktails ARE NOT ALLOWED.
- Please record your **coffee and tea consumption** throughout the day. Indicate type and amount of consumed. Please keep your coffee and tea intake consistent during the feeding

study. **DO NOT add** sugar, any other sweetener, milk, creamer, etc., to your coffee and tea – those will be provided by the metabolic kitchen.

- Please record any consumed food and/or beverage that was not provided by the metabolic kitchen.
- Please do not take any dietary supplements (vitamins, minerals, bioactive compounds, fatty acids, herbal supplements, etc.) during the 15-day feeding study and 5 weeks following the completion of the feeding study until the 3rd blood collection is collected!!!!

Body Weight (kg): 65.5

Date	Meal	Consumed? (Check the appropriate box when you eat your meal)	Time of Meal:	Notes - specify type and amount of any unconsumed food that you did not return to us	Notes - specify variations from Menu Plan	Alcohol Consumption (Indicate type of drink and amount consumed in ounces)		Tea and coffee consumption (Indicate type of drink and amount consumed in cups)	Did you consume any food and/or beverage that was not provided by the metabolic kitchen? (If yes, please specify the food and the approximate amount)
						Type of drink (i.e., beer, wine, liquor)	Ounces		
07/12/2016 Monday	Pre Breakfast	☒ Yes ☐ No ☐ N/A	5:30 AM / PM						
	Breakfast	☒ Yes ☐ No ☐ N/A	7:30 AM / PM					1 single espresso	
	Morning Snack	☒ Yes ☐ No ☐ N/A	9:30 AM / PM	½ apple					
	Lunch	☒ Yes ☐ No ☐ N/A	12:00 AM/ PM						1 Hershey's Dark Chocolate Kiss
	Afternoon Snack	☐ Yes ☒ No ☐ N/A	___:___ AM/PM						
	Dinner	☒ Yes ☐ No ☐ N/A	6:00 AM/ PM		Drank Coke from Morning Snack	Red Wine	10 oz		

	Evening Snack	☒ Yes ☐ No ☐ N/A	10:00 AM/ PM					I cup of chamomile tea	
	Late Night Snack	☐ Yes ☐ No ☒ N/A	___:___ AM/PM						

Complete these questions the following morning:
How long did you sleep last night? (hours:minutes) __7:15_____
Yesterday, how long did you sleep/nap during the day? (if you did not, select 0) (hours:minutes) _____0:45_____

APPENDIX F

DIRECTIONS FOR COLLECTING 24-HOUR URINE SAMPLE

As part of our study, we are asking you to collect EIGHT 24-hour urine samples over the 15-d feeding period (Day 1, 3, 5, 7, 9, 11, 13, and 15). Please note that on two out of eight days, we ask you to collect each of your urine voids in a separate container (see "*Directions for Collecting Multiple Spot Urine Sample"* for more details). In this kit, you will find all of the materials needed to collect your 24-hour urine sample (six in total) and temporarily store it until it is retrieved by a courier.

This URINE COLLECTION KIT contains the following:

- Two 3.0 L or 3.5 L containers each containing 4 g boric acid powder in a drawstring plastic bag.
- Urinal for males or collection 'hat' for females.
- POTABA Tablets (3 x 102 mg)
- Directions for Collecting 24-Hour Urine Sample
- 24-Hour Urine Collection Log
- Safety Pin
- Trolley Cooler bag (with HOBO Temperature Data Logger included)
- Seven ice packs
- Seven ziploc bags
- Sharpie pen

IF YOU MUST TAKE ANY MEDICATIONS CONTAINING ACETAMINOPHEN (TYLENOL), SULPHONAMIDES, FUROSEMIDE (LASIX) OR ANY OTHER PRESCRIPTION OR NON-PRESCRIPTION MEDICATIONS DURING THE 24-HOUR URINE COLLECTION PERIOD, PLEASE RECORD THIS IN YOUR 24-HOUR URINE COLLECTION LOG.

TO COLLECT THE 24-HOUR URINE SAMPLE, PLEASE FOLLOW THE DIRECTIONS BELOW:

1. Please place the seven ice packs in your freezer the evening before you start your 24-hour urine collection.
2. When you first get up in the morning on the day of the 24-hour urine collection, **DISCARD** your first urine. Enter the **DATE** and **TIME** of this first morning void for Question #1 and Question #2 on the 24-Hour Urine Collection Log, respectively.
3. At this point, please take out three frozen ice packs from your freezer and place them upright on three sides of your urine containers in the cooler bag. Please, keep the lid of the cooler closed at all times. Please do not remove the HOBO Temperature Data Logger from the cooler bag at any time.
4. Take one **POTABA** tablet with one full glass of water at breakfast time or within one hour after you wake up, whichever occurs first. **Please ensure that you take the first POTABA tablet AFTER you have voided your first morning urine.** Enter the time you took this first POTABA tablet for Question #4 on the 24-Hour Urine Collection Log.
5. Pin the safety pin to your undergarments. This is a visual cue to remind you to collect your urine each and every time you use the bathroom during the 24-hour collection period.
6. Record the **TIME** of your second morning urine of the day, which is the first urine of your 24-h urine collection. For females: place the hat on the toilet seat and collect the entire amount. For males: urinate directly into the urinal, collecting the entire amount. If possible, pass urine before passing stool. Pour the urine from the hat/urinal into the 3.0 L container, avoiding any spillage. Please ensure that the lid of the urine container is in closed position, and that the container remains in the cooler between voids at all times.

7. For the next 24-hour time period, you must collect ALL of your urine. Take care to not overfill the collection container. Stop filling when the urine reaches the **2800ml** and 3500 ml line on the measurement side of the 3 L and 3.5 L container, respectively. Begin using the second container.
8. If you accidentally miss collecting a sample or part of a sample, please make a note for Question #5 and enter the time and approximate amount of the missed amount for Question #5a on the 24-Hour Urine Collection Log. It is very important that we know if any urine has been missed. Continue your collection.
9. Take the **second POTABA** tablet with one full glass of water sometime between 12 - 2 pm (with lunch). Enter the time you took this second POTABA tablet for Question #4 on the 24-Hour Urine Collection Log.
10. Take the **third POTABA** tablet with one full glass of water sometime between 5 – 7 pm (with dinner). Enter the time you took this second POTABA tablet for Question #4 on the 24-Hour Urine Collection Log.
11. In the evening, please take out another two frozen ice packs from your freezer and insert them upright on two sides of your urine containers in the cooler bag. Please, make sure that the lid of the cooler is kept closed at all times.
12. Upon awakening in the morning the next day, <u>COLLECT your first morning urine</u>. This will be your last collected void of this 24-hour urine collection. Enter the **TIME** of this final urine void for Question #6 on the 24-Hour Urine Collection Log.
13. Please take out the last two frozen ice packs from your freezer and add them upright on two sides of your urine containers in the cooler.

14. Please record all prescription and nonprescription medications you took during the 24-hour urine collection period and the previous day in Question #7 on the Log. Please specify the name, brand and dose of medication, and day you took it.

15. To prepare your urine collection for pick-up, please make sure that the urine collection containers have been secured in a tightly closed bag in the trolley cooler with all seven ice packs. You do not need to return the hat/urinal. If you do use the paper form of the 24-Hour Urine Collection Log, please bring it with you next time you visit the metabolic kitchen. A courier will be scheduled to pick-up the cooler from your home (please talk with the Project Coordinator to make arrangements).

Tips to help you remember to collect all of your urine:

* Attach the safety pin provided to your underclothes.
* When at home, leave the hat/urinal on top of the toilet seat.
* When away from home, keep your supplies close by at all times.

If you have any questions or concerns, please contact

Project Coordinator

THANK YOU FOR PROVIDING THIS SAMPLE!

APPENDIX G

24-HR URINE LAB LOG

ID	Date of collection	Feeding Day when it was collected	Day of the Week	Urine processing in the lab	Container 1				Container 2				Cooler 8 hours T°C>22°C	Total BA (g)	Urine Volume (ml/d)	Freezer boxes					Notes in the lab	Notes at collection	POTABA (take a note only if more than 1 h difference to SOP	medication (Name, Brand, Dose)
					Boric Acid (g)	EMPTY (w/BA) (g)	FULL (g)	Urine (ml)	Boric Acid (g)	EMPTY (w/BA) (g)	FULL (g)	Urine (ml)				4.5 ml		1.8 ml							
																-20°C	f -80°C: #(	-20°C	-20°C	f -80°C: #(				
																A01-04	A05-07	A08-10	A11	A12-14				
		1	Tuesday																					
		3	Thursday																					
		5	Saturday																					
		7	Monday																					
		9	Wednesday																					
		11	Friday																					
		13	Sunday																					
		15	Tuesday																					
		1	Tuesday																					
		3	Thursday																					
		5	Saturday																					
		7	Monday																					
		9	Wednesday																					
		11	Friday																					
		13	Sunday																					
		15	Tuesday																					

APPENDIX H

24-HOUR URINE COLLECTION LOG

SUBJECT ID _____

24-HOUR URINE COLLECTION _____

INSTRUCTIONS:

- Please collect all urine for the entire 24-hour period into the urine bottles. Make sure that all collections are complete (see "Directions for Collecting 24-hour urine sample" for more details).
- When you first get up in the morning on the day of the 24-hour urine collection, **DISCARD** your first urine, and record the TIME you did this. Then, collect all the urines up to **AND INCLUDING** the first urine you pass on the following morning, and record the TIME.
- Take three POTABA tablets at evenly spaced intervals throughout the 24-h urine collection day, starting after discarding the first urine (see "Directions for Collecting 24-hour urine sample" for more details).
- Keep the urine bottles cool at all times.

1. Please enter the DATE when you start this 24-h urine collection.

   Date: |___|___| |___|___| |_2_|_0_|_1_|__|

   MO DAY YEAR

2. Please enter the TIME of your first morning urine that you have discarded.

   Time: |___|___|:|___|___| AM/PM

3. Please enter the TIME of your second morning urine of the day, which is the first urine of your 24-h urine collection.

   Time: |___|___|:|___|___| AM/PM

4. Please record the time you take each POTABA tablet.

   Did you forget to take a tablet?

Check box if YES

Tablet 1: Time: |___|___|:|___|___| AM/PM □

Tablet 2: Time: |___|___|:|___|___| AM/PM □

Tablet 3: Time: |___|___|:|___|___| AM/PM □

5. Did you miss collecting any urine during this 24-hour period?

|___| Yes |___| No → Skip to Question #6

5a. If you missed collecting any urine, please record the time and approximate amount of the missed void.

Time: |___|___|:|___|___| AM/PM estimated amount: _____ oz.

Time: |___|___|:|___|___| AM/PM estimated amount: _____ oz.

6. Please enter the TIME of your <u>first morning urine you pass the following day</u>, which is <u>the last urine of your 24-h urine collection</u>.

Time: |___|___|:|___|___| AM/PM

7. Please record all prescription and nonprescription medications you took during the 24-hour urine collection period and the previous day in the space provided below. Please specify the name, brand and dose of medication, and day you took it:

_____

_____

_____

THANK YOU FOR PROVIDING THIS SAMPLE!

APPENDIX I

PHYSICAL ACTIVITY LOG BOOK

Physical Activity Log Book

Instructions

We would like you to keep a log of all physical activities you engage in over the next 15 days.

It's extremely important that you don't make any changes in your physical activity when you are completing the log.

You will complete a log for each day throughout the 15-day feeding study. Fill the log out at the end of the day.

You will be asked about number of popular activities you may have done each day, including home, transportation, occupational, conditioning, sports and leisure activities.

For each activity, circle *yes* if you did the activity and *no* if you did not do the activity.

For each activity you did, write down the number of hours and/or minutes you were actually moving and the time you began the activity (am or pm).

If you did an activity many times during the day, write down the total time you did that activity during the day.

If you did any activities that are not on this list, please write them on the line labeled "other," circle *yes* and write in the hours and/or minutes.

Remember to record only the hours and/or minutes you were actively engaged in the activity.

Day 1: Date ___/___/20__			Day of the Week: _____	
Did you do this activity today?	**Yes** (circle one)	**No**	**How Long?** Hours: Minutes	Time started activity **AM or PM**
Home Activities				
Sweep, scrub floors, vacuum, washing clothes, etc	Yes	No	____:____	_____ AM/PM
Carpentry	Yes	No	____:____	_____ AM/PM
Gardening or Yard Work	Yes	No	____:____	_____ AM/PM
Transportation				
Walk to work, school, shopping	Yes	No	____:____	_____ AM/PM
Bicycle to work, school, shopping	Yes	No	____:____	_____ AM/PM
Occupation				
Sitting at work	Yes	No	____:____	_____ AM/PM
Standing at work	Yes	No	____:____	_____ AM/PM
Walking at work	Yes	No	____:____	_____ AM/PM
Lift or carry 10-20 lbs at work	Yes	No	____:____	_____ AM/PM
Lift or carry 20+ lbs at work	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Conditioning Activities				
Aerobic Exercise, Aerobic Dance	Yes	No	____:____	_____ AM/PM
Bicycling	Yes	No	____:____	_____ AM/PM
Calisthenics or gymnastics	Yes	No	____:____	_____ AM/PM
Jogging or running	Yes	No	____:____	_____ AM/PM
Hiking with pack or in mountains	Yes	No	____:____	_____ AM/PM
Martial arts (judo, karate, tai chi)	Yes	No	____:____	_____ AM/PM
Rowing a boat, canoeing	Yes	No	____:____	_____ AM/PM
Swimming	Yes	No	____:____	_____ AM/PM
Walking for exercise	Yes	No	____:____	_____ AM/PM
Weight lifting, body building	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Sports Activities				
Baseball or softball	Yes	No	____:____	_____ AM/PM
Basketball, European Handball	Yes	No	____:____	_____ AM/PM
Surfing	Yes	No	____:____	_____ AM/PM
Cross-country skiing	Yes	No	____:____	_____ AM/PM
Handball, racquetball, or squash	Yes	No	____:____	_____ AM/PM
Ice or roller skating, ice-hockey	Yes	No	____:____	_____ AM/PM
Rugby, football	Yes	No	____:____	_____ AM/PM
Soccer	Yes	No	____:____	_____ AM/PM
Tennis	Yes	No	____:____	_____ AM/PM
Volleyball	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Leisure Activities				
Bowling	Yes	No	____:____	_____ AM/PM
General Dancing	Yes	No	____:____	_____ AM/PM
Golf	Yes	No	____:____	_____ AM/PM
Fishing	Yes	No	____:____	_____ AM/PM
Table Tennis	Yes	No	____:____	_____ AM/PM
Walking for pleasure or social	Yes	No	____:____	_____ AM/PM
Yoga	Yes	No	____:____	_____ AM/PM
Watching television	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
Other: _____	Yes	No	____:____	_____ AM/PM
End of Day 1				

APPENDIX J

PHYSICAL ACTIVITY MET LIST

Activity	5-digit code	MET	Type of activity	Notes
Home activities				
Sweep, scrub floors, vacuum, washing clothes, etc.		3.2	Moderate	Average
Sweep – cleaning, sweeping carpet or floors, general	05010	3.3		
Vacuum	05043	3.3		
Washing clothes – light effort	05090	2.0		
Washing clothes-moderate effort	05092	4.0		
Scrub-moderate effort	05130	3.5		
Carpentry	06040	3.0	Moderate	General
Gardening or Yard Work		3.0	Moderate	Average
	08135	2.0		planting, potting, transplanting seedlings or plants, light effort
	08239	3.5		weeding, cultivating garden, light-to-moderate effort
	08215	3.5		trimming shrubs or trees, power cutter, using leaf blower, edge, moderate effort
Transportation				
Walk to work, school, shopping	16060	3.5	Walking Moderate	Walking for transportation
Bicycle to work, school, shopping	01011	6.8	Vigorous	
Occupation				
Sitting at work	09060	1.3	Sedentary	
Standing at work	09050	1.8	Standing	
Walking at work	11791	2.0	Walking Light	walking on job, less than 2.0 mph, very slow speed, in office or lab area
Lift or carry 10-20 lbs at work	11615	4.5	Moderate	
Lift or carry 20+ lbs at work	11820	5.0	Moderate	25 to 49 pounds
Conditioning Activities				
Aerobic Exercise, Aerobic Dance	03015	7.3	Vigorous	Aerobic dancing, general
Bicycling		7.3	Vigorous	Average
Bicycling, general	01015	7.5		
Bicycling, stationary, general	02010	7.0		
Calisthenics or gymnastics		3.8	Moderate	Average
Calisthenics, moderate effort	02022	3.8		
Gymnastics, general	15300	3.8		
Jogging or running		7.5	Vigorous	Average
Jogging, general	12020	7.0		
Running	12150	8.0		
Hiking with pack or in mountains	17012	7.8	Vigorous	Backpacking, hiking or organized walking with a daypack

Activity	5-digit code	MET	Type of activity	Notes
Martial arts (judo, karate, tai chi)	15425	5.3	Moderate	Slower pace
Rowing a boat, canoeing		4.7	Moderate	Average
Moderate effort	18050	5.8		
For pleasure, general	18070	3.5		
Swimming	18310	6.0	Vigorous	Swimming, Leisurely
Walking for exercise	17302	4.8	Walking Moderate	Moderate pace
Weight lifting, body building	02054	3.5	Moderate	resistance (weight) training, multiple exercises, 8-15 repetitions at varied resistance
Sports Activities				
Baseball or softball	15620	5.0	Moderate	
Basketball, European Handball	15055	6.5	Vigorous	General
Surfing	18220	3.0	Moderate	General
Cross-country skiing	19090	9.0	Vigorous	General
Handball, racquetball, or squash		8.8	Vigorous	Average
Handball	15320	12.0		
Racquetball	15530	7.0		
Squash	15652	7.3		
Ice or roller skating, ice-hockey	19030	7.0	Vigorous	Skating, ice, general
Rugby, football		7.2	Vigorous	Average
Rugby	15562	6.3		Noncompetitive
Football, general	15230	8.0		
Soccer	15610	7.0	Vigorous	
Tennis	15675	7.3	Vigorous	
Volleyball	15710	4.0	Moderate	
Leisure Activities				
Bowling		3.4	Moderate	Average
Bowling	15090	3.0		
Bowling, Indoor, bowling alley	15092	3.8		
General Dancing	03031	7.8	Vigorous	
Golf	15255	4.8	Moderate	General
Fishing	04001	3.5	Moderate	General
Table Tennis	15660	4.0	Moderate	Table tennis, ping pong
Walking for pleasure or social	17160	3.5	Walking Moderate	Walking for pleasure
Yoga		3.0	Moderate	Average
Yoga, Hatha	02150	2.5		
Yoga, Power	02160	4.0		
Yoga, Nadisodhana	02170	2.0		
Yoga, Surya Namaskar	02180	3.3		
Watching television	07020	1.3	Sedentary	
Other Activities				
Driving	16015	1.3	Sedentary	
Computer work/Online/Sitting at computer/sitting online/screens	09055	1.5	Sedentary	
Study and paperwork/study	09060	1.3	Sedentary	Sitting, studying, general including reading/writing, light effort

Activity	5-digit code	MET	Type of activity	Notes
prep/homework/projects with kids/Reading/Writing				
Sitting in class, sitting at home, sitting at ASU, sitting in car, working at home, Board meeting (Sitting)	09060	1.3	Sedentary	Same as sitting @ work
Standing at home, Standing around house, Standing @ home, Standing (Massage), Standing (event), Standing at party	09050	1.8	Standing	Same as standing @ work
Walking around house	17150	2.0	Walking Light	Walking, household
Eating/meals, eating, eating meals, Sit/eat dinner, Meals, Eating dinner	13030	1.5	Sedentary	Eating, sitting
Getting ready , Getting ready (Standing/Walking), Getting ready (Walking), Getting ready/Party (Standing/Light walking), Getting ready (Standing/light walking), Miscellaneous(getting ready, walking around the house)		2.3	Standing	Average
	13020	2.5		Dressing
	17150	2.0		Walking, household
Knitting	05080	1.3	Sedentary	
Sewing	05082	2.8	Sedentary	
Painting	09020	1.8	Standing	
Cooking	05052	2.5	Standing	
Cooking (reheating)	05050	2.0	Standing	cooking or food preparation - standing or sitting or in general (not broken into stand/walk components), manual appliances, light effort
Meals/Cooking, Eating, preparing food		2.0	Standing	Average
	05052	2.5		Cooking
	13030	1.5		Eating, sitting
Loading stuff on bike	05146	3.5	Moderate	standing, packing/unpacking boxes, occasional lifting of lightweight household items, loading or unloading items in car, moderate effort
Socializing		1.65	Sedentary	Average
Sitting and talking/socializing/Picnic/ Hanging out	09055	1.5	Sedentary	
Standing and talking/socializing, Standing (at dinner party-talking)	09050	1.8	Standing	

Activity	5-digit code	MET	Type of activity	Notes
Socializing Sitting/Standing, Party (Sitting 30 min/Standing 3 hours)		1.7	Standing	Average of 09055, 09050
Field work	11875	4.0	Moderate	Teaching physical education at elementary school
Pitching machine	09071	2.5	Standing	Standing (feeding machine with baseballs) 09071-standing, miscellaneous
Shopping	05065	2.3	Light	
Grocery	05060	2.3	Light	
Errands	05065	2.3	Light	Shopping
Playing cards	09010	1.5	Sedentary	
Playing with kids, playing with kids (trampoline, hide and go seek) low to moderate intensity, playing with children, playing with son (picking up son 26 lbs, playing catch running around), Chasing toddler, Chasing toddler around, Playing with toddler, Play with kids (bikes, running, playground)		3.2	Moderate	Average
	05171	2.8		standing, playing with child(ren) light effort, only active periods
	05175	3.5		walking/running, playing with child(ren), moderate effort, only active periods
activities with kids-hanging out (throwing baseball)	15235	2.5	Standing	football or baseball, playing catch
Lego building	09000	1.5	Sedentary	Board game playing, sitting
Nap, rest, relax, massage, lay down	07010	1.0		Equivalent to sleep/ lying quietly. **Ignore**
Soaking in tub				**Ignore** Sitting/laying down/relaxing
Floating in pool				**Ignore**
Stretch	02101	2.3	Light	
Stationary bike interval training/intervals on bike	02010	7.0	Vigorous	Bicycling, stationary, general
Riding bikes	01018	3.5	Moderate	Bicycling, leisure
Packing/unpacking/cooler repack/ errands for camp, Packing (standing and walking) light intensity	05090	2.0	Standing	laundry, fold or hang clothes, put clothes in washer or dryer, packing suitcase, washing clothes by hand, implied standing, light effort
Strength training/core training	02054	3.5	Moderate	resistance
Elliptical	02048	5.0	Moderate	
Circuit training	02035	4.3	Moderate	Circuit training, moderate effort
Playing video game	09045	1.0	Sedentary	
Arcade games	09071	2.5	Standing	Standing, miscellaneous
Setting up	05146	3.5	Moderate	Setting up her booth in the state fair standing, packing/unpacking boxes, occasional lifting of lightweight household

Activity	5-digit code	MET	Type of activity	Notes
				items, loading or unloading items in car, moderate effort
Setting up tent	09110	2.5	Standing	
Decorating	06126	2.5	Standing	home repair, general, light effort
Shooting	04130	2.5	Standing	Shooting while Standing
Watch movie	07025	1.5	Sedentary	
Trail running	12150	8.0	Vigorous	Running
Eating/reading	13030/09060	1.4	Sedentary	Average
Skiing, downhill		4.8	Moderate	Average
Skiing, light effort	19150	4.3		skiing, downhill, alpine or snowboarding, light effort, active time only
Skiing, moderate effort	19160	5.3		skiing, downhill, alpine or snowboarding, moderate effort, general, active time only
Trampoline	15700	3.5	Moderate	Trampoline, recreational
Wiggling	09050	1.8	Standing	Standing
P90x Boxing class (light squats)	02052	5	Moderate	Resistance (weight) training, squats, slow or explosive effort
Jump rope	02068	11.0	Vigorous	Rope skipping, general
Running on stairs	12170	15.0	Vigorous	Running, stairs, up (estimated value)
Skydiving	15600	3.5	Moderate	
Longboard	15580	5.0	Moderate	skateboarding, general, moderate effort
Washing dishes	05041	1.8	Standing	wash dishes, standing or in general (not broken into stand/walk components)
Standing/Walking around the house, Garage work (light walking/standing), Garage work(light standing/walking), Kid activities(Walking/Standing), Standing/Walking (Watching super bowl)		1.9	Standing	Average of 09050 and 17150
Child care, Mom duties (Standing/Walking around the house), Mum duties, Getting ready for bed (self and kids showers diapers), Getting ready for day/bed, Geting ready - self and kids	05185	2.0	Light	child care, sitting/kneeling (e.g., dressing, bathing, grooming, feeding, occasional lifting of child), light effort, general
Cleaning at work	05011	2.3	Light	cleaning, sweeping, slow, light effort
Home painting	11514	3.3	Moderate	painting, house, furniture, moderate effort
Pump bike tires				**Ignore**
Wash car	05020	3.5	Moderate	cleaning, heavy or major (e.g. wash car, wash windows, clean garage), moderate effort
Moving, Lifting light items/Carying, Moving (Lifting 10-20 lbs),	05121	5.0	Moderate	Moving, lifting light loads

Activity	5-digit code	MET	Type of activity	Notes
Moving - Lift/carry 10-20 lbs				
Moving furniture, Moving (boxes and furniture),Moving furniture (20+ lbs), Moving - Lifting 20+ lbs	05120	5.8	Moderate	moving furniture, household items, carrying boxes
Garage work (light standing/walking)		1.9	Standing	Average of 09050, 11791
Washing dishes/chores (light - laundry, cleaning etc)		2.1	Light	Average
	05041	1.8		wash dishes, standing or in general (not broken into stand/walk components)
	05011	2.3		cleaning, sweeping, slow, light effort
	05095	2.3		laundry, putting away clothes, gathering clothes to pack, putting away laundry, implied walking
Chores (tidying, folding laundry, picking up, dishes light)		2.1	Light	Same as above
Errands/chores - dishes, laundry, make beds, run errands, put away		2.4	Light	Average
	05041	1.8		wash dishes, standing or in general (not broken into stand/walk components)
	05095	2.3		laundry, putting away clothes, gathering clothes to pack, putting away laundry, implied walking
	05100	3.3		making bed, changing linens
Errands	05065	2.3		Shopping
Chores (dishes, picking up, packing coolers, etc - light)		1.9	Light	Average
	05041	1.8		wash dishes, standing or in general (not broken into stand/walk components)
	05090	2.0		laundry, fold or hang clothes, put clothes in washer or dryer, packing suitcase, washing clothes by hand, implied standing, light effort
Light unpacking (setting up new house)		3.3	Moderate	Average
	05146	3.5		standing, packing/unpacking boxes, occasional lifting of lightweight household items, loading or unloading items in car, moderate effort

Activity	5-digit code	MET	Type of activity	Notes
	05147	3.0		implied walking, putting away household items, moderate effort
Strolling (Park), Art museum	17151	2.0	Walking Light	walking, less than 2.0 mph, level, strolling, very slow
Watching TV/computer		1.4	Sedentary	Average
	07020	1.3		Watching television
	09055	1.5		sitting, talking in person, on the phone, computer, or text messaging, light effort
Shower	13050	2.0	Standing	showering, toweling off, standing
Lifting 10-20 lbs	11615	4.5	Moderate	Lift or carry 10-20 lbs. at work
Lift or carry 20+ lbs	11820	5.0	Moderate	Lift or carry 20+ lbs. at work, 25 to 49 pounds
Pool party				**Ignore**
Auto repair(Bending/Standing)	06030	3.3	Moderate	automobile repair, light or moderate effort
Bday event (light walk/stand)		1.9	Standing	Average of 09050 and 17150
Watching football (Standing)		1.7	Standing	Average
	09115	1.5		sitting at a sporting event, spectator
Standing	09050	1.8		
Watch track meet, Watching kids play ball, Suns game (Sitting in arena)	09115	1.5		sitting at a sporting event, spectator
Washing clothes, dishes		1.9	Light	Average
Washing clothes – light effort	05090	2.0		
	05041	1.8		wash dishes, standing or in general (not broken into stand/walk components)
Pickle ball (Racquetball/tennis/badminton - hitting/running - moderate intensity)		5.8	Moderate	Average
Racquetball	15530	7.0		
Tennis	15695	5.0		tennis, hitting balls, non-game play, moderate effort
Badminton	15030	5.5		badminton, social singles and doubles, general
throw a ball around	09050	1.8	Standing	

Activity	5-digit code	MET	Type of activity	Notes
Reading/talking/laying on couch	07070	1.3	Sedentary	reclining, reading
Reading/Phone/Sitting, Phone (Sitting), Reading news/phone, News/Read/Phone (Sitting), News reading/phone, Read news	09030	1.3	Sedentary	sitting, reading, book, newspaper, etc.
Jumping, Jumping (high intensity)	02040	8.0	Vigorous	circuit training, including kettlebells, some aerobic movement with minimal rest, general, vigorous intensity
Commute by bus, Commute by bus to work	16016	1.3	Sedentary	Riding in a bus or train
Suns game (Walking around arena)	17161	2.5	Walking Light	walking from house to car or bus, from car or bus to go places, from car or bus to and from the worksite
Kickball	15450	7.0	Vigorous	
Temple services (Sitting & Standing)		1.3	Sedentary	Average
	20000	1.3		sitting in church, in service, attending a ceremony, sitting quietly
	20015	1.3		standing quietly in church, attending a ceremony
Loading, unloading kayaks (~25 pounds)	11820	5.0	Moderate	Lift or carry 20+ lbs. at work, 25 to 49 pounds
Waxing truck	06225	2.0	Light	Washing and waxing car
Pilates	02105	3.0	Moderate	Pilates, general
Installing electricity in the attic, Installing flooring in the attic	06072	4.0	Moderate	carpentry, home remodeling tasks, moderate effort
Mountain biking	01009	8.5	Vigorous	Bicycling, mountain, general

APPENDIX K

BASELINE AND DESCRIPTIVE CHARACTERISTICS OF THE STUDY POPULATION (N = 57)

	Mean ± SD/Median ± IQR (Range)
Mean Age (years)‡	35.0 ± 21 (20 – 68)
Gender n (%) Males Females	 21 (36.8) 36 (63.2)
Mean BMI (kg/m^2) †	26.3 ± 4.0 (18.7 – 35.2)
Ethnicity n (%) White/Non-Hispanic/Caucasian Hispanic/Latino Other	 45 (78.9) 6 (10.5) 6 (10.5)
Education n (%) Some college Associate's/Bachelor's Degree Master's / Terminal Degree	 12 (20.3) 21 (39) 24 (40.7)
Marital status n (%) Single/never married/divorced Married/Living with a partner	 29 (50.9) 28 (49.1)
15-day mean physical activity measures	
Total MET- hours/day ‡	28.7 ± 7.5 (14.7 - 37.1)
Active MET-hours/day †	11.9 ± 5.4 (1.5- 24.9)
Walking time (hours/day) ‡	0.5 ± 0.7 (0 - 1.7)
Moderate activity time (hours/day) ‡	0.9 ± 1.6 (0 - 5.1)
Vigorous activity time (hours/day) ‡	0.3 ± 0.6 (0 - 2.0)
15-day mean dietary intake15-day mean dietary intake	
Total energy (kcal/d) †	2693.1 ± 625.8 (1516.7 – 5172.8)
Total sugars (g/d) †	112.2 ± 33.1 (47.4 – 203.7)
Added sugars (by total sugars) (g/d) †	65.8 ± 29.0 (10.5 – 159.1)
Added sugars density (%EI) †	9.7 ± 3.8 (1.8 – 19.3)
Naturally-occurring sugars (g/d) ‡	31.2 ± 28.4 (11.5 – 90.0)
Sucrose intake (g/d) †	58.6 ± 21.5 (14.6 – 104.0)
Fructose intake (g/d) ‡	17.0 ± 12.9 (5.6 – 51.4)
Total fructose intake (g/d) †	49.3 ± 15.9 (19.0 – 97.8)

Carbohydrate (g/d) ‡	280.1 ± 78.8 (185.7 – 517.5)
Total fiber (g/d) ‡	30.0 ± 13.4 (14.2 – 86.0)
Protein (g/d) ‡	102.5 ± 35.2 (59.8 – 275.6)
Fat (g/d) ‡	112.5 ± 34.8 (50.7 – 268.2)
8-day mean urinary sugars	
24-hour urinary sucrose (mg/d) †	26.6 ± 16.1 (4.9 – 81.4)
24-hour urinary fructose (mg/d) ‡	23.1 ± 20.9 (2.6 – 103.3)
24-hour urinary sucrose and fructose (mg/d) ‡	51.0 ± 32.3 (10.0 – 152.1)

† - Means and SD

‡ - Medians and IQR