Childhood Obesity and Its Relation to Beverage Consumption, Dental Caries and Salivary Biomarkers

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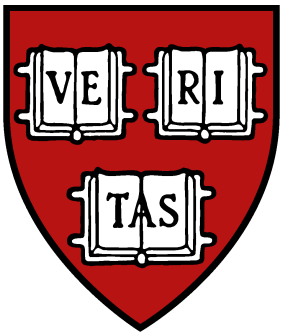

Childhood Obesity and Its Relation to Beverage Consumption, Dental Caries and Salivary Biomarkers

A Thesis Presented by
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Table of Contents

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ABSTRACT

Introduction: Children of Kuwait have both a high obesity and dental caries prevalence, as well as excessive consumption of sugar-sweetened beverages (SSB). Obesity is a major risk factor for serious health conditions such as diabetes, cancer, and cardiovascular disease. Metabolic syndrome (SMets), the occurrence of 3 out 5 variables (obesity, hypertension, hyperglycemia, lowered high-density lipoprotein cholesterol (HDLC), and hypertriglyceridemia) is a risk factor that predicts future cardiovascular disease and type 2-diabetes. The aim of this analysis was to assess the impact of the consumption of three beverages (soda, milk, and juice) on the obesity and dental caries in Kuwaiti children. We also aim to investigate the changes overtime of the salivary biomarkers; insulin, CRP, phosphate, and uric acid. This study followed a group of children before having developed SMets and after its development, and compared them to children with no SMets characteristics.

Methods and Materials: Data were obtained from the Kuwait Healthy Life Study. For the impact of beverage consumption on the severity of dental caries, 8,317 children were included from the baseline visit in 2012-13 (age =10). For the obesity 6,305 children were included, from both baseline and follow-up visit in 2014-15 (age =12). A subset of 94 children were included in the study of the salivary biomarker change over time, all the children had no SMets at baseline, 51 became SMets positive and 43 remain healthy at follow-up. A multivariate logistic regression analysis was conducted to examine the relation between developing obesity as a dependent binary variable, and beverages

consumption amount as a categorical independent variable. For the severity of dental caries, a multivariate logistic regression was used to evaluate this association between the severity of dental caries as a binary dependent variable (low and high), and the categorical consumption (amount and pattern) of the three beverages as independent variables. For the salivary biomarkers, the changes in the salivary biomarker measurements from baseline to follow-up for each group were tested using Wilcoxon matched-pairs signed-ranks test.

Results: A significant association between high soda and milk consumption with developing obesity (OR=1.68, P= 0.004, CI =1.19- 2.39) (OR=1.77, P=0.019, CI =1.10-2.87), respectively. For the severity of dental caries, high soda consumption was significantly associated with severe dental caries (OR=1.20, P= 0.041, CI =1.01- 1.42). Moderate milk drinking showed a protective effect from having severe dental caries (OR=0.88, P= 0.007, CI =0.81- 0.97). The change in salivary biomarkers from baseline to follow-up, children in the healthy group had no significant change. Children who developed SMets had a significant increase in all salivary biomarkers; insulin became double the baseline levels (p =0.014), CRP were 120% higher (p =0.005), phosphate became 11% higher (p =0.030) and uric acid showed 17% elevation (p =0.009).

Conclusion: High soda consumption was significantly associated with obesity and dental caries. High milk consumption was significantly associated with obesity but not with dental caries. Moderate milk consumption was protective from having severe dental

caries, even if it was occasional use of flavored milk. Children who developed SMets showed a significant elevation in all the four salivary biomarkers while there was no significant change noted in the levels for children who did not develop SMets.

GENERAL BACKGROUND

Sugar consumption is currently one of the most notorious public health issues. Sugar sweetened beverages (SSB) are the greatest source of added sugar in the average diet.[1] SSB consumption has been linked to several health concerns and even deaths.[2] In a study conducted by *Singh et al,* [2] looking at the burden of disease associated with SSB consumption, they estimated that 184,000 deaths/year globally were attributed to SSB consumption. Diabetes accounted for 133,000 of these deaths; cardiovascular disease was responsible for 45,000, and an additional 6,450 were from various cancers. [2] The authors noted that 24.1% of these deaths occurring in high-income countries, 70.9% in middle-income, and 5% in low-income countries. [2]

High SSB consumption has contributed to the rise of obesity globally[3] and is now a growing pandemic. [4] According to World Health Organization (WHO), the prevalence of obesity in 2014 has doubled compared to what it was in 1980.[4] The overall global prevalence of individuals who are overweight and obese is 39% and 13%, respectively.[4] Obesity during childhood is a major public health crisis associated with a wide range of diseases and serious health complications including premature death.[5] As in adults, childhood obesity is linked with several non-communicable diseases that persist into adulthood; particularly diabetes,[6, 7]cholesterol[8] and cardiovascular diseases.[9] The prevalence of childhood obesity is rapidly increasing in both developing and developed countries.[10] Between 1970s and 1990s, the childhood obesity prevalence in the United States increased three fold and during this same period Egypt's childhood obesity prevalence increased 3.9 times.[5]

Children who consume SSB in large quantities, eat less fiber, minerals, vitamins and calcium than those who consume low amounts of added sugar, despite their greater caloric intake.[11] Due to the excessive amount of sugar and non-nutritive calories, SSB are strongly associated with several health problems mainly through obesity.[3, 12, 13] SSB contain high amounts of sucrose and fructose that may lead to diabetes and metabolic syndrome (SMets) apart from obesity. [14 15] The added sugar (sucrose and fructose) in SSB increases the dietary glycemic load that may lead to pancreatic β - cells dysfunction and insulin resistance. [14 15]

Regular consumption of SSB is also linked to oral health concerns, particularly dental erosion[16] and dental caries.[17] The carcinogenicity of certain food items has been the subject of study for a number of authors for a long time.[18, 19] Historically, early studies on the carcinogenicity of soft drinks compared soft drink consumption within individual US states to statewide caries prevalence. [18-20] Early investigations did not reveal any associations; in fact, they showed an inverse association and states with higher consumption had a lower prevalence of dental caries.[18-20] However, several more recent studies have confirmed a strong positive association between soft drink consumption and dental caries. [21-25]

It has been well established in the literature that individuals continue many of their dietary habits from childhood and adolescence into adulthood. Some of these habits place them at risk of cardiovascular diseases[26], obesity[26], diabetes[27], and dental caries.[28] In a cohort study included 1,586 children, *Webber et al,* [29] found that two of the SMets serum lipid indicators (high density lipid and triglyceride) could be tracked from childhood to adulthood and this was especially true when tracking SMets combined risk factor

clusters.[29]

This highlights the importance of early intervention programs by public health experts who may identify children at risk of obesity-related at an early age. This would help to minimize the lasting effect of negative dietary habits and prevent the onset of obesity related diseases. In order to identify people at risk, simple non-invasive diagnostic tools can be of great benefit, especially with children. Children are often fearful of hospitals, medical procedures, needles and vaccines.[30] This fear can be a barrier for both health care providers and families to obtain the necessary health cares services or seek preventive care. [30] For regular screenings, blood biomarkers can be used to give information about disease status. However, blood drawing is invasive and many children fear having blood drawn. Saliva offers an ideal non-invasive alternative[31] since it is easier to collect than plasma and urine. In addition, saliva contains biomarkers that can be stored and used for multiplexed assay screening.[31] Saliva may serve for diagnosis, monitoring and follow up for patients with systemic disease.[31, 32] The use of salivary tests can help to reach a greater number of at risk patients. An example for the success of salivary diagnostic tools is the increase of participation in Human Immunodeficiency Virus (HIV) salivary antibody tests after it was offered as an approved alternative for blood test.[33] Salivary biomarkers have been studied for several systemic disease such as; hypertension, [34] cardiovascular diseases, [35] renal diseases, [36] cancers, [37] and diabetes. [38]

As discussed above, several studies were cited to show the significant relationship between SSB and obesity [3, 12] as well as dental caries[17, 25] The wealth of knowledge about the role of sugar in obesity led to a recent recommendation for policy to be implemented to reduce the individual sugar intake and the amount added to foods .[39-42] In 2015, WHO

made the following recommendations concerning the role sugar plays in obesity and dental caries[43]:

- "WHO recommends a reduced intake of free sugars throughout the life course (*strong recommendation)"* [43]
- "In both adults and children, WHO recommends reducing the intake of free sugars to less than 10% of total energy intake (*strong recommendation)"* [43]
- "WHO suggests a further reduction of the intake of free sugars to below 5% of total energy intake (*conditional recommendation)"*[43]

Kuwait

Kuwait is a small country (17,818 km^2)[44]; with a population of 4,416,094, of which only 30% are Kuwaiti nationals.[45] Nearly half of Kuwaitis are under 19 years of age. [45] It is among the world's richest countries, with $46,342.045 GDP per capita.[46] Since this wealth started with the discovery of oil in the1960s, there has been a surge of nutrition related health issues.[47, 48] Rapid increase of several chronic health conditions accompanied this sudden wealth: obesity and metabolic syndrome,[49-52] diabetes,[53] and dental caries[54] were on top of the list and Kuwait ranked second in overweight population after the United States.[55] In 2005, Kuwait's Ministry of Health survey showed the prevalence of the combined categories of obesity and overweight as 73.6% for male and 77.4% for female adults. Obesity alone accounted for 36.4% and 47.9% for men and women respectively.[56] In a more recent survey (2010) the prevalence of overweight and obese adults had risen to 80.4%, with obese comprising 47%. An additional 36% had metabolic syndrom.[52] Childhood obesity is also alarmingly high in Kuwait. Children 10-14 years old had a prevalence of 30.7% overweight and 14.6% obese,[50] placing them at risk of having metabolic syndrome and diabetes. [57]

Metabolic syndrome is a risk factor for type 2 diabetes, and according to the International Diabetes Federation (IDF), Kuwait has one of the highest percentages of individuals with diabetes in the world.[58] The "age adjusted comparative prevalence" of diabetes in Kuwait reach 20%.[58] Even more alarming is the rapid rise of the diabetes prevalence in Kuwait. The "national prevalence" of diabetes in Kuwait leaped from 7% in 2000 to 14% by 2014.[58]

The change in life style has also affected the oral health of Kuwait's population.

Although no-cost medical and dental treatment are available for all Kuwaiti citizens in government-owned hospitals, the latest dental survey showed 87% of 5-year olds had at least one decayed primary tooth, and 78% of 12-year olds had at least one decayed or missing permanent tooth. [54]

The consumption of SSB in Kuwait is high. Thirteen years old Kuwaiti adolescents showed the highest proportion (75%) of every day soft drink consumption, when compared to the 34 countries that participated in the Health Behavior in School-Aged Children (HBSC) collaborative study.[59] In another study, primary and secondary school students, 44.6% reported drinking soft drinks with dinner, 35% as a snack, and 27.7% with lunch. [60]

If sugar intake is strongly associated with obesity and dental caries, it would be very reasonable to expect individuals with a high-added sugar intake to have a higher risk of obesity, as well as a higher level of dental caries. The association between dental caries and obesity does not always follow this expected logic; some studies have shown an inverse relationship between obesity and dental caries[61-65]. In fact, one cross sectional study by *Goodson et al,*[65] on Kuwaiti children showed less caries in overweight children compared to normal and underweight children.[65] These latest results by *Goodson et al,* are part of the first survey of the collaborative work between the Dasman Diabetes Institute and the Forsyth Institute (2012). The larger study, known as *The Kuwait Healthy Life Study* included dental examinations, saliva collection, body measurements, as well as lifestyle and diet intake interviews for 8,319 children between 9 to 12 years of age. In 2014, the survey and exams were repeated with the same population, of which 6,316 children participated.

Dasman Diabetes Institute and The Kuwait Healthy Life Study

With growing concerns about obesity and diabetes, the Kuwaiti government established Dasman diabetes institute in 2006.[66] The institute mission is *"To prevent, control and mitigate the impact of diabetes and related conditions in Kuwait through effective programs of research, training, education, and health promotion and thereby improve quality of life in the population."*[66] In 2012, collaboration between The Forsyth Institute in Cambridge, Massachusetts and Dasman institute lunched the Kuwait Healthy Life study, with a goal to study risk factors for type 2-diabetes on school-aged children in Kuwait.

Details about healthy life study and data management:

Sample:

In 2012, data was collected from 8,317 Kuwaiti public school children. Only 4th and 5th grade Kuwaiti students were included in the sample, and they were selected from 39 out of 250 primary schools in Kuwait (a total of 52,687 children are enrolled in these primary schools). All children included in the study had an Arabic written parental informed consent, with child assent obtained prior to enrollment in the study as well. In 2014, 6,316 children from the original sample participated in the follow-up study. The survey process was reviewed and approved by both the Dasman Institute Human Ethical Review committee in Kuwait and the Forsyth Institutional Review Board in Cambridge, Massachusetts.

Inclusion Criteria:

- Child in the 4th or 5th grade studying in a Kuwaiti public school, with willingness to participate.

Exclusion Criteria:

- Child without assent and/or parental consent.
- Any existing condition that would hinder proper examination.

Data Collection:

The data collected and transferred to a database are the followings:

Demographic and Basic data	
Age at the time of visit	Obesity category by WHO percentile
Sex	Obese by WHO percentile (Yes or No)
Waist circumference	Diastolic blood pressure
Sublingual temperature	Systolic blood pressure
Weight	Heart rate
Height	Salivary flow rate
BMI	Fitness level
Oral examination	
Number of teeth (permanent + Primary)	Percent of deciduous teeth decayed
Number of permanent teeth	Number of primary teeth
Number of filled permanent teeth	Number of filled deciduous teeth
Number of permanent teeth with decay	Number of deciduous teeth with decay
Percent of permanent teeth decayed	Number of decayed or filled primary teeth
Number of decayed or filled perm teeth	Percent of teeth with decay or filling (normalized measure of dental caries severity)
Percent of deciduous teeth decayed	Percent of sites that were red (normalized measure of periodontal disease severity)

Diet and nutrition (beverages with each meal)	
Breakfast	**Lunch**
Juice	Juice
Milk	Milk
Tea with sugar	Carbonated Soft drink
Tea with milk	
Dinner	**Snack**
Juice	Juice
Milk	Milk
Carbonated Soft drink	Carbonated Soft drink
Tea with sugar	Diet Carbonated Soft drink (Yes/No)
Sugared tea with milk	Flavored milk (Yes/No)

Oral examination:

Using a portable dental chair, an external halogen light source, and disposable dental mirrors, previously trained and calibrated licensed dentists performed clinical oral examinations, assisted by trained dental assistants. No radiographic images or explorers were used during the examinations. The number of presenting primary and permanent teeth were recorded, as well as the number of teeth with fillings and unfilled carious teeth. Gingival health was recorded by identifying the number of areas with red or swollen dental papilla, the number of buccal and lingual gingival margins that were red and swollen, as well as gingival areas with spontaneous bleeding. Areas with erupting teeth were excluded from examination.

Diet Questionnaire and Analysis:

An electronic questionnaire was created by the teams at Forsyth and Dasman that could be administered using an iPad in English and Arabic (Figure 1). Children were asked to select what they usually eat with each meal. The list of food items were based on responses from a pilot study conducted on 95 Kuwaiti schoolgirls prior to launching the survey.[67] The dietary preference questions included 79 food and beverage items with accompanying pictures, and food selection options were modified to reflect the regularly consumed foods in Kuwait. Interviewers queried the children on the food items they usually ate for breakfast, lunch, dinner and snacks. Following the questions on food preferences, questions on quantity were presented, with pictures provided to assess the difference between portion sizes: for example, one can, two cans, and three cans.

Beverage scoring (independent variable for paper 1 and 2):

Two portions of the electronic questionnaire were designed to determine the amount of drinks consumed:

1.Beverage selected for this specific meal or snack (Yes or No) figure 1.

2. Number of servings of the beverage selected with the meal or snack (ranging from 1-3)

After the questionnaire was completed, total drinks per day were tallied for each item as seen in this chart.

Example: Beverage = (soda, juice, milk, tea with sugar and tea with milk)

	Do you drink (beverage) with lunch	How many servings with lunch	Do you drink (beverage) with dinner	How many servings with dinner	Do you drink (beverage) snacks	How many servings with snacks	Total number of (beverage) servings
Child A	1	1	0	0	1	2	1 + 0 +2 = 3 beverage/day

Every child had a total number of servings for each beverage/day, as shown in the example below.

Beverage	Total servings/day
Total milk	1 serving
Total soda	3 servings
Total tea with sugar	0
Total tea with milk	0
Total juice	5 servings

Figure 1. Screen capture of the administered iPad dietary option page.

Following, three beverages (soda, juice and milk) were changed to be in categorical form:

Beverage (soda, juice and milk)	Total servings/day
No consumption	0 servings / day
Moderate consumption	1-2 servings / day
High consumption	3+ servings / day

Innovation

To our knowledge there are no longitudinal studies conducted to test the impact of beverage consumption on children and the development of obesity in Middle East and North African countries (MENA). With such an extremely alarming percentage of obesity as well as high SSB consumption in Kuwait, we aimed to use data from a longitudinal observational cohort study in the MENA region to investigate the relationship between SSB and the incidence of obesity among Kuwaiti children over a period of 2 years. In the first two papers, we are assessing the roles of SSB, which are widely consumed by Kuwaiti children, weighing its effect on two pressing community health problems in Kuwait (obesity and dental caries).[59] It describe the frequency and pattern of consumption and providing insight on how to help tailor the final public health recommendations to better serve the Kuwaiti community. This study proposes to use data from a longitudinal survey to present a more precise examination of the SSB consumption effect on obesity. It also uses cross sectional data from the first visit, to examine the dental caries severity association with these beverages on Kuwaiti children. Moreover, it may shed light on the reality behind the inverse association between obesity and dental caries found in both surveys. [65, 68] The aim is to clarify the role of the abundant sugar source (SSB) on both obesity and dental caries, which will help us recognize areas in which sugar may have a lesser effect that could indirectly explain such inverse associations.

As we stated earlier, tools to identify children at risk of diabetes can be of great value in a community with an epidemic of obesity and diabetes as in Kuwait. In the third paper, we continue on the previous work conducted by the research team at the Forsyth

institute and Dasman diabetes institute.[32, 69-71] We aim to investigate the changes in four crucial salivary biomarkers (insulin, C-reactive protein, phosphate, uric acid) over time, before developing SMets and after its development. We also aim to compare the change in levels of these biomarkers with another group that did not develop SMets

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BEVERAGE CONSUMPTION BY KUWAITI CHILDREN: IMPACT ON OBESITY BY ANALYSIS OF LONGITUDINAL COHORT DATA

ABSTRACT

Background:

Obesity is a major risk factor of serious health conditions such as diabetes, cancer and cardiovascular disease. The aim of this analysis was to assess the association between obesity and consumption of three beverages (soda, milk and juice).

Methods

Longitudinal cohort data were obtained from the Kuwait Healthy Life Study for 6,305 children, seen first in 2012-13 (age =10) and again in 2014-15 (age =12) years. Multivariate logistic regression analysis was conducted to examine the relation between developing obesity during the period from 2012 to 2014 as a dependent binary variable, and soda, juice and milk consumption as a categorical independent variable. Model selection was based on clinical relevant covariates and potential confounders with a predetermined significance level of α=0.05. Variables included in the final model were based on stepwise model selection keeping only significant variable or variable with effect modification.

Result:

During the two years study period, 378 (6%) of participants became obese. Of those subjects, 47 (12.4%) reported high soda consumption compared to children who did not become obese. Multivariate logistic regression model showed significant association

between soda drinking and developing obesity during the two years. Milk drinking also showed significant association with developing obesity. Covariates and potential confounders included in the final model were age, sex, governorates and fitness level. None of the covariates were found to be significant confounders for the association between obesity and any of the three beverages.

Conclusion:

Findings of this study suggest that those children who reported high consumption of soda had significantly higher prevalence of obesity (OR=1.68, P= 0.004, CI =1.19- 2.39) as was also found with milk consumption (OR=1.77, P=0.019, CI =1.10- 2.87) but not in those with high juice consumption (OR=1.11. p=0.494, CI=0.82-1.50)

INTRODUCTION

The impact of sugar-sweetened beverage (SSB) consumption on the development of obesity is a growing healthcare concern. [1] Although epidemiologic data for adults convincingly associates sugar consumption with type 2 diabetes, very little comparable information is available for children.

One would expect an effect due to beverage consumption would be greatest for those living in desert conditions. Kuwait, the target population for this analysis is a desert environment with an average temperature of 27.1ºC (80.8ºF) and a yearly average rainfall of 113.9 mm. [2] Kuwait has one of the world's highest percentile of obese adults.[3] [4] In 2005, a survey showed 75.5% of Kuwaiti adults to be overweight and 42.1% obese.[5] These percentages were high but what the more alarming observation is the rapid increase to 80.4% overweight and 47% obesity for both sexes of by 2010.[6] Adolescents were not an exception 10-14 years old Kuwaiti children had an overall prevalence of 30.7% overweight and 14.6% obese.[7] Obesity is strongly associated with heart diseases, [8] and diabetes. [9, 10] Currently, Kuwait has one of the world's highest age adjusted diabetes comparative prevalence rates (20.0%)[11], placing the country at highest risk even in Middle East and North Africa (MENA) countries.[12]

In 2015, the World Health Organization (WHO) made several recommendations to reduce sugar intake, mainly concerning the role it plays in obesity and dental caries.[13] Several studies were examining the relationship between SSB and obesity.[14, 15] Soft drinks in particular have been under scrutiny as they represent the number one source of added sugar in the diet, accounting for around 36% of the total added sugar consumed.[16] The consumption of these beverages is usually found to accompany fast foods and

certain high-calorie, low-nutritional value food items such as pizza, chips, French fries and hamburgers. [17] This is potentially due to the fact that these added sugar drinks may promote excess food intake, due to their high glycemic index. [18] Also, due to the excessive amount of sugar and unnecessary extra calories, these drinks have also been found to be associated with several health problems. [19]

Kuwait is one of the world's richest countries. Since the wealth started with the discovery of oil in the1960s, there has been a surge of nutrition related health issues in Kuwait.[20, 21] The dietary habits of Kuwaitis have undergone major changes as a result of their lifestyle transformation, particularly after the Gulf War in 1990.[22] *Honkala et al,* showed that 13 year-old Kuwaiti adolescents have a higher proportion of soft drink consumption compared to adolescents in 34 countries participating in their study.[23] Approximately 75% of the Kuwaiti adolescents in his study drank soft drinks every day. [23] To our knowledge there are no longitudinal studies conducted to test the impact of beverage consumption on children and the development of obesity in MENA.

With such an extremely alarming percentages of obesity as well as high soft drink consumption, we aimed to conduct a longitudinal observational cohort study in the MENA region to investigate the relationship between and the incidence of obesity among Kuwaiti children over a period of 2 years. The objective of our study is to investigate the role of the three most commonly consumed beverages, (soda, milk and juice) on the development of obesity in children at this critical age of life.

MATERIAL AND METHODS

Sample and study design:

Study data were obtained from Kuwaiti children enrolled in Kuwait public schools. All participants are Kuwaiti nationals and only 4th and 5th grade Kuwaiti students were included in the study. All participants were equally representing the six Kuwait governorates. All children provided a signed parent/guardian written informed consent in Arabic, and adolescent assent obtained the day of the school visit. The first school visit (baseline) occurred in 2012, and there was 8,317 students participated. In 2014, a total of 6,316 adolescent from the original sample participated in the follow-up visit; longitudinal data was collected from this second group. Data from 11 subjects were excluded due to incomplete information, to end up with a total of 6,305 subjects in the sample. Both the Dasman Institute Human Ethical Review committee in Kuwait and the Forsyth Institutional Review Board approved the study. The study is a longitudinal observational prospective analysis. Further details of the study are described elsewhere [24-26].

Beverage scoring:

A questionnaire was created and administered on an iPad in Arabic and English. Children were asked to select what they usually eat and drink with each meal and as snack. The list of food items were based on responses from a pilot study conducted on 95 Kuwaiti schoolgirls prior to launching the survey. [27] The dietary preference questions included 79 food and beverage items with accompanying pictures, and food selection options were modified to reflect the regularly consumed foods in Kuwait. Interviewers queried the children on the food items they usually ate for breakfast, lunch, dinner and snacks. Following the questions on food preferences, questions on portion were

presented, with pictures provided to assess the difference between portion sizes: for example, one can, two cans, and three or more cans of soda with each meal. At the end of food selection, subjects were asked if they preferred diet or regular soda and if they drink flavored or unflavored milk. The total for each beverage from breakfast, lunch, dinner and snacks were added to give a total number of servings. At the end we had total servings per day for each of the 3 commonly used beverages; soda, juice and milk. Coffee and tea were excluded due to a very small number of subjects consuming these two beverages. The servings for each of the three beverages (soda, juice and milk) were changed to be in serving per day categories (0, 1-2 and 3 or more) servings per day. Those who reported 0 servings a day were considered to have no consumption for the beverage being considered. Children who reported 0 serving a beverage are considered non-consumers. Children that consumed 1-2 servings per day are considered moderate consumers. Consumption of three or more servings is defined as high beverage consumers.

Obesity measurements:

During the two visits both weight and height were measured. Weight in kilograms, height in centimeters and age were used to identify the body mass index for each participant and categorize them as obese or not using the World Health Organization (WHO) definition of obesity using BMI Z-scores obesity cut-off. [28] At each visit, participants were categorized as either obese or not. If the participant had a Z-score greater than 2 SD they are considered obese. Children were either obese or non-obese at baseline and at follow-up. Based on these two visits participants were placed in 4 groups shown in Table 1.

Data management and statistical analysis:

The four groups created for obesity status at both visits were used to identify the children who developed obesity during the study period. The children who were non obese at baseline and became obese at follow-up were identified as the "became obese group" (Group 1). If the adolescent was in this (Group 1), it means the adolescent is in the interest group and he/she developed obesity during the two years period. Participants who were non-obese at baseline and remain non-obese at follow-up were defined as "remain non-obese group" (Group 2). Children who were obese at baseline and remain obese at follow-up were defined as "remain obese group" (Group 3). Finally, participants who were obese at baseline and became non-obese at follow up were defined as "became non obese group" (Group 4). To analyze the effect of age, children were divided into two groups around the median age of the participants at the baseline visit. Subjects were in the younger age group if the age was <9.9 and older age group if they were ≥9.9 years old. The two age groups were used for the descriptive Table 2. Fitness was measured by the Queens College step test [29] as the increase in heart rate (beasts/minute) following a standard exercise. Chi-square analysis was used to determine the significance level between the children who developed obesity and the other groups combined. Comparisons of binary group differences in sex, age and fitness, are presented and consumption percentages of different levels of soda, juice and milk consumption are indicated.

To examine the association between the three beverages consumed and developing obesity within the two years study period, initially, univariate logistic

regression was performed. The univariate logistic regression was used to report the crude association between each beverage and the odds of developing obesity. To identify confounders the followings variables were tested separately in the logistic regression based on the clinical relevance and possibility of being confounders; age, gender, governorate, blood pressure, fitness level, salivary glucose and salivary HDLC (cholesterol) level. None of these variables had an odds ratio with 10% difference compared to the crude association odds ratio with any of the three beverages. Stepwise selection was performed and variables were added to the model keeping only significant variables at *P=0.05* significance level. Age, governorate and fitness were significant, but gender was not. All other variables tested were not statistically significant and therefore were not added to the fully adjusted multivariate model. Interaction terms were created for all variables and no effect modification were significant. The goodness of fit was examined for all the three beverages using Hosmer-Lemeshow test and all three had sufficient goodness of fit. The final model was used to report the fully adjusted model used in Table 3. The children who developed obesity "became obese" (Group 1) were the group of interest and were tested against all the other 3 groups combined, Table 3 demonstrates the crude association and the fully adjusted model. Then (Group 1) was tested against each group separately, first against the children who "remain non-obese" (Group 2) alone. Followed by the "remain non-obese" (Group 3) alone, and finally against "the remain obese" (Group 4) alone. To test for the trend between the categories for each beverage in both crude and adjusted models, we used the categorical beverage variable codded as continuous variable. If the P value was <0.05 then we have a significant trend between the categories.

Two supplementary descriptive tables were added to describe the participants by beverage consumption and by baseline obesity status. Another supplementary analysis was added to evaluate the role of the three beverages on the remission of obesity including only group 3 and 4 and compare it to the incidence of obesity comparing group 1 to group 2. Also, Additionally, a cross sectional analysis on the baseline data was conducted using multivariate logistic regression adjusting for age, sex, governorate and fitness to compare cross sectional and longitudinal analysis.

RESULTS

There are 6,305 children included in this analysis, at baseline 4,171 (66.1%) were non obese and 2,134 (33.9%) were obese. Three hundred and seventy eight (6%) of the study population developed obesity between the baseline (2012) and the follow-up visit (2014) under group 1. Three thousands seven hundred and ninety three (60.2%) were non obese at baseline and remain non obese at the follow-up to be in group 2. The third group had 1,827 (28.9%) who were obese at baseline and remained obese at follow up. While group 4 had 307 (4.9%) categorized obese at baseline and became non obese at the follow-up. Table 1 and Figure 1, illustrate the percentage of the population that that changed or remained the same in obesity status between baseline and the follow up visit.

Table 2 illustrates the characteristics of participants who became obese (Group 1) and those who did not (Groups 2, 3 & 4). Considering sex, boys had a higher tendency to develop obesity than girls ($p < 0.001$). Considering age, younger children were significantly more likely to become obese ($p < 0.001$). Fitness did not differ significantly between those who developed obesity and those who did not ($p = 0.143$). None of the comparisons at consumption levels of moderate consumption were significantly different between those who became obese and those who did not. Children who developed obesity during the study period reported highest percentage of individuals that high consumption of soda (12.43% v 7.4%, $p = 0.001$) or milk (5.6% v 3.3%, $p = 0.057$). Children consuming juice did not differ significantly in the percentage becoming obese (17.2% v 15.7%, $p=0.116$). Supplementary Table S1 and Table S2, shows further description for the study participants by the beverage consumption and by obesity status at baseline, respectively.

The association of the different beverages and the odds of becoming obese (Table 3) demonstrates that participants who reported drinking high soda consumption had 1.68 times the odds of becoming obese compared to who reported no soda drinking ($p = 0.004$). Subjects who reported high consumption of milk had odds of 1.78 times that of becoming obese compared to those who reported no milk drinking ($p = 0.018$). Adjustment for potential confounders did not alter this association between obesity and soda consumption. Milk had similar effect, as those who reported high consumption of milk had 1.77 times the odds of becoming obese compared to who reported no milk drinking at all. Consumption of juice (OR=1.11) did not significantly affect the percentage of children who became obese ($p = 0.494$).

A comparison of children who became obese (Group 1) with each of the other three groups is shown in Table 4. In this analysis, we found children who reported high soda consumption showed significantly higher odds of being obese than those who reported no soda drinking for every group comparison. The highest odds of becoming obese were apparent when we compared the subjects who became obese with the children who were obese at baseline and became non obese at follow-up with (OR = 2.42, $p = 0.005$). Milk was only significant when children who became obese (Group 1) were compared to children who were non obese at both visits (OR= 1.87, $p = 0.013$). Consumption of milk was not significantly associated with any of the other groups tasted. Consumption of juice was not significantly associated with any group. None of the tests in Table 3 and Table 4, showed any significant trend to be reported.

Figure 2, summarizes the association between high consumption of any of the three beverages and the odds of being obese comparing group 1, to all and each of the

other 3 groups. Figure S1; summarize the mean for the BMI change from baseline to follow-up for each group by beverage consumption.

Supplementary Table S3 is showing an analysis for the study participants to compare incidences of obesity and remissions of obesity. Children in group 1 are the children who became obese in the two years study period (incidences), in comparison to children in group 2 (remain non obese). For the remission, children in group 4 are the children who became non obese (remission) compared to children in group 3 (remain obese). None of the three beverages had significant association with remission of obesity.

In the supplementary analysis studying the association between the beverages and being obese at baseline only, we found no significant association between any of the beverages consumed and being obese. Supplementary Table S4 shows the association between being obese at baseline and the consumption of the three beverages.

DISCUSSION

Our findings suggest that soda or milk but not juice consumption in Kuwaiti children is strongly associated with children who became obese (Table 3). It is of interest to note that only high beverage consumption was associated with an effect on obesity. That is to say, individuals may consume 1-2 beverages per day without effect on their becoming obese. This association was only evident when we studied the longitudinal data, not when we investigated the cross-sectional data (Table S4).

Some of the effects described in this analysis are likely associated with the climate of Kuwait. The majority of longitudinal observational studies have been conducted in North America [14, 30-38] and European countries [39-42]. One would expect that values obtained in Kuwait, a desert country, would result in relatively higher beverage consumption levels. We were unable to find any published observational studies investigating beverages association with development of obesity or weight gain in Kuwait. Also, we believe this is the first time that this association has been investigated in one of the MENA countries that have a high prevalence of both sugary drink consumption [23] and obesity [6]. The majority of studies investigating SSB and weight gain in children and adolescents, shows positive association between SSB and weight gain.[15] SSB include wide spectrum of beverages as; sugar added soft drinks/sodas, energy drinks, flavored juice beverages, sports drinks, coffee and tea with added caloric sweeteners, and electrolyte replacement drinks. [43] Some of the studies investigated these beverages combined as one group, [14, 33, 35, 39] and some separated these beverages investigating each beverage separately accounting for different source of sugar [30, 37, 38, 40]. We find segregating the beverages beneficial as we found juice not to be associated with

developing obesity, but soda and milk were. In other studies that segregated different SSB, soda was constantly associated with weight gain more than the other beverages investigated. [37, 42] *Striegel-Moore et al,* [37] found that high soda consumption was the strongest predictive of weight gain compared to all beverages including; diet and regular soda, milk, coffee/tea fruit juice and fruit-flavored drinks. *Viner et al*,[42] also showed significant weight gain in children who reported high consumption of soda. We had the same finding as children who had high consumption of soda, demonstrated higher odds of becoming obese. Soda is a great source of phosphate in diet, and high phosphate or phosphorus intake association to obesity was observed in other epidemiological studies[44, 45] but the mechanism is still unclear.[46] In our research group, *Hartman et al,* [47] found on a cross sectional investigation on 77 children, that salivary phosphate was significantly elevated in obese children compared to normal weight children. Giving our results and the last paper observation, it raises the hypothesis, that phosphorus combination with sugar in soda may have contribution in the strong association between soda and developing obesity. This hypothesis may need further investigation.

High consumption of milk in the other hand, provided significantly higher odds to become obese but only when compared to children who were non obese and remain non obese (Group 2). Our finding about milk is opposite to findings by some longitudinal studies on the relationship between milk and weight. [48-50] Also, some studies did not find any association between milk and changes in weight.[36, 51] On the other hand, our findings were consistent with the findings by *Berkey et al,* [52] as they found in a longitudinal study on 12,829 US adolescents that reported drinking 3 or more servings of milk associated with weight gain. In their findings they also found that the weight gain is attributed to the

added calories from the milk, as the association attenuated when they adjusted for the total energy intake. In our study we were unable to adjust for the total energy intake, to identify if the association between milk and weight gain will remain the same or it may disappear. Similar to the suggested contribution of phosphorus in the soda, we also, may claim here the hypothesis, that the fat content in milk in combination to sugar added may attribute to the association between weight gain and obesity. Strong recommendation to limit sugar intake for adults and children were published by WHO[13] in 2015 due to the widely documented association with obesity and dental caries. We find it important to investigate some other ingredients added in some beverages that make some more constantly associated with obesity. Phosphorus in soda beverages, and fat in flavored or unflavored milk drinks are two good examples of some of these additives.

Considering that juice consumption is described to be associated with obesity by many [53] it was a surprise that compared to soda and milk, fruit juice was not found to have a significant effect on obesity. However, our findings about fruit juice are in agreement with other studies investigating fruit juice and obesity. [54]

As the vast majority of studies concerning dietary intake, are usually self-reported; therefore, they are subject to reporter accuracy, bias and recall especially in children and adolescents. [55] Our study is no exception, but we believe using the pictures for both selecting the beverages and selecting the portion size may help in improving the accuracy. As bottles, cans and cups come in different sizes the servings computed in the survey are utilized as a proxy for consumption pattern expected.

The questions about beverage consumption did not discriminate between regular soda or diet, but in a follow up question at the end of the survey, children were asked if

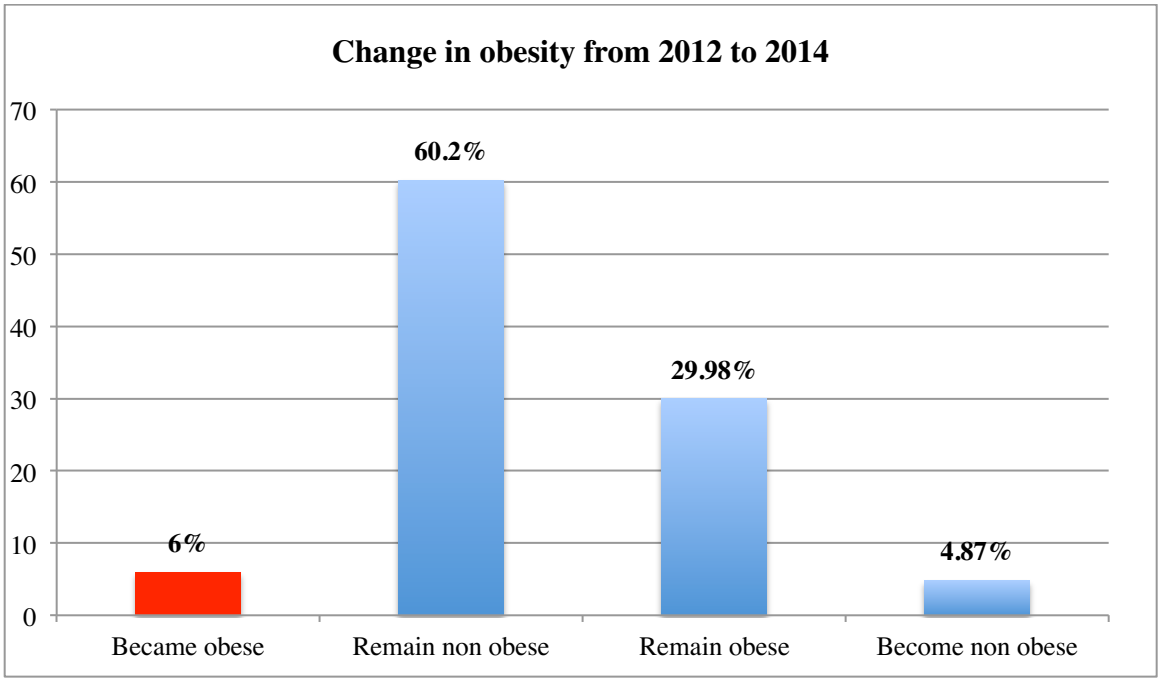
they chose regular or diet soda and only 283 children (4.5%) reported drinking diet soda. Since the percentage was too small, we decided to keep these 283 children in the sample. Questions regarding milk, did not inquire about type of milk consumed (whole, low fat, skim milk, flavored or unflavored). But at the end of the survey children were asked if they drink flavored milk (chocolate, strawberry etc.) and 3,152 (49.9%) children responded yes to this question. Therefore, it is important to consider that milk category definitely contain flavored (with added sugar) milk.

The extremely high percentage of beverage consumption and the extremely high prevalence of obesity were alarming. It is important to emphasize the role of beverages on the obesity crisis in Kuwait. National public health effort should be encouraged to control the crisis and effort to limit consumption should be directed to younger children as we noted that they have significantly more tendency to become obese.

CONCLUSION

We found significant association between high consumption (3 or more servings) of soda or of milk but not fruit juice in developing obesity of Kuwaiti children. The odds ratio for association with obesity was associated with increased consumption of soda (OR=1.68) and milk (OR= 1.77). High and moderate juice consumption was not associated with obesity. Consumption of moderate amounts of these beverages (1-2 drinks/day) was not associated with significant increased obesity.

TABLES & FIGURES



Change in obesity status from 2012 to 2014			N (%) 6,305
Non obese at baseline 4,171 (66.1%)	→ Obese 2014 (become obese)	Group 1*	378 (6%)
	→ Non obese 2014 (remain non obese)	Group 2	3,793 (60.2%)
Obese at baseline 2,134 (33.9%)	→ Obese 2014 (remain obese)	Group 3	1,827 (28.9%)
	→ Non obese 2014 (become non obese)	Group 4	307 (4.9%)

Table and figure 1. Change in obesity status from 2012 to 2014. Children in group 1 are our interest group, these are children who developed obesity during the 2 years study period. All the other groups are compared to the interest group.
*Interest group

Variable	Those who became obese (Group 1, n= 378, 6%)$^{\Delta}$	Those who did not become obese (Group 2,3 and 4, n=5927, 94%)$^{\Delta}$	P value
Gender			
Female 3,958 (62.8%)	229 60.6 %	3729 62.9%	<0.001*
Male 2,347 (37.2%)	149 39.4%	2198 37.1%	
Age $^{\alpha}$			
Younger (≤9.9 years) 3,157 (50.1%)	217 57.4%	872 47.7%	<0.001*
Older (>9.9 years) 3,148 (49.9)	161 42.6%	955 52.3%	
Fitness $^{\beta}$			
Low fitness (≥23.5 bpm) 3,149 (49.94%)	175 46.3%	2,974 50.18%	0.143
High fitness (<23.5 bpm) 3,156 (50.06)	203 53.7%	2953 49.82%	
Soda Consumption			
No soda 2,170 (34.4%)	130 34.39%	2040 34.4%	
Moderate consumption 3,649 (57.9%)	201 53.17%	3448 58.2%	0.001*
High consumption 486 (7.7%)	47 12.43%	439 7.4%	
Juice Consumption			
No juice 2,670 (42.3%)	158 41.8%	2512 42.4%	
Moderate consumption 2,640 (41.9%)	155 41.0%	2485 41.9%	0.116
High consumption 995 (15.8%)	65 17.2%	930 15.7%	
Milk Consumption			
No Milk 2,766 (43.9%)	157 41.5%	2609 44.0%	
Moderate consumption 3,322 (52.7%)	200 52.9%	3122 52.7%	0.057
High consumption 217 (3.4%)	21 5.6%	196 3.3%	

Table 2. Characteristics of Kuwaiti children by obesity change status from 2012 to 2014, by chi square analysis.

P values listed are computed for the 3+ serving group for soda, juice and milk consumption.

α=Age divided to younger and older age groups around the median age at baseline (9.9 years).

β=Fitness divided to low and high fitness groups around the median fitness level at baseline (23.5bpm).

Δ= Obesity as defined by the WHO obesity Z-score.

(bpm = beats/minute)

* Significantly associated at 0.05 level using Chi-square test.

N= 8317	Crude			Adjusted (Model 1) α			Adjusted (Model 2) β		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Soda	Trend P value = 0.502			Trend P value = 0.356			Trend P value = 0.337		
No soda	-	-	-	-	-	-	-	-	-
Only with meals	1.07	0.97 - 1.18	0.152	1.07	0.97 - 1.18	0.149	.92	0.75 - 1.14	0.455
With and between meals	1.02	0.90 - 1.16	0.716	1.04	0.92 - 1.19	0.517	.89	0.70 - 1.12	0.321
Juice	Trend P value = 0.668			Trend P value = 0.610			Trend P value = 0.532		
No Juice	-	-	-	-	-	-	-	-	-
Only with meals	0.96	0.85 - 1.08	0.503	0.96	0.85 - 1.08	0.465	0. 95	0.78 - 1.14	0.562
With and between meals	0.98	0.89 - 1.08	0.676	0.98	0.89 - 1.08	0.619	0. 96	0.78 - 1.19	0.717
Milk	Trend P value = 0.025*			Trend P value =0.029*			Trend P value = 0.139		
No consumption	-	-	-	-	-	-	-	-	-
Only drink Flavored milk	0.99	0.87 - 1.13	0.897	0.98	0.86 - 1.11	0.732	0.98	0.86 - 1.11	0.731
Only plain milk	0.90	0.79 - 1.01	0.076	0.89	0.79 - 1.01	0.074	0.69	0.52 – 0.91	0.010*
Both plain and flavored milk	0.89	0.79 - 1.00	0.047	0.88	0.78 - 1.00	0.043*	0.70	0.52 – 0.93	0.013*

Table 4. The logistic regression models for the associations between beverage consumption patterns and the severity of dental caries.

α Model 1: adjusted for sex, age, governorates (geographic location), obesity and gingival redness.

β Model 2: adjusted for sex, age, governorates (geographic location), obesity, gingival redness and consumption amount.

Test of trend using the categorical variable codded as continuous

OR=odds ratio, 95% CI=95% confidence interval.

* Significantly associated at 0.05 le

	Crude			Adjusted α		
	OR	95% CI	P value	OR	95% CI	P value
Soda						
a) Only with meals N=6841	Trend P value = 0.132			Trend P value = 0.105		
No consumption	-	-	-	-	-	-
Moderate consumption	1.07	0.97 - 1.18	0.194	1.07	0.97 - 1.18	0.205
High consumption	1.12	0.91 – 1.38	0.276	1.15	0.93 - 1.43	0.188
b) With and between meals N=4302	Trend P value = 0.396			Trend P value = 0.232		
No consumption	-	-	-	-	-	-
Moderate consumption	0.99	0.87 – 1.13	0.893	1.00	0.87 – 1.15	0.988
High consumption	1.20	0.93 – 1.56	0.164	1.27	0.97 – 1.65	0.078
Juice						
a) Only with meals N=5117	Trend P value = 0.513			Trend P value = 0.439		
No consumption	-	-	-	-	-	-
Moderate consumption	0.96	0.85 – 1.08	0.517	0.96	.85 – 1.08	0.473
High consumption	0.95	.64 – 1.41	0.816	0.93	.62 – 1.38	0.718
b) With and between meals N=6716	Trend P value = 0.771			Trend P value = 0.720		
No consumption	-	-	-	-	-	-
Moderate consumption	0.97	0.87 – 1.09	0.647	0.97	0.87 – 1.08	0.574
High consumption	0.99	0.87 – 1.13	0.862	0.99	0.86 – 1.13	0.830

Table 5. The association between severe dental caries and the amount and pattern combined including only subset of children who drink the beverage (soda or juice) with meals only and another subset of children who drink it with and between meals.
α adjusted for sex, age, governorates (geographic location), obesity and gingival redness.
OR=odds ratio, 95% CI=95% confidence interval.

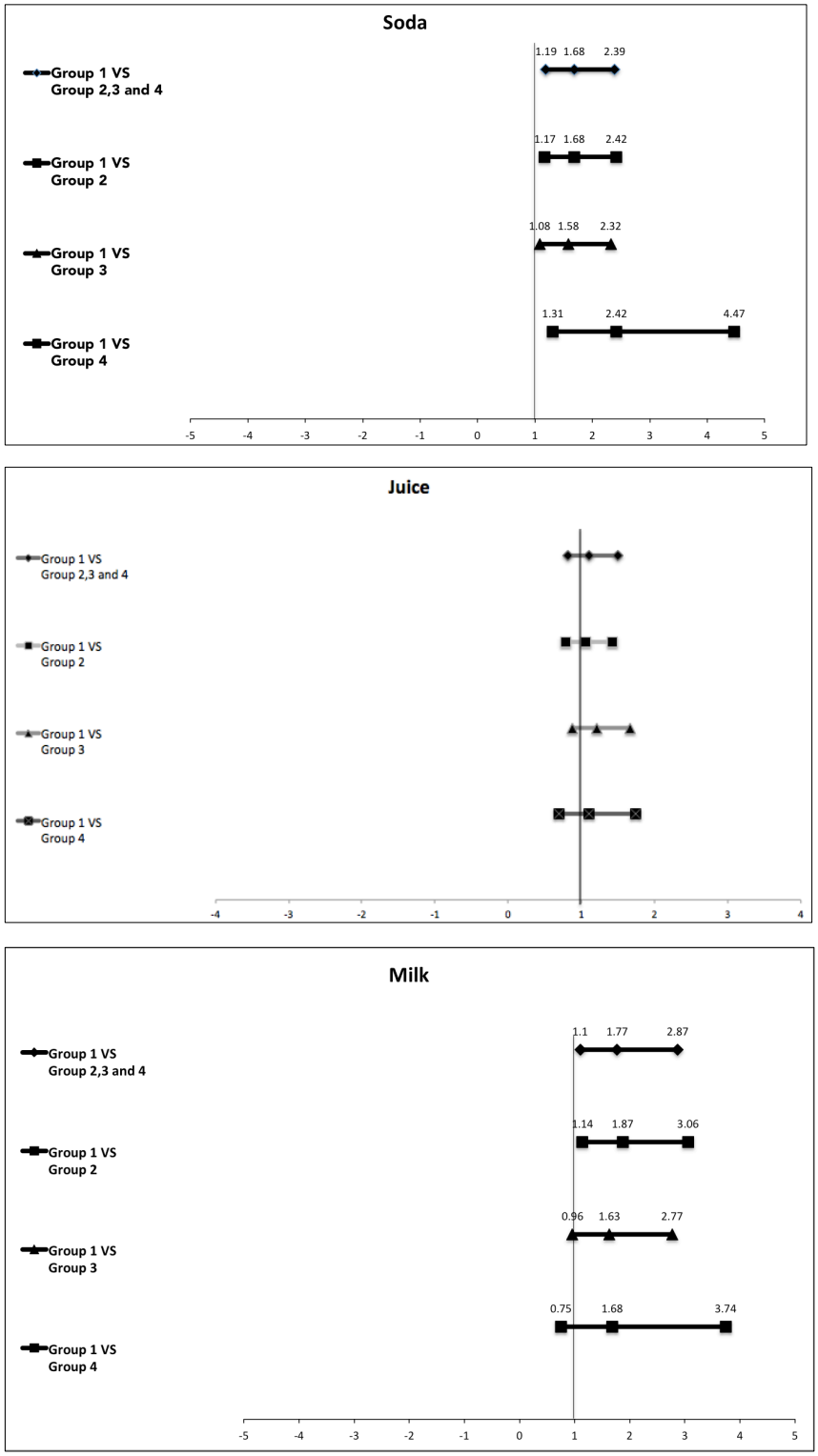


Figure 2. The odd ratios and 95% confidence intervals showing the association between high soda, juice and milk consumption comparing children who developed obesity and all the other 3 groups.

-If the bar passes through the solid vertical line it mean there is no significant association

Group 1: Became obese Group 2: Remain non obese

Group 3: Remain obese Group 4: Became non obese

SUPPLMENTARY TABLES & FIGURES

Variable	Soda			Juice			Milk		
	No soda 2,170 (%)	Drink soda 4,135 (%)	P-value	No juice 2670 (%)	Drink Juice 3635 (%)	P-value	No milk 2,766 (%)	Drink milk 3,539 (%)	P-value
Sex									
Female 3,958 (62.8%)	1359 (62.6%)	2599 (62.8%)	0.859	1621 (60.7%)	2337 (64.3%)	0.004*	1751 (63.3%)	2207 (62.3%)	0.443
Male 2,347 (37.2%)	811 (37.4%)	1536 (37.2%)		1049 (39.3%)	1298 (35.7%)		1015 (36.7%)	1332 (37.6%)	
Age [α]									
Younger 3,157 (50.1%)	1,088 (50.1%)	2,069 (50.1%)	0.939	1,364 (51.1%)	1,793 (49.3%)	0.167	1,386 (50.1%)	1,771 (50.0%)	0.958
Older 3148 (49.9%)	1,082 (49.9%)	2,066 (49.1%)		1,306 (48.9%)	1,842 (50.7%)		1,380 (49.9%)	1768 (50.0%)	
Fitness [β]									
Low fit 3149 (50%)	1,072 (49.4%)	2,077 (50.2%)	0.532	1,338 (50.1%)	1,811 (49.8%)	0.819	1,366 (49.4%)	1,783 (50.4%)	0.432
High fit 3156 (50%)	1,098 (50.6%)	2,058 (49.8%)		1,332 (49.9%)	1,824 (50.2%)		1,400 (50.6%)	1,756 (49.6%)	
Obesity [Δ]									
Non obese 4,171 (66.1%)	1,437 (66.2%)	2,734 (66.1%)	0.935	1,726 (64.6%)	2,445 (67.3%)	0.030*	1,853 (67.0%)	2,318 (65.5%)	0.214
Obese 2,134 (33.9%)	733 (33.8%)	1,401 (33.9%)		944 (35.4%)	1,190 (32.7%)		913 (33.0%)	1,221 (43.5%)	
Governorates									
Al Ahmadi 1301 (20.6%)	479 (22.1%)	822 (19.9%)	0.001*	494 (18.5%)	807 (22.2%)	<.001*	570 (20.6%)	731 (20.7%)	0.128
Al Farwaniyah 960 (15.2%)	311 (14.3%)	649 (15.7%)		459 (17.2%)	501 (13.8%)		453 (16.4%)	507 (14.3%)	
Hawali 601 (9.5%)	209 (9.6%)	392 (9.5%)		245 (9.2%)	356 (9.8%)		258 (9.3%)	343 (9.7%)	
Al Jahra'a 1454 (23.1%)	481 (22.2%)	973 (23.5)%		632 (23.7%)	822 (22.6%)		647 (23.4%)	807 (22.8%)	
Al Asimah 1547 (24.5%)	570 (26.3%)	977 (23.6%)		630 (23.6%)	917 (25.2%)		641 (23.2%)	906 (25.6%)	
Mubarak Alkabeer 442 (7.0%)	120 (5.5%)	322 (7.8%)		210 (7.9%)	232 (6.9%)		197 (7.1%)	245 (6.9%)	

Table S1. Characteristics of participants by beverage consumption

* Significantly associated at 0.05 level using Chi-square test.

Variable	Non obese 4,171 (%)	Obese 2,134 (%)	P value
Sex			
Female 3958 (62.8%)	2,744 (65.8%)	1,214 (56.9%)	<0.001
Male 2347 (37.2%)	1,427 (34.2%)	920 (43.1%)	
Age [α]			
Younger 3157 (50.1%)	2,160 (51.8%)	997 (46.7%)	<0.001
Older 3148 (49.9%)	2,011 (48.2%)	1,137 (53.3%)	
Fitness [β]			
Low fit 3149 (49.9%)	2,298 (55.1%)	851 (39.9%)	<0.001
High fit 3156 (50.1%)	1,873 (44.9%)	1,283 (60.1%)	
Governorate			
Al Ahmadi 1301 (20.6%)	953 (22.9%)	348 (16.3%)	<0.001
Al Farwaniyah 960 (15.2%)	621 (14.9%)	339 (15.9%)	
Hawali 601 (9.5%)	378 (9.1%)	223 (10.5%)	
Al Jahra'a 1454 (23.1%)	1,014 (24.3%)	440 (20.6%)	
Al Asimah 1547 (24.5%)	904 (21.7%)	643 (30.1%)	
Mubarak Alkabeer 442 (7.0%)	301 (7.2%)	141 (6.6%)	
Soda			
No soda 2170 (34.4%)	1,437 (34.45%)	733 (34.35%)	0.996
Moderate consumption 3649 (57.9%)	2,413 (57.85%)	1,236 (57.9%)	
High consumption 486 (7.7%)	321 (7.7%)	165 (7.73%)	
Juice			
No juice 2670 (42.4%)	1,726 (41.4%)	944 (44.2%)	0.075
Moderate consumption 2640 (41.9%)	1,767 (42.4%)	873 (40.9%)	
High consumption 995 (15.8%)	678 (16.3%)	317 (14.9%)	
Milk			
No milk 2766 (43.9%)	1,853 (44.4%)	913 (42.8%)	0.461
Moderate consumption 3322 (52.7%)	2,176 (52.2%)	1,146 (53.7%)	
High consumption 217 (3.4%)	142 (3.4%)	75 (3.5%)	

Table S2. The characteristics of participants by obesity (WHO z-score obesity) status at baseline.

α= Age divided around the median age at baseline (9.9 years).

β= Fitness divided to low and high fitness groups around the median fitness level at baseline (23.5bpm).

* Significantly associated at 0.05 level using Chi-square test.

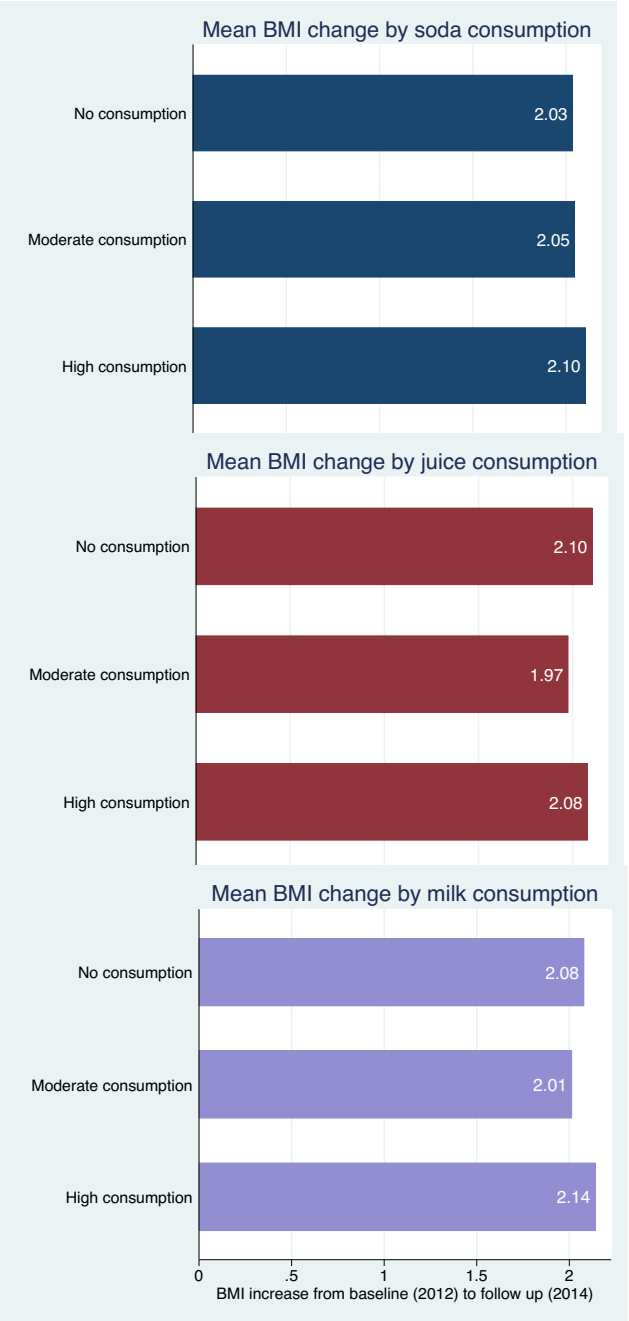
Variable	a) Incidences			b) Remission		
	Group 1 VS Group 2 (n=4171)			Group 3 VS Group 4 (n=2134)		
	OR	95% CI	P Value	OR	95% CI	P Value
Soda	Trend P value = 0.162			Trend P value = 0.353		
No soda	-	-	-	-	-	-
Moderate consumption	.91	.72 – 1.15	.432	.97	.75 - 1.3	0.848
High consumption	1.68	1.17– 2.42	0.005*	.71	.42 - 1.2	0.211
Juice	Trend P value = 0.646			Trend P value = 0.428		
No juice	-	-	-	-	-	-
Moderate consumption	.98	.78 – 1.24	0.864	1.26	.97-1.65	0.083
High consumption	1.06	.78 – 1.43	0.720	1.04	.71-1.52	0.844
Milk	Trend P value = 0.059			Trend P value = 0.485		
No milk	-	-	-	-	-	-
Moderate consumption	1.08	.87 – 1.35	0.489	.91	.71-1.17	0.455
High consumption	1.87	1.14- 3.06	0.013*	.92	.46-1.85	0.820

Table S3. The association between the four beverages and incidences or remission of obesity over the two-year study period:

a) This tests children who had incidences of obesity (group 1) with children who remained non obese (group 2).
   OR= Odd Ratio to develop obesity.
b) This tests children who had remission of obesity (group 4) with children who were obese and remained obese (Group 3).
   OR= Odd Ratio to have remission of obesity.
   * Significantly associated at 0.05 level

Variable	OR	95% CI	P Value
Soda			
No soda	-	-	-
Moderate consumption	1.00	.89 - 1.12	0.974
High consumption	.97	.78 – 1.19	0.752
Juice			
No juice	-	-	-
Moderate consumption	.92	.82 – 1.03	0.145
High consumption	.86	.73 – 1.01	0.058
Milk			
No milk	-	-	-
Moderate consumption	1.07	.96 – 1.19	0.222
High consumption	1.07	.80 – 1.44	0.642

Table S4. Logistic regression model for the relation between the obesity status at baseline and different beverages adjusting for age, sex, governorates and fitness



Soda	Mean BMI change	95% CI
No soda	2.03	1.93 -2.14
Moderate consumption	2.05	1.96 -2.13
High consumption	2.10*	1.88 -2.33

Juice	Mean BMI change	95% CI
No juice	2.10	2.01 -2.20
Moderate consumption	1.98	1.87 -2.08
High consumption	2.08	1.93 -2.23

Milk	Mean BMI change	95% CI
No milk	2.08	1.98 -2.17
Moderate consumption	2.01	1.92 -2.10
High consumption	2.14*	1.79 -2.49

Figure S2. Summary of the mean BMI change from the baseline (2012) to the follow up (2014) for each beverage.

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THE IMPACT OF BEVERAGE CONSUMPTION ON THE SEVERITY OF DENTAL CARIES IN KUWAITI CHILDREN

ABSTRACT

Background: Children of Kuwait have both a high dental caries prevalence and excessive consumption of sugar-sweetened beverages (SSB). In this analysis we investigate the association between consumption of beverages (soda, juice and milk) by children and the severity of their dental caries.

Materials and methods: Cross sectional data were collected from 8,317 fourth and fifth grade Kuwaiti children (Age=9.9). Multivariate logistic regression was used to evaluate this association between the severity of dental caries as a binary dependent variable of caries severity (low and high), and consumption (amount and pattern) of the three beverages as categorical independent variables.

Results: Soda was the most consumed beverage 6,841 (66%) reported drinking at least one serving a day, followed by juice 4,801 (61.5%), plain and flavored milk 2,430 (29.2%), plain milk only 2,222 (26.7%), and only flavored milk 1,691 (20.3%). Multivariate logistic regression adjusting for age, sex, governorates and gingival redness revealed significant association between high soda consumption and having severe dental caries (OR=1.20, P= 0.041, CI =1.01- 1.42). Moderate consumption of soda, however, did not have an effect on dental caries (OR=1.05, p=0.315, CI=0.95-1.15). Moderate milk drinking showed a protective effect from having high dental caries (OR=0.88, P= 0.007, CI =0.81- 0.97). Drinking milk including flavored milk between meals was also borderline protective from having high dental caries (OR=0.88, P= 0.043, CI =0.78-1.00).

Conclusion: High soda consumption was associated with a significantly increased severity of dental caries. This was not seen with moderate soda consumption. Increased dental caries severity was not seen with high juice consumption or high milk consumption. Moderate consumption of milk protected children from having severe dental caries. Milk consumption between meals was also protective from having severe dental caries. These data indicate that only high soda consumption contributed to dental decay an effect not seen with either juice or milk.

INTRODUCTION

Added sugar has become one of the most notorious public health topics. Beverages are the greatest source of added sugar in the average person diet particularly sugar-sweetened beverages (SSB).[1] The definition of SSB varies among researchers, but the Center for Disease Control (CDC) in 2010 defined SSB as any beverage with added caloric sweetener.[2] SSB include wide range of beverages as; soft drinks (sodas, pops), fruit drinks (punches), energy drinks, vitamin water (sport drinks), coffee or tea (with added caloric sweetener) and sweetened milk/milk alternatives.[2] The reason behind the growing concerns about SSB is the large amount of added calories with low nutritional values. [3] SSB have been linked to several health problems including weight gain [3], diabetes [4], and dental caries. [5] The consumption of these sugary beverages is particularly alarming since it is showing a rapid increase. [6]

Soft drinks are the most commonly consumed SSB.[7] Among 75 countries of different income levels, the average consumption of soft drinks leaped to 11.4 gallons per person per year in 2010, from 9.5 gallons per person per year in 1997.[8] Considering the US in 1977, soft drinks represented 2.8% of the total daily caloric intake for all age groups and increased to 7.0% in 2001.[9] SSB has now become the most consumed food item in the US.[10] The carbonated soft drink consumption of 6-17 year-olds showed a 48% increase in the periods from 1977-79 to 1994-98. Thirty-seven percent of boys and girls in 1977-78 consumed soft drinks; this percentage leaped to 56% by 1994-1998. [7] Not only was the overall percentage of consumption increasing, but the amounts consumed individually were rising as well. Teenage boys between 12-19 years showed more than a two-fold increase in consumption, from 7 ounces per day in 1977-79 to 19 ounces per day

in 1994-96. Girls showed an average consumption of 6 ounces per day in 1977-78, which increased to 12 ounces per day by 1994-1996.[11] Even during the transition years from childhood to teenage, the amounts consumed have been shown to double. [12] Six to eleven year-old girls consume about 7.2 ounces per day, while boys consume 9.6 ounces.[12] Teenagers between 12-19 years of age consume 21.3 and 13.8 ounces per day for boys and girls, respectively. [12] Even more alarming is that by the end of the 1990s, children at 8 years of age were regularly consuming more carbonated soft drinks than both milk and fruit juice combined.[13] Despite the high nutritional values of milk and diary products, there is a distinct reduction in the consumption.[14] The decline in consumption has been linked to an increase incidence of some health problems as well as dental caries. [14]

In Kuwait after the Gulf War in 1990, lifestyle and dietary habits experienced major changes.[15] For primary and secondary Kuwaiti school children, 35% reported drinking a soft drink as a snack, 27.7% reported drinking it with lunch and 44.6% reported drinking it with dinner.[15] In the Health Behavior in School-Aged Children (HBSC) collaborative study with the World Health Organization (WHO), *Honkala et al,* [16] showed that 13 year-old Kuwaiti adolescents with a reported 75% drinking soft drinks every day consumed the highest proportion of soft drinks of all 34 countries who participated in the study. Kuwait has a desert-like climate with temperatures may reach up to 48ºC (114.8ºF) during summer months. [17] Therefore, it is realistic to expect higher beverage consumption for the population under study.

It is well understood that for the development and initiation of dental caries, cariogenic bacteria require simple carbohydrates to produce acids that initiate the caries process. [18] As SSB were found to be the leading source of the total added sugar in the

average diet[19] SSB, have had their share of the attention and were investigated for both adults [5, 20, 21] and children.[22-25] Most of these studies found a significant positive association between SSB, soft drinks in particular, and dental caries. [5, 22-30] A few studies have failed to capture such an association.[31-33]

Milk impact on dental caries was one of the first beverages to be studied. [34] Milk has shown cariostatic properties in both animals [35] and human studies. [36] Although Milk contains 4% lactose as a potential cariogenic threat, it contains several other cariostatic components. [35] Several protective products for treatment of dental caries have been commercially developed from dairy and bovine milk using its casein phosphopeptides (CPP) proteins. [37] These products have shown both caries re-mineralization and caries prevention properties. [38] To our knowledge, no data on milk consumption in Kuwait have been published.

In Kuwait, despite the availability of free medical and dental treatment for all Kuwaiti citizens, the prevalence of dental caries is high. [39] In the latest dental survey in 2006, nearly 87% of 5-year old Kuwaiti children had at least one decayed or filled primary tooth. For permanent dentition among 12 year olds, almost 78% of adolescents had at least one decayed, filled or missing tooth due to caries.[39] With an extremely high dental caries prevalence and excessive consumption of SSB, this encourages us to investigate the association between commonly consumed beverages among children including soda, juice and milk, and the severity of dental caries. We hypothesize that due to the high-added sugar of soda beverages, an association exists between high consumption of soda and dental caries may be found among the children of Kuwait.

MATERIAL AND METHODS

The data used for this study was from the Kuwait Healthy Life Study. The data were collected on 8,317 fourth and fifth grade students in Kuwaiti public schools where only Kuwaiti citizens are enrolled. Schools selected were equally distributed among the six governorates (geographic regions) in Kuwait. Prior to entry, informed consent was obtained from parents. Child assents were obtained on the day of examination. Further details about this study have been described in previous publications. [40, 41]

Dental Examination

During the visits, trained and calibrated licensed dentists performed clinical oral examinations, assisted by trained dental assistants. The dental exam was performed using a portable dental chair, an external halogen light source, and disposable dental mirrors. The number of existing primary and permanent teeth were recorded, as well as the number of teeth with fillings and unfilled carious teeth. No radiographic images or explorers were used during the examinations. Decayed and filled teeth were scored for every subject examined.

Beverage consumption

An electronic questionnaire was created by the teams at Forsyth and Dasman that was administered on an iPad (Apple® Cupertino, California) in both English and Arabic. Children were asked to choose what they typically eat with each meal. The questionnaire food items were based on responses from a previous pilot study conducted prior to launching the study on 95 Kuwaiti schoolgirls.[42] Approximately 80 food and beverage items with accompanying pictures were included in the questionnaire. The food options included were tailored to reflect the commonly consumed foods in Kuwait. Trained

interviewers queried the participants on the food and beverage items they usually ate for each meal and as a snack. Following, questions about portions were asked using pictures representing variety of portion sizes. Four commonly consumed beverages were included in the survey: milk, juice, tea, and soda. At the end of the questionnaire, an additional item asked about diet or regular soda. Only 371 (4.5%) of children reported drinking diet soda, and due to this small percentage, all soda entries were coded as regular soda. A question addressing flavored milk consumption revealed that a high proportion of children drink flavored milk (49.5%). Therefore all milk amount selections are expected to include large amounts of flavored milk. The total number of servings from breakfast, lunch, dinner and snacks were computed for each type of beverage. We also totaled the servings per day for each of the three most commonly consumed beverages: soda, juice, and milk. We decided to exclude tea because of the relatively small number 1,860 (22.4%) of children reporting drinking tea. The beverage data was transformed to a categorical format with three consumption amount categories; no consumption if they reported zero consumption, moderate consumption for 1-2 servings and high consumption for three or more servings per day. To further investigate the effect of drinking beverages, only with meals or with and between meals on dental caries, a new categorical variable was created for soda and juice (consumption pattern). Children who reported no consumption scored 0, children who reported drinking beverages only with meals scored 1, and children who reported consumption both with and between meals scored 2. Milk was categorized by consumption of flavored milk. Milk consumption was labeled 0 for those who did not drink plain or flavored milk, 1 for only flavored milk, 2 for both plain and flavored milk and 3 for only plain milk.

Data Analysis

Most of the participants with mixed dentition and missing teeth were not counted due to clinical examination without radiographs. The percentage of carious and filled teeth was calculated as follows: [number of primary and permanent teeth with unfilled caries + number of primary and permanent filled teeth / number of primary and permanent teeth in the mouth] X 100. Subjects were divided into two groups based on the severity of dental caries; low dental caries if the percentage of caries and fillings was equal to or lower than the median for caries percentage, and high dental caries if it was above the median (8.7%). For the binary analysis of the age effect, age for participating subjects was divided into older and younger adolescent groups around the median age (9.97 years). Obese and non-obese participants were classified according to the WHO obesity growth reference data for school-aged and adolescents. [43] A subject with a Z-score greater than two standard deviations was classified as obese. Gingival redness was also divided into two groups: low and high based on the median for areas with gingival redness percentage (78.2%). This variable was measured by identifying areas that are abnormally red and divided by the number of all measured areas. For this analysis, we consider the gingival redness variable to be a proxy for oral hygiene status, as children who have a high gingival redness percentage may be likely to have poor oral hygiene. [44]

To test how consumption patterns combined with the amount consumed related to dental caries severity, we repeated the association test two more times. First, we excluded subjects who reported consumption of beverages with and between meals to limit the analysis to those who reported moderate and high consumption with meals only and compared them to those who reported no consumption. Second, we excluded subjects

who reported consumption only with meals to limit the analysis to those who reported moderate and high consumption of beverages with and between meals and compared them to those who reported no consumption.

Statistical Analysis

The participating children were described using the two dental caries severity groups: low and high. Gender, age, obesity, gingival redness, soda, juice, and milk consumption were used as covariates and a two-tailed chi-square association test was performed. A p-value of <0.05 was considered statistically significant.

For the association between severity of dental caries and the three beverages, we used logistic regression. Model building was based on the clinical relevance of the covariates and potential confounders. Initially, the crude association between each beverage consumption category and dental caries was tested. Then, each variable was added gradually to the model and only variables with a significant p-value (<0.05) were kept in the model. Several variables were added but only age, governorates (geographic location), and gingival redness were significant. Obesity and sex were not statistically significant but both remained in the models. Obesity was kept in the model due to its known association with dental caries [41] and with beverage consumption. [45] Sex was kept as a precision variable. All possible interaction terms were created and tested, but no effect modification was evident. Therefore, no interaction terms were added to the final model. Even after adding several interaction terms, the model had significant specification error and we used the "boxtid" Stata command that transforms the variables to find the best model fit. The variable gingival redness was significantly correlated with dental caries, and the variable was transformed to the 2.65 power. Finally, a Hosmer-

Lemeshow test was performed for the fully adjusted model for each beverage to examine the goodness of fit.

To examine the association between dental caries and consumption amount (Step 1, Figure 1), logistic regression was used and included the consumption variables created. Both the crude and adjusted odds ratios were reported. Two models were created to report the adjusted odd ratios, model 1, included the adjustment variables (age, sex, governorate, obesity and gingival redness). Model 2 included all adjustment variables in model 1 and the consumption pattern variable created.

For the association between dental caries severity and consumption pattern (Step 2, Figure1), both crude and adjusted models were reported. The same variables included for adjustment in the previous test between beverage consumption amount and caries severity were used for the adjusted model 1. For the adjusted model 2, all adjustment variables included in model 1 were added plus the consumption amount variable created.

Trend test between categorical beverage consumption amount and pattern was conducted for both the crude and the adjusted models. Codding the categorical beverage consumption variable as continuous variable was used for the trend test, if the P value was <0.05, then we have significant trend between the categories.

For analyzing the association between dental caries and the amount of consumption combined with the consumption pattern, two subsets of the data were analyzed (Step 3, Figure 1). The first subset included only participants who reported no consumption of the beverage, or they reported moderate or high consumption but only with meals, and excluded any subjects who reported beverage consumption between meals. In the second data set, we excluded those who reported consumption only with

meals and limited our analysis to subjects who reported no consumption or moderate and high consumption both with and between meals. The two subsets were analyzed using logistic regression and both crude and adjusted odd ratios are reported. The same variables adjusted for in model 1 in the two previous tests were used for adjustments for the adjusted model. The variables included for adjustments are age, sex, obesity, governorate and gingival redness.

RESULTS

Of the total 8,317 participating children (Table 1), 4,094 (49.2%) had a dental caries percentage lower than the median (low dental caries), and 4,223 (50.8%) had a dental caries percentage higher than the median (high dental caries). Younger children had significantly higher percentages of high dental caries (*P-Value <0.001*). Also, children with high levels of gingival redness were more likely to have high dental caries (*P-Value <0.001*). High milk consumption was the only one of the three beverages to show an initially significant association with having high dental caries (*P-Value =0.008*).

The consumption pattern was markedly different between the three beverages (Table 2). More than half the subjects (66%) drank soda; the majority (48.3%) only drank soda with meals. Fewer children reported drinking juice every day (57.8%) and the majority drank juice with and between meals (38.5%). The milk consumption pattern was collected to obtain information on flavored and plain milk. Approximately three quarters of participants reported drinking some kind of milk; 20.3% drank only flavored milk, 26.7% drank only plain milk and 29.2% drank both flavored and plain milk.

For the association between dental caries severity and the three beverages, the crude association for the amount of consumption (Table 3) shows that moderate consumption of milk has 22% lower odds of high dental caries compared to no milk consumption (*P-Value = 0.005*). After adjusting for possible confounders in model 1 (age, sex, obesity, governorates and gingival redness), children who reported moderate milk consumption still had lower odds of high dental caries compared to those who reported no milk consumption (OR=0.88 *P-Value = 0.007*). On the contrary, high soda consumption showed significantly greater odds of high dental caries. Children who

reported high soda consumption had 1.20 times the odds of having high dental caries compared to those who reported no soda consumption in the adjusted model 1 (*P-Value =0 .041*). Gingival redness in particular, was the variable that made this association significant after adjustment. After adjusting on the consumption pattern (model 2), the association between high soda consumption and severe dental caries became borderline significant (P-Value =0.057). However, there was a significant trend in the odd ratios reported in model 2 (P-Value for trend =0.054). Juice did not show any association with severity of dental caries in both the adjusted and unadjusted models.

The associations between beverage consumption patterns and the severity of dental caries are displayed in Table 4 and show a weak protective effect of consumption of both plain and flavored milk compared no consumption of milk in the crude and adjusted model 1. The crude association (*P – value = 0.047*) and the adjusted model 1 association (*P – Value = 0.043*) were both border line significant. However, after adjusting for the milk consumption amount (model 2 in Table 4), the milk protective effect on dental caries became more significant. Children who reported drinking only plain milk had lower odds to have high dental caries (OR=0.69 P-Value= 0.010). Also, the children who reported drinking both plain and flavored milk had lower odds of having high dental caries (OR=0.70 P-Value=0.013). Both the crude and adjusted model 1 had significant trend for the reported odd ratios (crude: P-Value for trend= 0.025) and (adjusted model 1: P-Value for trend= 0.029).

The effects of amount and consumption pattern combined for soda and juice are shown in Table 5. For soda, two subsets were analyzed. In the first subset, every subject who reported consumption of soda with and between meals was excluded (1,476

subjects), leaving subjects who reported no soda consumption or consumption with meals (6,841 subjects). There was no significant association between dental caries and high consumption of soda, and the odds ratio (1.15) was lower than the odds ratio that included all subjects (1.20) as reported in Table 3 adjusted model 1. In the second subset, those who reported drinking soda only with meals (4,015 subjects) were excluded to limit the analysis to children who reported no soda consumption or only consumed soda with and between meals (4,302 subjects). The association between high soda consumption and dental caries was not statistically significant *(P – Value =0.078),* but the odds ratio (1.27) was higher than the odds ratio for all subjects (1.20). Juice did not show any significant association with dental caries in both subsets, and the odds ratios for the subsets (Table 5) were the same as the odds ratio for all subjects in Table 3.

DISSCUSSION

This cross-sectional study is the first to examine the association between commonly consumed sweetened beverages and the severity of dental caries in one of the Arabian Gulf States where both consumption of sugary beverages and dental caries are prevalent. Our findings suggest a significant association between high soda consumption and the severity of dental caries. Children who reported high soda consumption had significantly higher odds of high dental caries compared to those who reported no soda consumption. Although we saw no significant association between patterns of soda consumption and dental caries, when we combine amount with consumption patterns, the odds for high dental caries increased for children who drink soda with and between meals. This subset equaled nearly half of the total subjects and this could explain why the association did not reach significance compared to the one that includes all subjects.

Participants who reported moderate milk consumption had significantly lower odds of high dental caries. Children who drank both plain and flavored milk appear to have a protective effect from severe dental caries. We may have expected to observe this only with those who drank plain milk exclusively and not with those who also drank sweetened flavored milk.

The role of sugar in dental caries has been extensively investigated. Recently, *Moynihan and Kelly* (2014)[46] conducted a thorough systematic review to assist in the formation of WHO guidelines on the intake of sugar and dental caries. Fifty-five papers were included in this systematic review, 50 studies on children and 5 on adults. All the studies on adults showed at least one positive association between sugar and dental caries, and 42 out of 50 studies on children showed at least one positive association.

Despite the wide variability of methodological approaches among the included studies in the review, as well as some of the weaknesses in the included studies, a consistent, positive association between sugar and dental caries was observed. The positive association included different age groups, countries of different development levels with varying access to fluoridation and covered the decades from the 1950s up until 2010.

As sweetened beverages are considered one of the main sources of dietary sugars, [19] the association between dental caries and SSB has received careful scrutiny. As part of the requirement for the Master of Public Health degree, *Du Yuerong* conducted a systemic review on SSBs and childhood dental caries. [47] Fourteen studies, which varied from cross-sectional to cohort, were included in the review. A positive association between SSB and caries was found in 11 studies and a null association was seen in the remaining three studies. Some of the studies were limited to only carbonated soda and did not include all SSB. For example, the study by *Heller et al,* in 2001[21] investigated sugar carbonated soda and dental caries with data from the 1988-94 Third Health and Nutrition Examination survey (NHANES III). The study failed to show an association between soda and dental caries in subjects under the age of 25. The same data was re-analyzed by *Sohn et al*, in 2005[23] limiting the analysis to primary teeth and children between 2-10 years of age. In the analysis, *Sohn et al* used clustering analysis rather than conventional analysis and found a significant association between dental caries and carbonated soda beverages.

Studies of the association between beverages and dental caries differ on how they account for sweetened beverages; some studies combine the intake of all beverages in one group defined as sugar sweetened beverages (SSB), [5, 25, 29, 30, 48] while many others

investigate soda alone.[20-24, 26, 27, 31, 32] In our study, we examined all commonly consumed beverages rather than one item.

Soda is one of the most commonly studied beverages and the vast majority of studies show a significant association with dental caries in both children[5, 23, 24, 26, 29, 49] and adults. [21, 30] We had the same positive association between severity of dental caries and consumption of soda. Furthermore, we noticed that that the association is more prominent in those who have high consumption of soda both between meals and with meals. This is similar to the findings by *Palmer et al* [50], and *Marshal et al* [27], that show that limiting sugary beverages to meals do not make them a major risk factor for caries. On the contrary, the consumption of SSB as snacks and between meals can increase caries risk. [51] The American Academy of Pediatric Dentistry (AAPD) concluded in recommendations to practicing dentists, that frequent intake as well as the prolonged surface contact between sugary snacks and beverages with teeth is a risk factor for the development of carious lesions. [52]

As noted earlier, the study by *Heller et al,* [21] only observed a positive association in an older age group rather than subjects younger than 25 years of age. They suggested that older individuals had less exposure to water fluoridation and less benefit from fluoridated toothpastes. Other findings support this suggestion. *Gibson et al,* [53] found that a positive association was not found in individuals who had better oral hygiene and used fluoridated toothpastes. *Burt* [54] concluded in a systematic review that the role of sugar in dental caries was weaker after fluoridation. Unfortunately, in our study we had no information about oral hygiene or fluoride exposure, but we had the gingival redness variable that served as a proxy for oral hygiene and we know there is no community

water fluoridation in Kuwait. The association between gingival redness and dental caries severity was significant and adding gingival redness to our analyses improved the association between dental caries severity and high soda consumption. This highlights the importance of accounting for additional important factors as oral hygiene habits.

Despite the high sugar content of juice, it was not associated with caries. Using the same subjects, *Goodson et al* [55] studied the macronutrients in their diets and showed a significant positive association between high dental caries and high dietary phosphorus intake regardless of sugar intake. He concluded that high phosphorus intake alone could stimulate the dental caries process. However, the strongest positive association was found in children who had high intakes of both sugar and phosphorus. Since soda contains both sugar and phosphorus, this suggests that soda-consuming children may be more prone to dental caries.

Studies show that plaque rich with Calcium (Ca) and Phosphorus (P) was found to be protective against dental caries.[56] *Stanton* in 1969 [57], suggested that the balance of dietary intake of Ca and P in form of Ca/P ratio is associated with dental caries. He found both the high and low Ca/P ratios to be associated with dental caries and 0.57 was the intake ratio for caries unsusceptible subjects. However, *Rugg-Gunn et al* [58], and *Marques et al* [59], refuted this association. Later, in 2013, *Lin et al* [60], after studying the association between several micro and macronutrients, found only the Ca/P ratio was associated with dental caries.

Since dental caries is a constant dynamic relationship between the demineralization and re-mineralization processes[61], several investigators suggested that dairy products rich with calcium (Ca) and phosphate (PO4), such as cheese, yogurt and

milk, may tip this dynamic process in favor of re-mineralization either by preventing, delaying or stopping the demineralization and dental caries development process. [36, 62-66] In our study, moderate milk consumption seemed to be protective for dental caries. According to *Grenby et al,* [67] milk has two advantages over the other caries protective agents; it is natural and it does not raise toxicological concerns. Laboratory studies on milk date back to the 1940s [68] and continued many years, [35, 69] showing the protective effect of milk. These were followed by epidemiological studies in different countries supporting milk's role in caries protection. In Italy, *Petti et al* [36] reported that milk was only protective to children with high sugar intake and poor oral hygiene. In the US, *Palmer et al* [50] found children who drank milk between meals had less tendency to develop new carious lesions. The protective quality of milk with dental caries is not completely well-defined due to its colloidal complex nature and wide variability in its components.[67] Some milk components such as lactose are known to be cariogenic, [70] but others have been found to have caries protective properties, such as Ca, P, proteins, casein fats [35, 37, 38 67] and high buffering capacity. [71]

Children who reported consumption of both flavored and plain milk had lower odds of severe dental caries. This finding was the opposite of our expectations since we expected lower caries protection properties in flavored milk due to the added sugar. However, studies on rats showed that milk maintained its cariostatic properties even with a cariogenic food source. [67] Also, a two year clinical trial showed a small, non-significant increase in dental caries after adding sugar and chocolate to whole milk compared to plain whole milk.[72] The question about flavored milk consumption in our survey was limited to its consumption as a snack. Therefore children who reported consumption of

both plain and flavored milk are those who drank milk products between meals. This group had lower odds of high dental caries which matches the observation by *Palmer et al* [50]. Moreover, with the increased consumption of flavored milk and growing concerns about its cariogenic potential, some experts conclude that it is does not pose a risk for teeth and have accepted its occasional consumption.[71]

This study is similar to most nutrition studies in that dietary intake was self-reported by participants. Self-reported food intake is often subject to some level of diminished accuracy as well as recall bias. This is of special concern when the subject is a child or adolescent as children knowledge about food items and ability to estimate portion size are limited.[73] However, many studies have suggested that children as old as 8-10 years of age can still provide a reliable report about their food intake.[74-76] That in our study, we used pictures to depict foods and beverages as well as serving amounts, which may help limit the impact of inaccuracy and bias.

The questionnaire did not include options to select regular or diet soda for each meal. However, one item at the end of the questionnaire asked about the overall consumption of diet soda. Fewer than 5% of participants reported consuming diet soda. Therefore, due to the small percentage of diet users we included those subjects in the soda consumers groups, expecting no impact in the overall results.

CONCLUSIONS

The results of our study confirmed our hypothesis that high consumption of soda is associated with dental caries. We found high soda consumption to be associated with high severity of dental caries. This association did not exist with high juice consumption or high milk consumption. Oral hygiene status is an important component in this association. We found moderate consumption of milk to be protective from having high dental caries. Also, the consumption of milk between meals was significantly protective from having high dental caries, even if it was the occasional use of flavored milk.

TABLES & FIGUERS

Variable	Low dental caries 4,094 (49.2%)	High dental caries 4,223 (50.8%)	P-value
Gender			
Female 5,098 (61.3%)	2,509 49.2%	2,589 50.8%	0.983
Male 3,219 (38.7%)	1,585 49.2%	1,634 50.8%	
Age[α]			
Younger (≤9.9) 4,159 (50.1%)	1,935 46.5%	2,224 53.5%	<0.001*
Older (>9.9) 4,158 (49.9)	2,159 51.9%	1,999 48.1%	
Obesity[β]			
Non-obese 5,473(65.8%)	2,722 49.7%	2,751 50.3%	0.196
Obese 2,844(34.2)	1,372 48.2%	1,472 51.8%	
Gingival redness[Δ]			
Low Gingival Redness 4,136 (49.7%)	2,270 54.9%	1,866 45.1%	<0.001*
High Gingival Redness 4,181(50.3%)	1,824 43.6%	2,357 56.4%	
Soda consumption			
No consumption 2,826 (34%)	1,418 50.2%	1,408 49.8%	0.238
Moderate consumption 4,839 (58.2%)	2,372 49.0%	2,467 51.0%	
High consumption 652 (7.8%)	304 46.6%	348 53.4%	
Juice consumption			
No consumption 3,516(42.3%)	1,717 48.8%	1,799 51.2%	0.801
Moderate consumption 3,500(42.1%)	1,737 49.6%	1,763 50.4%	
High consumption 1,301 (15.6%)	640 49.2%	661 50.8%	
Milk consumption			
No consumption 3,665 (44.1%)	1,747 47.7%	1,918 52.3%	0.008*
Moderate consumption 4,359(52.4%)	2,214 50.8%	2,145 49.2%	
High consumption 293 (3.5%)	133 45.4%	160 54.6%	

Table 1. Characteristics of the participants by the dental caries severity categories.

[α] Age= divided around the median age 9.97.

[β] Defined as obsess using the BMI Z-Score, WHO growth chart.

[Δ] Gingival redness = divided around the median gingival redness 78.26%.

*Statistically significant using Chi square test at a 0.05 significance level.

N= 8317	N	%
Soda		
No Soda	2,826	34.0%
Only with meals	4,015	48.3%
With and between meals	1,476	17.7%
Juice		
No Juice	3,516	42.3%
Only with meals	1,601	19.3%
With and between meals	3,200	38.5%
Milk		
No consumption	1,974	23.8%
Only drink flavored milk	1,691	20.3%
Only plain milk	2,222	26.7%
Both plain and flavored milk	2,430	29.2%

Table 2. The number of children and percentages for different beverage consumption frequency pattern for each beverage.

N= 8317	Crude			Adjusted (Model 1) α			Adjusted (Model 2) β		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Soda	Trend P value= 0.104			Trend P value= 0.056			Trend P value= 0.054*		
No consumption	-	-	-	-	-	-	-	-	-
Moderate consumption	1.05	0.95 - 1.15	0.328	1.05	0.95 - 1.15	0.315	1.10	0.92 - 1.32	0.290
High consumption	1.15	0.97 - 1.37	0.102	1.20	1.01 -1.42	0.041*	1.26	0.99 -1.60	0.057
Juice	Trend P value= 0.682			Trend P value= 0.620			Trend P value= 0.916		
No consumption	-	-	-	-	-	-	-	-	-
Moderate consumption	0.97	0.88 – 1.06	0.506	0.96	0.88 – 1.06	0.460	0.94	0.75 – 1.18	0.587
High consumption	0.99	0.87 – 1.12	0.825	0.98	0.86 – 1.12	0.768	0.95	0.72 – 1.26	0.718
Milk	Trend P value= 0.072			Trend P value= 0.099			Trend P value= 0.056		
No consumption	-	-	-	-	-	-	-	-	-
Moderate consumption	0.88	0.81 - 0.96	0.005*	0.88	0.81 - 0.97	0.007*	0.89	0.73 – 1.08	0.250
High consumption	1.10	0.86 – 1.39	0.453	1.12	0.88 – 1.43	0.362	1.13	0.84 – 1.52	0.435

Table 3. The logistic regression models for the associations between dental caries severity and beverage consumption amount.
α Model 1: adjusted for sex, age, governorates (geographic location), obesity and gingival redness.
β Model 2: adjusted for sex, age, governorates (geographic location), obesity, gingival redness and consumption pattern.
Test of trend using the categorical variable codded as continuous
OR=odds ratio, 95% CI=95% confidence interval.
* Significantly associated at 0.05 level.

N= 8317	Crude			Adjusted (Model 1) α			Adjusted (Model 2) β		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Soda	Trend P value = 0.502			Trend P value = 0.356			Trend P value = 0.337		
No soda	-	-	-	-	-	-	-	-	-
Only with meals	1.07	0.97 - 1.18	0.152	1.07	0.97 - 1.18	0.149	.92	0.75 - 1.14	0.455
With and between meals	1.02	0.90 - 1.16	0.716	1.04	0.92 - 1.19	0.517	.89	0.70 - 1.12	0.321
Juice	Trend P value = 0.668			Trend P value = 0.610			Trend P value = 0.532		
No Juice	-	-	-	-	-	-	-	-	-
Only with meals	0.96	0.85 - 1.08	0.503	0.96	0.85 - 1.08	0.465	0. 95	0.78 - 1.14	0.562
With and between meals	0.98	0.89 - 1.08	0.676	0.98	0.89 - 1.08	0.619	0. 96	0.78 - 1.19	0.717
Milk	Trend P value = 0.025*			Trend P value =0.029*			Trend P value = 0.139		
No consumption	-	-	-	-	-	-	-	-	-
Only drink Flavored milk	0.99	0.87 - 1.13	0.897	0.98	0.86 - 1.11	0.732	0.98	0.86 - 1.11	0.731
Only plain milk	0.90	0.79 - 1.01	0.076	0.89	0.79 - 1.01	0.074	0.69	0.52 – 0.91	0.010*
Both plain and flavored milk	0.89	0.79 - 1.00	0.047	0.88	0.78 - 1.00	0.043*	0.70	0.52 – 0.93	0.013*

Table 4. The logistic regression models for the associations between beverage consumption patterns and the severity of dental caries.

α Model 1: adjusted for sex, age, governorates (geographic location), obesity and gingival redness.

β Model 2: adjusted for sex, age, governorates (geographic location), obesity, gingival redness and consumption amount.

Test of trend using the categorical variable codded as continuous

OR=odds ratio, 95% CI=95% confidence interval.

* Significantly associated at 0.05 le

	Crude			Adjusted α		
	OR	95% CI	P value	OR	95% CI	P value
Soda						
a) Only with meals N=6841	Trend P value = 0.132			Trend P value = 0.105		
No consumption	-	-	-	-	-	-
Moderate consumption	1.07	0.97 - 1.18	0.194	1.07	0.97 - 1.18	0.205
High consumption	1.12	0.91 – 1.38	0.276	1.15	0.93 - 1.43	0.188
b) With and between meals N=4302	Trend P value = 0.396			Trend P value = 0.232		
No consumption	-	-	-	-	-	-
Moderate consumption	0.99	0.87 – 1.13	0.893	1.00	0.87 – 1.15	0.988
High consumption	1.20	0.93 – 1.56	0.164	1.27	0.97 – 1.65	0.078
Juice						
a) Only with meals N=5117	Trend P value = 0.513			Trend P value = 0.439		
No consumption	-	-	-	-	-	-
Moderate consumption	0.96	0.85 – 1.08	0.517	0.96	.85 – 1.08	0.473
High consumption	0.95	.64 – 1.41	0.816	0.93	.62 – 1.38	0.718
b) With and between meals N=6716	Trend P value = 0.771			Trend P value = 0.720		
No consumption	-	-	-	-	-	-
Moderate consumption	0.97	0.87 – 1.09	0.647	0.97	0.87 – 1.08	0.574
High consumption	0.99	0.87 – 1.13	0.862	0.99	0.86 – 1.13	0.830

Table 5. The association between severe dental caries and the amount and pattern combined including only subset of children who drink the beverage (soda or juice) with meals only and another subset of children who drink it with and between meals.
α adjusted for sex, age, governorates (geographic location), obesity and gingival redness.
OR=odds ratio, 95% CI=95% confidence interval.

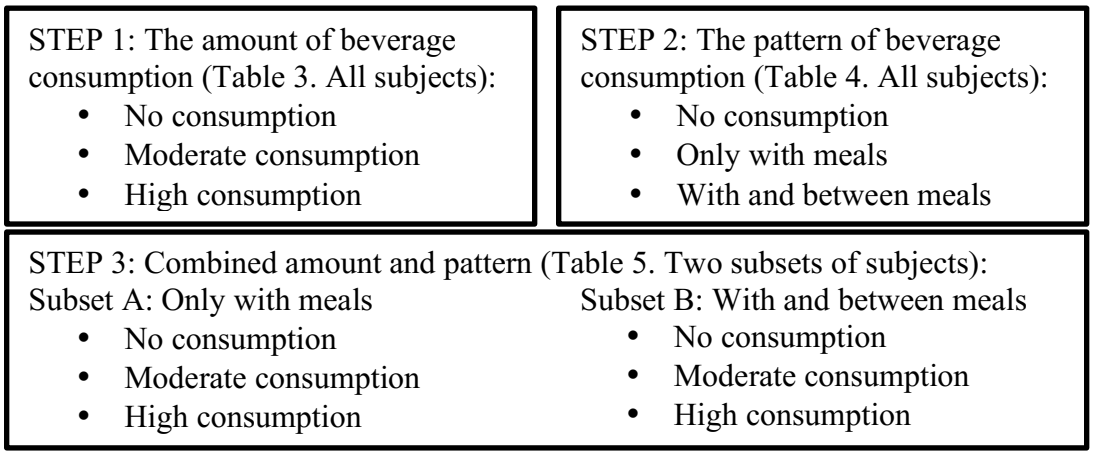

Figure 1. Summary for each data analysis step.

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PROMINENT SALIVARY BIOMARKER CHANGES RELATED TO RAPID DEVELOPMENT OF ADOLESCENT OBESITY

ABSTRACT

Background:

The development of obesity within a few years is a phenomenon often occurring in children. In this analysis we investigate longitudinal salivary biomarker changes in two groups of children; one changing from normal to obese in two years and the other maintaining normal healthy status over the same period.

Metabolic syndrome, the occurrence of 3 out 5 variables (obesity, hypertension, hyperglycemia, lowered high density lipoprotein cholesterol (HDLC), and hypertriglyceridemia) is a risk factor that predicts future cardiovascular disease and type 2-diabetes (T2D) in adults. In this study, in addition to clinical measures, we use salivary assay of glucose and HDLC to evaluate changes. This measure included 3 of the 4 variables obesity, hypertension , salivary glucose and salivary HDLC.

The aim of this study is to investigate the changes overtime of the salivary biomarkers; insulin, CRP, phosphate, and uric acid. This study followed a group of children before having developed SMets and after its development, and compared them to children with no SMets characteristics.

Methods:

Ninety-four Kuwaiti children (mean age=9.8 year old) were included from the Kuwait Healthy Life longitudinal cohort study. At baseline in 2012, none of the children had three or more SMets characteristics and therefore none were SMets positive. At follow-

up, in 2014, 51 children had developed SMets and 43 children did not develop SMets. The children who did not develop SMets were considered healthy. The anthropomorphic characteristics and salivary biomarkers for the two groups at both visits were compared using two-sample t-test and Wilcoxon rank-sum test. The changes in the salivary biomarker measurements from baseline to follow-up for each group were tested using Wilcoxon matched-pairs signed-ranks test. All statistical tests were at 0.05 significant level.

Result:

At baseline there was no significant difference between the salivary levels comparing the SMets positive group and the healthy group. At the follow-up all the four salivary biomarkers were significantly higher in the SMets positive group compared to the healthy group; Insulin ($p < 0.001$), CRP ($p < 0.001$), phosphate ($p = 0.008$) and uric acid ($p < 0.001$). When comparing salivary biomarker levels at baseline to follow-up, children in the healthy group had no significant change. To the contrary, the SMets positive group had significant increase in all salivary biomarkers; insulin increased to almost double the baseline levels *($p = 0.014$),* CRP levels were 120% higher than the baseline levels *($p = 0.005$),* phosphate became 11% higher *($p = 0.030$)* and Uric Acid showed 17% elevation *($p = 0.009$).*

Conclusion:

Children who developed SMets showed significant elevation in all the four salivary biomarkers while there was no significant change noted in the levels for children who did not develop SMets. CRP showed the strongest elevation increasing almost 120%. Insulin

doubled at follow-up compared to baseline and uric acid and phosphate showed 17% and 11% increase, respectively.

INTRODUCTION

The term Metabolic syndrome (SMets) was introduced in the 1970s to tell of the association between obesity and type 2 diabetes (T2D).[1,2] SMets currently is described as a cluster of risk factors used to predict future cardiovascular disease and T2D.[3] In 2006, the International Diabetes Federation (IDF), determined that in order to define a person with SMets the individual must have central obesity for his/her specific ethnicity, Body Mass Index (BMI) >30kg/m2, and two of these four factors: [4]

Triglycerides ≥150mg/dl (1.7 mmol/L),

High Density Lipid (HDLC) <40mg/dl (1.03 mmol/L) in men or <40mg/dl (1.29 mmol/L) in women,

High blood pressure (BP) systolic ≥130 or diastolic ≥85 mmHg, and

Fasting blood glucose (FBG) ≥100 mg/dl (5.6mmol/L). [4]

Applying this definition to children was controversial as children experience many changes in their anthropometric measurements, blood pressure and lipid levels during puberty.[5] However, research showed that SMets indicators during the transition from childhood to young adulthood were stable overtime. [6] Furthermore, childhood SMets was a strong predictor for the development of T2D and cardiovascular disease in adulthood in longitudinal cohort studies. [7] [8,9]

As intervention during adolescence can be a great opportunity for preventing future systematic diseases,[10] tools to identify or monitor children at risk are valuable. Blood biomarkers are most commonly used to give reliable information about disease status. Due to blood biomarkers relative invasiveness, saliva offers an ideal easy non-invasive alternative as it carries several biological biomarkers, which also may serve for

diagnosis, monitoring and follow up for patients with a systemic disease. [11,12] A study noted that US dentists preferred salivary tests over blood test, by way of finger needle sticks.[13] The same was true for patients. This support the idea that simple non-invasive diagnostic tools can be useful for diagnosis,[14] progression, follow-up and large epidemiological studies especially in children. [15] *Goodson and Welty,*[11] suggested that saliva could offer an ideal screening tool to identify children at risk of obesity, SMets and eventually T2D.

Due to the epidemic of T2D, finding a salivary test to diagnose and monitor T2D is attractive.[12] Such salivary test could be of paramount benefit for communities currently suffering from high incidences of T2D. As childhood SMets known as a strong predictor of T2D,[16] early identification of children with SMets can be very useful in identification or monitoring children at risk.

Insulin resistance is defined as a decreased biological response to the body's demand of insulin and is strongly linked to obesity.[17] Before reaching the level of insulin resistance, Insulin increase (hyperinsulinemia) occurs until the failure of β - cells. [18] Insulin resistance is essential in the process of developing SMets in adults[19,20] and in children. [21] *Hartman et al,* [22] analyzed salivary biomarkers from 8,245 Kuwaiti children, and found salivary hyperinsulinemia to be more associated with obesity than high salivary glucose (hyperglycemia). [22] In an eight year longitudinal study, *Bao et al,* [23] reported that subjects who had constant high plasma insulin levels also had a strong association with being overweight or obese.

Recently, obesity has been viewed as a chronic inflammatory disease.[24] C-Reactive Protein (CRP) is produced by the liver as a protein and a marker for

inflammation.[25] It increases considerably as a response to an acute infection, and mildly for a chronic infection. [26] CRP is a predictor for the risk of developing coronary heart diseases.[27] Additionally, there is an association between CRP and being overweight or obese in adults [28] and in children as young as three years old.[29] Elevated plasma CRP was also a predictor for both T2D[30] and SMets.[31,32] Salivary CRP is detectable,[33 34] in Kuwaiti children, saliva analysis was made for 744 children from different body weight categories; obese children had 6 times higher salivary CRP compared to non obese children.[34]

Phosphate and obesity are recently becoming an area of interest, although there is no clear understanding of their association. Based on a study conducted on 6-12 year old obese and non-obese children, reduced phosphate level showed an inverse association with obesity and insulin resistance.[35] To the contrary, the salivary phosphate levels were significantly higher in obese children compared to non-obese. [36]

The association between uric acid (UA) and SMets has been extensively investigated mainly due to the increased consumption of fructose. [37] Fructose is the most appealing artificial sweetener for the food companies and became the most commonly consumed sweetener, due to its solubility in low temperatures, longer shelf live and cheep price. [38] Fructose is available in the most commonly consumed processed food products, table sugar, and soft drinks and was tightly connected to the epidemic of obesity. [38] One unique feature fructose has, is the ability to raise UA regardless of the energy intake.[39] A recent understanding of fructose ability to induce SMets is based on its ability to increase UA, by depleting the intracellular ATP.[37] A possible causative role of UA in the development of SMets has been suggested. [40] In a study on rats *Nakagawa et al,*[40] fed two

groups of rats with fructose; one had UA lowering medications and one did not. The rats received UA lowering medication did not develop SMets.[40] The association between the serum UA and SMets is well demonstrated in several studies,[41-43] however saliva UA was only documented in one pilot study.[44] *Soukup et al, [44]* collected salivary UA samples from 78 men and women (18-65 years old). The study reported a significantly higher salivary UA levels in participants who had SMets. However, per our knowledge there are no studies about salivary UA and SMets in children.

Kuwait have one of the highest percentages of SMets, obesity [45], and diabetes. [46] A series of cross sectional salivary biomarkers studies were conducted on Kuwaiti children investigating the changes in salivary biomarkers in obesity and SMets.[11,22,34,47] In this study, we aim to expand on the work from the previous studies and investigate the change in four crucial salivary biomarkers over time. We specifically aim to compare the levels before developing SMets and after its development. Finally, we aim to compare the changes in the levels of these biomarkers that occurred in the SMets positive group (having 3 or more SMets characteristics at follow-up) with healthy group that did not develop SMets.

We hypothesize that children who developed SMets will have significant changes in these four salivary biomarkers at follow-up after developing SMets, compared to their levels at the baseline before developing SMets. We also hypothesize that levels of the biomarkers in the children, who did not develop SMets, will not show a significant change.

MATERIAL AND METHODS

This study used data from the Kuwait healthy life longitudinal prospective cohort study that was conducted by Dasman Diabetes Institute in Kuwait. It comprised of two visits; the first visit, baseline, took place in 2012 and screened 8,317 fourth and fifth grade students in Kuwaiti public schools. The second visit, follow-up, included 6,316 students who participated at baseline. The follow-up visit took place two years after the baseline.

The country of Kuwait has six geographic locations (governorates). All public schools within each governorate may only accept Kuwaiti citizens for enrollment. For the study children were equally selected from schools of all six governorates. A total of 94 children were included to form two groups based on developing SMets at follow up or not. At the baseline, no children had three or more SMets characteristics. At follow-up, children who developed three or more SMets characteristics were allocated to the SMets positive group, while those who had two or less SMets characteristics were allocated to the healthy group. A total of 51 children were in the SMets positive, and 43 were in the healthy group. This study was approved by the Dasman Diabetes Institute Human Ethical Review committee in Kuwait and the Forsyth Institutional Review Board in Cambridge, Massachusetts. Parental informed consents were obtained on both visits, as were children's verbal assents. Further details of the study are available in previous publications. [11,22,34,47]

Data collection

During both visits each child had a unique identification number and data collected were entered into a programmed iPad (Apple, Cupertino, CA). Age and

anthropomorphic measurements were collected including; weight in Kilograms (Kg) using a digital scale, height in centimeters (cm) using a stadiometer, waist circumference in centimeters (cm) using measurement tape, blood pressure in millimeters of mercury (mmHg) using an automated cuff measuring device to record both systolic and diastolic blood pressure, and heart rate (beats/minute). The fitness was measured using Queens College step test.[48] This test records both the child's initial heart rate and their heart rate after stepping on and off of a platform for three minutes. The difference between the two measurements reflects the level of physical fitness; the higher the heart rate the lower the fitness level.

Saliva Collection and Oral Examination

The children who participated in the study were instructed to fast from midnight until the next day, prior to saliva collection, when they were offered 15 ml of water mouth rinse. Following, the children were asked to drool in a sterile 15 ml tube, providing approximately three ml of saliva. Each sample was weighted and centrifuged using 2800 RPM, at 4°C controlled temperature, for 20 min to remove any mucosal cells or debris remaining in the tube. The 15 ml tubes used to collect saliva were kept in a screw-cap 2D barcoded storage tubes (Matrix™ tube, Thermo Scientific Inc., Waltham, Massachusetts, USA). These tubes were maintained on ice until they arrived at the Dasman Institute in Kuwait. A barcode reader (VisionMate ST, Thermo Scientific Inc., Waltham, Massachusetts, USA) was used to read the barcode on the tube. To prepare for transfer, the tubes were sealed using torque-controlled tube capper (8-Channel Screw Cap Tube Capper, Thermo Scientific Inc., Waltham, Massachusetts, USA), and placed in a 96-vial rack (Thermo Scientific Latch Rack, Waltham, Massachusetts, USA). While in

transit to the Forsyth Institutes laboratories, , the racks were stored in chambers under dry ice (Biocair, Boston MA) to maintain a temperature of -80 °C until time for salivary assay. In a separate spreadsheet the participants' unique identification number and the scanned barcode were recorded.

At baseline, the salivary collection was performed for all children, although the analysis was only performed on 744 samples. The analysis samples were randomly selected, but designed to include all the 4 WHO body weight categories (under weight, normal weight, over weight and obese) with equal sex distribution. [34] At the follow-up, salivary analysis was made for 94 children only based on the development of SMets or not. Calibrated and previously trained dentists performed the oral examination to record the existing carious teeth and the gingival health status. The steps provided here about saliva collection and oral examination specifics are available in previous publications. [22,34,47,49]

Salivary Analysis

Salivary assay was carried out using 25 microliters of salivary supernatant and a Luminex 200 platform (Luminex, Austin, TX). Different magnetic bead panels were used during the assay; for metabolic hormones as insulin, a 3-plex metabolic hormone magnetic bead panel was used with no dilution (Millipore cat #HMHMAG-34AK; lot #2055724). [34] Salivary inflammatory CRP was measured using diluted 1:2 samples and a 3-plex human obesity magnetic bead panel (R&D cat #LOB000, LOB1065, LOB1707, LOB1359; lot #300710). [34]

Phosphate and UA were measured using mass spectroscopy. 120 μL samples of saliva from each participant at baseline and two years later were evaluated. Relative

amounts of each metabolite were obtained by integrating peaks detected on a untargeted metabolic profiling platform (Metabolon®, Durham, North Carolina) employing high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) optimized for basic species, and gas chromatography-mass spectroscopy (GC-MS) for volatile species.[50] Compounds were identified by matching chromatographic retention index and mass spectral fragmentation signatures with reference library data created from authentic standards.

Fluorescent assay (Glucose Colorimetric/Fluorometric Assay Kit #K606-100, BioVision, Inc., Mountain View, CA, USA) was used to measure the salivary glucose. Samples were implemented on an 8-channel liquid handling arm in Tecan Freedom EVO® 150 robotic processor (Tecan Group Ltd., Männedorf, Switzerland).[22] Further details about the salivary analysis are available in previous publications. [22,34,47,49]

Participants' Allocation

To improve children and parents willingness to participate in the study, no blood samples were drawn from children and only saliva samples were collected. [11 34] Since both salivary high-density lipoprotein (HDLC) and salivary glucose have shown moderate to strong correlation to plasma levels, the saliva was used as a surrogate for these two plasma biomarkers.[51,52] Cut off levels for salivary HDLC and salivary glucose were approximated from correlation findings of different studies.[22,47] Salivary HDLC of 0.6mg/dl is approximately equal 50mg/dl plasma HDLC level,[47] and 1.13 mg/dl salivary glucose is approximately equal to 100 m/dl fasting plasma glucose level. [22] Due to measurement discrepancies triglycerides were excluded and SMets was limited to the presence of three or more of these four criteria; i) waist circumference ≥90th percentile; ii)

systolic blood pressure (SBP) ≥130mmHg or diastolic blood pressure (DBP)≥85mmHg; iii) Salivary HDLC≤0.6mg/dl; and iv) salivary glucose≥1.13 mg/dl. At baseline, none of the 94 children had three or more of the SMets characteristics. During the follow-up visit, if the child had three or more SMets characteristics, he/she was allocated to be in the SMets positive group. If the child had zero to two SMets criteria, he/she was allocated to the healthy group.

Statistical Analysis

Children were included in the statistical analysis based on whether or not they developed the outcome SMets during the two years study period. The physical and anthropomorphic characteristics of children were presented for both visits. The mean and standard deviation (SD) was reported if the variable was normally distributed. Two-sample t-test was performed to compare the mean for the two groups. If the variable was not normally distributed we reported the median and interquartile range (IQ), and used a Wilcoxon rank-sum test (Mann–Whitney U test) for the comparison between the two groups. The p-value of <0.05 was used as a cut off for statistical significance in comparison between the two groups.

Before analyzing the four salivary biomarkers of interest, their values were investigated for possible outliers using the upper/ lower inner and outer fences for the IQ for each of the salivary biomarkers. Only eight values of the salivary biomarkers were considered as possible outliers. To correct for outlying values, the median value for each specific biomarker was used to replace the eight outlying values. Following, a separate data set was created to confirm if the outliers might change the outcome or not.

We performed a Shapiro-Wilk W test for normal distribution on the four salivary biomarkers of interest for the SMets positive and the healthy group at each visit. All biomarkers were not normally distributed. To compare the baseline salivary biomarker scores between the two groups we preformed a non-parametric Wilcoxon rank sum (Mann–Whitney U) two-sample statistics. The same test was also performed at follow-up.

To examine the correlation between the four salivary biomarkers, we tested the data at baseline, follow-up and the difference between the two (the biomarker value at follow-up minus the value at baseline). We then divided these three groups further into three more categories; all 94 participants, those who were SMets positive and those who were healthy. The Normality test was made for all the values while the Spearman rank correlation test was used for baseline and follow-up. Pairwise correlation was used for the difference between follow-up and baseline as all the differences values were normally distributed.

To test if there was a significant change in the salivary biomarker levels after the two years study period, the salivary biomarkers were tested comparing the baseline levels to the follow up level for all 94 subjects. We used Wilcoxon matched-pairs signed-ranks test, as all biomarkers were not normally distributed. Lastly, to compare how each salivary biomarker has changed from the baseline to the follow-up for each group, the same tests were made for each group separately.

The entire analysis was repeated again using the modified data set in which the possible outliers were replaced with the biomarker specific medians. No changes in the general outcomes or associations were observed. Therefore, the data set with the

originally entered values was used to report the findings from this study. We used Stata13 Statistical software (StataCorp LP, College Station, TX, USA) packages for the statistical analysis in this study.

RESULTS

This study was designed to ensure that the salivary samples collected from the 94 participants represented those who developed SMets during the two years study period and those who did not. Fifty-one children developed three or more SMets characteristics (SMets positive) at follow-up, while 43 did not (healthy).

Table1 illustrates the basic and anthropomorphic characteristics as well as the salivary biomarker levels for the study participants at baseline. There were statistically significant differences between the two groups in the weight ($p < 0.001$), as children in the SMets positive group showed initially a significantly higher weight. Children in the SMets positive also had statistically larger waist circumference compared to the healthy children ($p < 0.001$). The Body Mass Index (BMI) for the SMets positive group was significantly higher compared to the healthy group ($p < 0.001$). Statistically, the SMets positive group was significantly taller children compared to the healthy group ($p = 0.006$). Both systolic ($p = 0.004$) and diastolic blood pressure ($p = 0.001$) showed an increase in the SMets positive group compared to the healthy group.

There was no significant difference between the salivary biomarker levels in the two groups at baseline. The test between the two groups for each of the four biomarker was as follow; Insulin ($p = 0.743$), CRP ($p = 0.585$), phosphate ($p = 0.967$) and UA ($p = 0.131$).

Table 2 demonstrates the same physical characteristics, demographic characteristics and salivary biomarker levels as Table 1; however the values in Table 2 represent those collected at the follow-up. All the variables that were significantly different at baseline visit remained significantly different at the follow-up except the

fitness level. Fitness level changed from being statistically significant to not being significantly different (*p=0.808*). Salivary HDLC was significantly lower for children in the SMets positive group (*p =0.012*).

In regards to the four salivary biomarkers at follow-up, there was a significant difference between the SMets positive group and the healthy group. Insulin (*p <0.001*), CRP (*p <0.001*), phosphate (*p =0.010*) and UA (*p <0.001*) were all significantly higher in the SMets positive children.

Table 3 illustrates the correlation between the four biomarkers as represented by the difference in salivary biomarker levels at follow-up and baseline (biomarker value at follow-up minus biomarker value at baseline). Phosphate and UA showed a significant positive correlation *r*=0.317 (*p =0.002*). However, after testing the correlation separately for each group, it was only significant for the SMets positive group *r*=0.386 (*p =0.006*). Figure 1 shows a scatterplot for the correlation between phosphate and UA. No significant correlation was found in any of the other salivary biomarkers. More details about the correlation between the biomarkers values at baseline and at follow-up are available in the supplementary tables S1a and S1b.

Comparisons between the changes in the biomarkers levels from baseline to follow-up were initially completed on all 94. Only UA was considered to have borderline significance (*p =0.065*). Insulin *(p =0.419),* CRP (*p =0.237*), and phosphate (*p =0.239)* were not significant. Yet, when the analysis was repeated for each group separately different results were achieved. Comparing the salivary biomarkers values at follow-up to the values at baseline in the healthy children group did not show any significant change; insulin *(p =0.073)*, CRP *(p =0.122)*, phosphate *(p =0.673)* and UA *(p =0.847).*

All the children in the SMets positive group showed significant elevation in all four of the salivary biomarkers at follow-up compared to their values at baseline. Insulin increased to almost double what it was at baseline *(p =0.014),* CRP levels were 120% higher than baseline levels *(p =0.005).* Phosphate became 11% higher *(p =0.030)* and UA showed 17% elevation *(p =0.009).* Table 4a 4b, 4c, d4 as well as Figure 2a, 2b, 2c, and 2b summarize the changes in the values for each salivary biomarker between baseline and follow-up.

DISSCUSSION

In our study, we found significant elevation of the four salivary biomarkers (insulin, CRP, phosphate and UA) in children who developed SMets, the SMets positive group. This elevation occurred concurrently with the development of SMets characteristics between the baseline and the follow-up. Salivary biomarkers showed no significant change in children who did not develop SMets, the healthy group.

Salivary insulin levels in the SMets positive group became approximately double the levels at baseline; while healthy group did not show significant change. Similar increase was reported in the studies from baseline visit studied salivary insulin level with obesity [22] and SMets.[34] Findings about increased insulin level during puberty are challenged by the biological changes rather than being due to disease process. [53,54] During puberty, the body responds to the increased insulin resistant and attenuated insulin sensitivity by increasing the insulin secretion.[53,54] In our study, we observed that the salivary hyperinsulinemia was associated with the development of SMets rather than a biological response to puberty, as it only occurred in children who developed SMets, while both groups from the same age group with an equal distribution of sex.

Our findings indicate that the significant increase in CRP level is associated with SMets, as the children in the SMets positive group showed significant elevation in salivary CRP levels after developing SMets at follow-up, despite having normal salivary CRP levels before developing SMets at baseline. On the other hand, children who did not develop SMets had a non-significant reduction in the salivary CRP level from baseline to follow up. Data about the correlation between plasma CRP and saliva CRP are contradicting. *Dillon et al,* [26] were among the first to study this correlation and showed no

correlation between the two. However, another study showed a strong to moderate correlation ($r = .72$, $p < 0.001$) between serum and salivary CRP. [55] In addition, *Dorothée Out et al,* [56] also found a moderate correlation between salivary and plasma CRP as they investigated the correlation from 107 women via both cross sectional and 2 years longitudinal analysis. In a study about the association between salivary CRP and obesity, cross-sectional data collected from 170 South African children showed that overweight children had a 2.5 times the odds of having an elevated CRP compared to normal weight children.[57]

The significant elevation in the phosphate levels for children in the SMets positive group is in agreement with the findings from a study which included 77 ten-years old children in USA.[36] This particular study found a significant increase of salivary phosphate in obese children as well as positive correlation between salivary phosphate and BMI. [36] Unlike salivary phosphate, serum phosphate showed a decrease in association with obesity in adults[58] and children.[35] It also showed decreases with glucose intolerance.[59,60]

The increase in salivary UA showed by children in the SMets positive group, is in line with the salivary UA increase in adults with SMets reported by Soukup *et al,* [44] after studying the association between the two on 78 adults. The fourth quartile for the salivary UA included 67% of the SMets subjects.[44] Due to the value UA has in detecting SMets, [38,40,43] and its strong correlation between salivary and serum UA[44,61] *Kim et al,*[62] developed a biosensor to provide a real time monitoring for the salivary UA using a wireless mouth guard.

Although children in the SMets positive group had a significantly increase in waist circumference, weight, and BP at baseline for children in the SMets positive group, none of the 94 children had three or more SMets characteristics. Children became SMets patients at follow-up only. The levels of the four biomarkers at baseline (before developing the SMets) were not different between the two groups. They only became different after one group became SMets positive. This is a limitation of our study, as both groups did not have matching blood pressure, weight, and waist circumference at baseline.

Some values for the four salivary biomarkers were found to be considered possible outliers; these values were replaced with the median from each biomarker. This was performed to confirm if the observation was due to some extremely high/low values or not. The analysis was repeated with these modified values to compare them to the originally entered values. No difference was noted in the outlier managed data analysis and the originally entered data. The results with the modified outliers are reported in the supplementary Tables S2 and S3.

CONCLUSIONS

Children who developed SMets showed significant elevation in all the four salivary biomarkers; insulin, CRP, phosphate and UA. There were no significant changes in the four biomarkers levels for children who did not develop SMets. At baseline no significant difference in the biomarker levels from either group was noted. For the children who developed SMets during the study, CRP showed the strongest elevation, increasing almost 120%. Insulin doubled at follow up compared to baseline and UA and phosphate showed 17% and 11% increase, respectively.

TABLES & FIGUERS

Table 1.

Table 1a Variables	Overall (n=94) Mean ± SD	SMets positive (n=51) Mean ± SD	Healthy (n=43) Mean ± SD	P-value
Age (year)	9.83 ±0.61	9.8 ±0.61	9.8 ±0.62	0.827
Sex (male)	0.45± 0.50	0.43 ±0.50	0.49 ±0.50	0.585
Waist circumference (cm)	64.63 ±6.93	68.4 ±5.43	60.13 ±5.75	<0.001*
Height (cm)	136.47 ±7.29	138.33 ±6.65	134.25 ±7.46	0.006*
Diastolic BP (mmHg)	72.98 ±12.76	77.43 ±13.00	67.70 ±10.33	0.001*
Systolic BP (mmHg)	108.66 ±15.74	112.88 ±14.90	103.65 ±15.40	0.004*
Heart rate (beats/min)	89.53 ±18.92	89.31 ±17.36	89.79 ±20.83	0.904
Week day sleep (Hr)	8.69 ±1.80	8.93 ±1.46	8.40 ±2.10	0.147
Salivary glucose (mg/dl)	0.18 ±0.14	0.19 ±0.18	0.16 ±0.08	0.301
Table 2b Variables	Overall (n=94) Median (IQ range)	SMets positive (n=51) Median (IQ range)	Healthy (n=43) Median (IQ range)	P-value
Weight (kg)	36 (11)	40 (6.5)	30 (8)	<0.001*
BMI (kg/m^2)	19.3 (4.5)	21.03 (2.7)	16.76 (2.33)	<0.001*
Saliva flow rate (ml/hour)	27.4 (20.24)	27.2 (19.77)	27.3 (23)	0.685
Fitness (Beats/min)	22.5 (33)	29 (39)	17.5 (31.5)	0.057
Red periodontal sites (%)	82 (37.9)	79.7 (40.2)	83.3 (35.9)	0.634
Salivary HDLC (mg/dl)	0.94 (0.60)	0.86 (0.56)	1.03 (0.68)	0.124
Insulin	94.79 (208.16)	88.83 (164.38)	138.86 (232.4)	0.743
CRP	148.6 (336.58)	156.66 (255.89)	129.65 (569)	0.585
Phosphate	0.99 (0.27)	0.97 (0.29)	1.01 (0.28)	0.967
Uric Acid	0.96 (0.52)	0.97 (0.47)	0.82 (0.56)	0.131

Table1. Children's physical characteristics and the salivary biomarkers at the baseline.
SMets positive: Children who had no metabolic syndrome (SMets) characteristics at baseline, but developed SMets at follow up.
Healthy: Children who did not have SMets at both visits.
SMets= Three or more Metabolic syndrome characteristics.
Table 1a. Normally distributed variables and t-test association was used to compare the groups.
Table 1b. Non-normally distributed variables and rank sum test was used to compare the groups.
*Indicate significant difference at *0.05* levels.
SD= Standard deviation, IQ= Interquartile range

Table 2.

Table 2a Variables	Overall (n=94) Mean ±SD	SMets positive (n=51) Mean ± SD	Healthy (n=43) Mean ± SD	P value
Age (y)	11.92 ±0.62	11.9 ±0.62	11.9 ±0.63	0.704
Sex (male)	0.45 ±0.50	0.43 ±0.50	0.49 ±0.51	0.585
Waist circumference (cm)	79.6 (12.60	89.08 ±4.64	68.40 ±9.24	<0.001*
BMI	22.6 (4.23	26.04 ±1.53	18.53 ±2.36	<0.001*
Height (cm)	149.6 (8.04	151.45 ±6.72	147.42 ±8.96	0.015*
Diastolic bp (mmHg)	83.63 ±14.19	89.82 ±11.69	76.28 ±13.46	<0.001*
Systolic bp (mmHg)	120.16 ±17.20	129.57 ±11.71	109 ±16.02	<0.001*
Heart rate (beats/min)	88.82 ±16.54	91.76 ±16.76	85.32 ±15.76	0.060
Week day sleep (Hr)	8.69 ±3.09	8.90±2.94	8.44 ±3.27	0.475
Red periodontal sites (%)	43.69 ±17.04	44.95 ±18.70	42.20 ±14.9	0.439
Salivary glucose (mg/dl)	0.15 ±0.30	0.18 ±0.34	0.11 ±0.24	0.244
Salivary HDLC (md/dl)	0.61 ±0.89	0.39 ±0.60	0.86 ±1.10	0.012*
Table 2b Variables	Overall (n=94) Median (IQ range)	SMets positive (n=51) Median (IQ range)	Healthy (n=43) Median (IQ range)	P-value
Weight (kg)	53.3 (21.2)	60 (8)	38.6 (10.0)	<0.001*
Saliva flow rate (ml/hour)	21.6 (21.3)	24.08 (20.72)	20.06 (15.6)	0.171
Fitness (Beats/min)	22.75 (22)	22.5 (23)	23 (21.5)	0.808
Salivary glucose (mg/dl)	.04 (0.11)	0.06 (0.11)	0.03 (0.09)	0.337
Insulin	114.1 (177)	181.29 (194.2)	72.71 (108.2)	<0.001*
CRP	185.91 (618)	347.8 (756.2)	57.5 (137.9)	<0.001*
Phosphate	1.00 (0.32)	1.08 (0.39)	0.98 (0.23)	0.010*
Uric Acid	1.06 (0.65)	1.19 (0.66)	0.82 (0.52)	<0.001*

Table2. Children's physical characteristics and the salivary biomarkers at the follow-up.
SMets positive: Children who had no metabolic syndrome (SMets) characteristics at baseline, but developed SMets at follow up.
Healthy: Children who did not have SMets at both visits.
SMets= Three or more Metabolic syndrome characteristics.
Table 2a. Normally distributed variables and t-test association was used to compare the groups.
Table 2b. Non-normally distributed variables and rank sum test was used to compare the groups.
*Indicate significant difference at *0.05* level.
SD= Standard deviation, IQ= Interquartile range.

All participants N=94	Insulin difference (P-value)	CRP difference (P-value)	Phosphate difference (P-value)	UA difference (P-value)
Insulin difference	1.0000			
CRP difference (P-value)	0.052 (0.621)	1.0000		
Phosphate difference (P-value)	0.175 (0.093)	-0.063 (0.546)	1.0000	
UA difference (P-value)	0.079 (0.450)	-0.132 (0.204)	**0.317 (0.002)***	1.0000

Healthy N=43	Insulin difference (P-value)	CRP difference (P-value)	Phosphate difference (P-value)	UA difference (P-value)
Insulin difference	1.0000			
CRP difference	-0.012 (0.940)	1.0000		
Phosphate difference	0.075 (0.631)	-0.147 (0.340)	1.0000	
UA difference	0.176 (0.252)	-0.183 (0.234)	0.220 (0.151)	1.0000

SMets positive N=51	Insulin difference (P-value)	CRP difference (P-value)	Phosphate difference (P-value)	UA difference (P-value)
Insulin difference	1.0000			
CRP difference	0.047 (0.746)	1.0000		
Phosphate difference	0.186 (0.196)	-0.037 (0.801)	1.0000	
UA difference	-0.106 (0.464)	-0.129 (0.373)	**0.386 (0.006)***	1.0000

Table 3. The pairwise correlation between the differences between biomarkers values from baseline to follow-up (follow-up minus baseline).
SMets positive: Children who had no metabolic syndrome (SMets) characteristics at baseline, but developed SMets at follow up.
Healthy: Children who did not have SMets at both visits.
SMets= Three or more metabolic syndrome characteristics.
*Indicate significant pairwise correlation at *0.05* level.

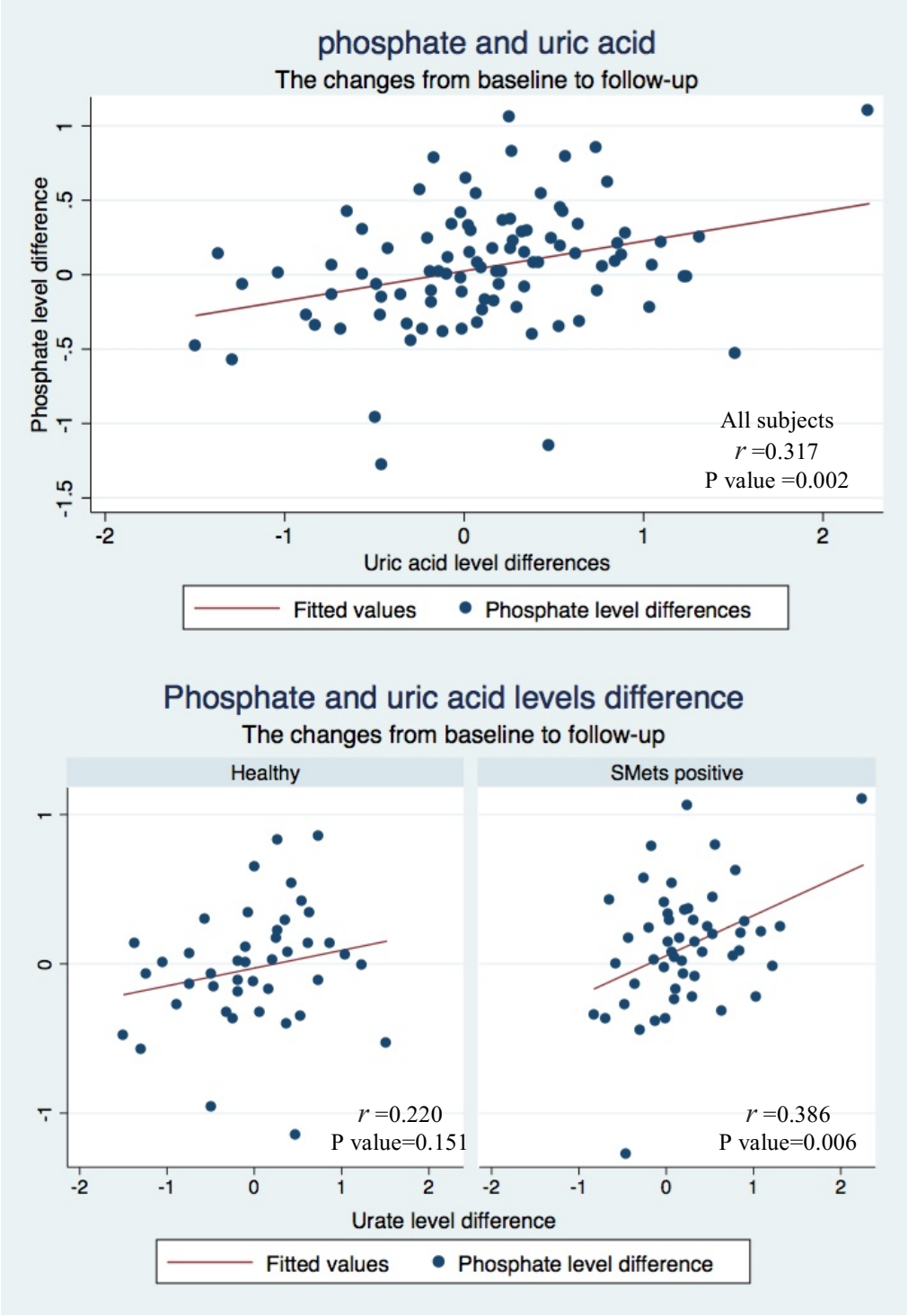

Figure 1. Scatter plot for the differences in phosphate and uric acid level between baseline and follow-up.

Fig 2a.

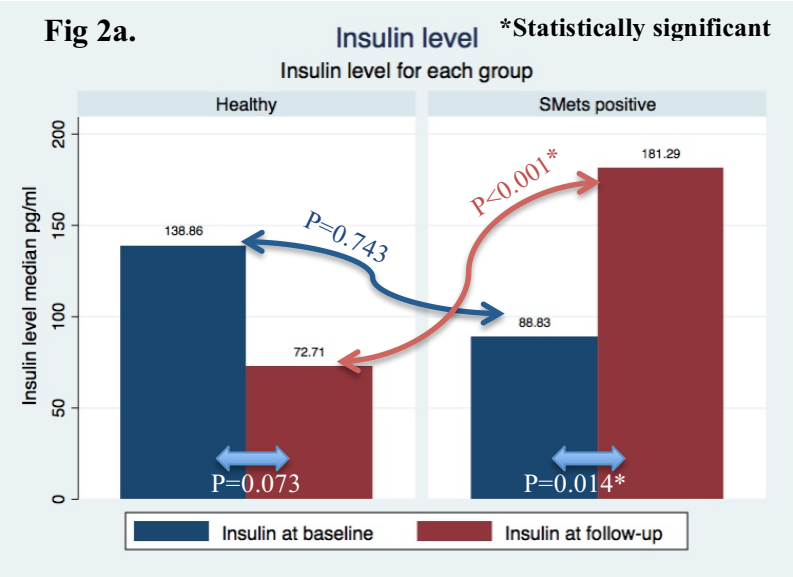

Table 4a.	Healthy n=43			SMets positive n=51		
Biomarkers	Baseline median (IQ Range)	Follow up median (IQ Range)	P value	Baseline median (IQ Range)	Follow up median (IQ Range)	P value
Insulin	138.86 (232.4)	72.71 (108.2)	0.073	88.83 (164.38)	181.29 (194.2)	0.014*

Fig 2b.

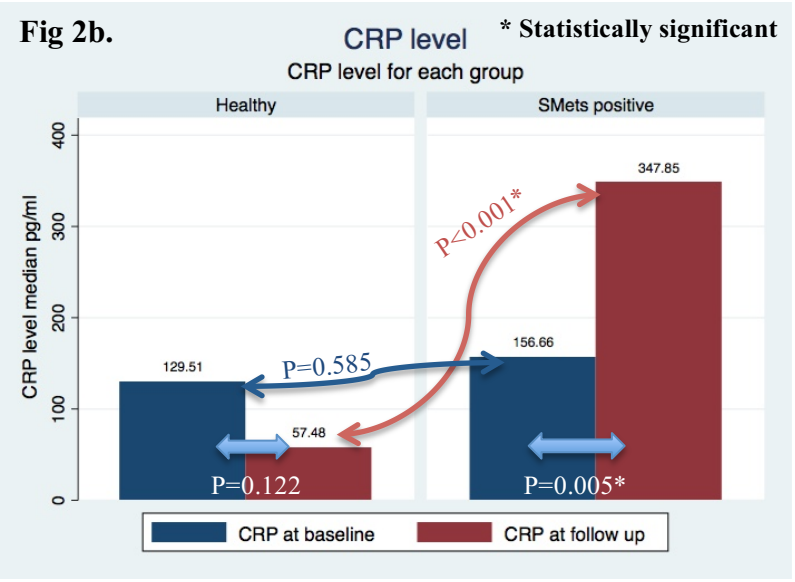

Table 4b.	Healthy n=43			SMets positive n=51		
Biomarkers	Baseline median (IQ Range)	Follow up median (IQ Range)	P value	Baseline median (IQ Range)	Follow up median (IQ Range)	P value
CRP	129.65 (569)	57.5 (137.9)	0.122	156.66 (255.9)	347.8 (756.2)	0.005*

Fig 2c. ***Statistically significant**

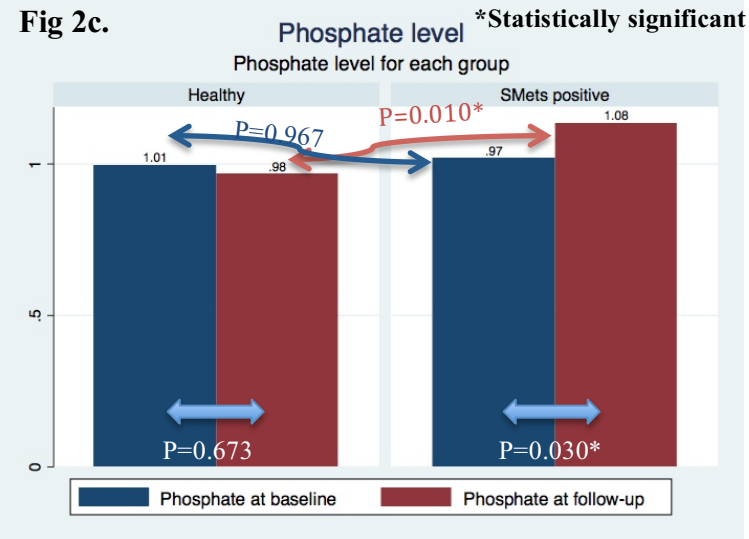

Table 4c.	Healthy n=43			SMets positive n=51		
Biomarkers	Baseline median (IQ Range)	Follow up median (IQ Range)	P value	Baseline median (IQ Range)	Follow up median (IQ Range)	P value
Phosphate	1.01 (0.28)	0.98 (0.23)	0.673	0.97 (0.29)	1.08 (0.39)	0.030*

Fig 2d. ***Statistically significant**

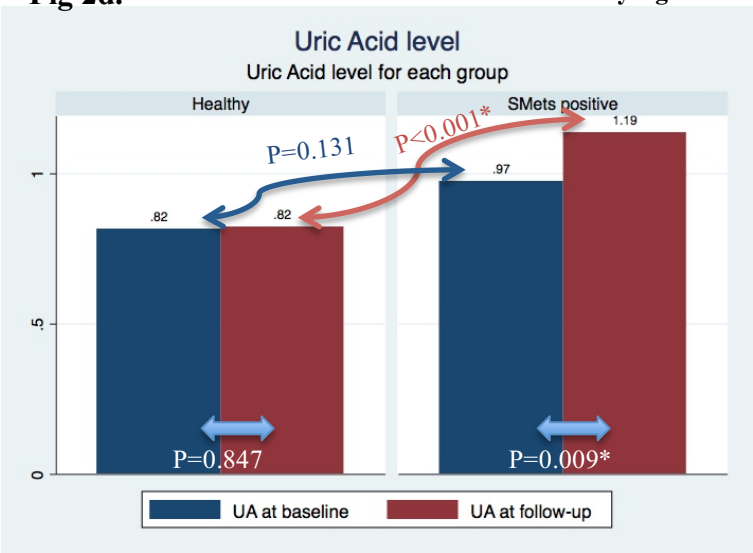

Table 4d.	Healthy n=43			SMets positive n=51		
Biomarkers	Baseline median (IQ Range)	Follow-up median (IQ Range)	P value	Baseline median (IQ Range)	Follow-up median (IQ Range)	P value
Uric acid	0.82 (0.56)	0.82 (0.52)	0.847	0.97 (0.47)	1.19 (0.66)	0.009*

Tables 4(a, b, c and d). The salivary biomarkers level change from baseline (2012) to follow up (2014) for each group.

Wilcoxon matched-pairs signed-ranks test at significant level *0.05.*

Figures 2 (a, b, c and d). Changes in each salivary biomarker for each group and in each visit.

SUPPLMENTARY TABLES & FIGURES

Table S1 (a) Baseline

All participants N=94	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	0.1483 (0.154)	1.0000		
Phosphate	**-0.2161 (0.037)***	-0.002 (0.988)	1.0000	
UA	-0.044 (0.450)	0.019 (0.854)	**0.304 (0.003)***	1.0000

Healthy N=43	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	0.0628 (0.685)	1.0000		
Phosphate	-0.007 (0.964)	-0.045 (0.773)	1.0000	
UA	-0.022 (0.886)	-0.048 (0.758)	**0.307 (0.042)***	1.0000

SMets positive N=51	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	0.202 (0.160)	1.0000		
Phosphate	**-0.396 (0.004)***	-0.034 (0.816)	1.0000	
UA	-0.060 (0.681)	0.074 (0.621)	**0.289 (0.042)***	1.0000

At baseline, the correlation between the biomarkers for all subjects shows that there is a positive correlation between phosphate and UA (r=0.304). After separating the subjects to two groups, both groups maintained the same correlation. The correlation between insulin and phosphate were negatively correlated (r=-0.216) for all participants, but after dividing the participants only those in the SMets positive had a significant negative correlation (r=-0.396).

Table S1 (b) Follow-up

All participants N=94	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	-0.043 (0.679)	1.0000		
Phosphate	**0.462 (<0.001)***	0.021 (0.844)	1.0000	
UA	**0.473 (<0.001)***	-0.126 (0.226)	**0.383 (<0.001)***	1.0000

Healthy N=43	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	-0. 297 (0.050)	1.0000		
Phosphate	**0.338 (0.025)***	0.243 (0.112)	1.0000	
UA	**0.603 (<0.001)***	-0.437 (0.003)*	**0.483 (<0.001)***	1.0000

SMets positive N=51	Insulin (P-value)	CRP (P-value)	Phosphate (P-value)	UA (P-value)
Insulin	1.0000			
CRP	-0.189 (0.189)	1.0000		
Phosphate	**0.445 (0.001)***	0.027 (0.854)	1.0000	
UA	-0.246 (0.085)	-0.234 (0.102)	0.257 (0.072)	1.0000

At follow up, the correlation between the biomarkers for all participants shows a positive weak correlation between phosphate and UA (r=0.383). After separating the participants, the correlation was only significant in the healthy group (r=0.483). On the other hand the correlation between insulin and phosphate were also positive (r=0.462), and after dividing the subjects the correlation was significant for both groups but stronger for the SMets positive group (r=0.445). The correlation between insulin and UA was positive weak correlation for all participants (r=0.473), but when subjects were divided children in SMets positive group had a significant moderate positive correlation (r=0.603), but the healthy group had non-significant negative correlation (r=-0.246).

Table S1 a and b. The correlation between the four salivary biomarkers at both visits.
*Indicate significant association at 0.05 level using spearman correlations test.

Biomarker	Overall (n=94) Median (IQ range)	SMets positive (n=51) Median (IQ range)	Healthy (n=43) Median (IQ range)	P-value
Baseline				
Insulin	94.7 (203.1)	88.83 (164.38)	106.56 (221.11)	0.885
CRP	147.4 (226.13)	148.62 (204.47)	129.51 (335.88)	0.727
Phosphate	0.99 (0.27)	0.97 (0.29)	1.01 (0.27)	0.805
UA	0.96 (0.50)	0.98 (0.45)	0.82 (0.49)	0.060
Follow up				
Insulin	114.01 (171.53)	166.64 (157.66)	72.71 (108.22)	<0.001
CRP	181.49 (459.08)	321.98 (639.6)	57.48 (137.9)	<0.001
Phosphate	1.03 (0.30)	1.08 (0.38)	0.99 (0.22)	0.014
UA	1.06 (0.58)	1.14 (0.53)	0.82 (0.50)	<0.001

Table S2. Children's salivary biomarkers at the baseline and at the follow up.
SMets positive: Children who had no metabolic syndrome (SMets) characteristics at baseline, but developed SMets at follow up.
Healthy: Children who did not have Mets at both visits.
*Indicate significant difference at 0.05 levels using Wilcoxon rank-sum test (Mann–Whitney U test) to compare SMets positive to healthy.
SD= Standard deviation, IQ= Interquartile range

	Healthy n=43			SMets positive n=51		
Biomarkers	Baseline median (IQ Range)	Follow up median (IQ Range)	P Value	Baseline median (IQ Range)	Follow up median (IQ Range)	P Value
Insulin	106.56 (221.11)	72.71 (108.22)	0.167	88.83 (164.38)	166.64 (157.66)	0.054*
CRP	129.51 (335.88)	57.48 (137.9)	0.299	148.62 (204.47)	321.98 (639.6)	<0.001*
Phosphate	1.01 (0.27)	0.99 (0.22)	0.744	0.97 (0.29)	1.08 (0.38)	0.022
UA	0.82 (0.49)	0.82 (0.50)	0.499	0.98 (0.45)	1.14 (0.53)	0.005

Table S3. The change in the salivary biomarkers levels from baseline (2012) to follow up (2014).
Wilcoxon matched-pairs signed-ranks test.
*Indicate significant difference at 0.05 levels.
SD= Standard deviation, IQ= Interquartile range.

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GENERAL DISCUSSION

In the first two papers, we studied the impact of commonly consumed beverages on two pressing health issues in Kuwait; obesity and dental caries. Sodas had a role in both obesity and severe dental caries. According to several news outlets, the Arabian Gulf Countries are in the process of applying taxes on tobacco, sugary drinks and energy drinks due to their harmful impact on health.[1, 2] The taxation is in accordance with the WHO recommendations urging countries to apply fiscal tools to curb the rise of non communicable diseases. [3] In our study about obesity we demonstrate the influence of sodas on the incidences of obesity during a relatively short period. The obesity definition used in our study is the WHO "BMI Z-score" [4]; it has a higher obesity cutoff compared to the CDC score. Many subjects may have been considered obese if the CDC Growth Chart [5] or International Obesity Task Force (IOTF) [6] scale were used. Few studies have used waist circumference to measure abdominal obesity [7], although we have the waist circumference measured at both visits, but due to lack inter rater reliability between baseline and follow-up visits there is a possibility of discrepancies between the two measurements. The position of the measuring tape can be the source of the discrepancy. We decided to use the WHO obesity definition, as it is not subject to such discrepancies. This is considered a strength in the study, as it showed the impact of soda using longitudinal data in such a short period using a very strict obesity definition.

In the third paper, we contribute to the knowledge base for the potential use of salivary biomarkers to diagnose and monitor metabolic syndrome SMets. The distinctiveness of this study is that it took place for some children at the critical time of puberty. There are certain periods of life when obesity diagnosis and intervention are

useful. Critical periods for obesity are the periods of increased risk of overweight or obesity onset, complications, or maintenance. [8] These important periods have two components; antenatal factors and postnatal factors.[8] In the postnatal factors, both the adiposity rebound (between 5 to 6 years of age) [9] and the adolescence period are fundamental for the development of obesity.[10]

During puberty period children in Kuwait reported high consumption of SSB. [11] SSB contain large amounts of fructose [12] as discussed in the third paper, fructose was associated with the elevation of UA, which is released after purine nucleotide breakdown. UA is the end product after the catabolic process of purine nucleotide.[13] Purine has an essential function as it form the monomeric precursors for the cell's nucleic acids DNA and RNA.[13] Over breaking down of purine leads to overproduction of UA from the liver. [14]High serum circulating UA were linked to several health conditions as renal diseases [15], cardiovascular disease [16], hypertension [17], diabetes [18], obesity[17] and SMets. [19] Several studies confirm the positive association between serum UA and SMets from different countries. [20-22] There is a high correlation between salivary and serum UA introducing salivary UA as a viable alternate for the serum UA. [23-25] Our findings report significantly higher salivary UA in children who developed SMets at follow up.

In a previous cross sectional study based on the data from the baseline visit, there was evidence of alteration of some salivary biomarkers in obese children. [26] Four salivary biomarkers (insulin, CRP, leptin and adiponectin) were significantly different in obese children compared to non-obese children. [26] Also, the same studied group of children showed a significant elevation of salivary insulin in the obese children but no significant elevation in salivary glucose levels.[27] This study continued in confirming that the

elevation that was identified from the cross sectional data analyzed from the baseline is in fact associated with the development of SMets, as the elevation was only noted in children who developed SMets. Having a group of children who did not develop SMets is helps to strengthen the argument that the elevation in these four biomarkers is associated with developing SMets not with puberty.

Limitations

Our second study on dental caries and beverages was a cross sectional study. Although oral examination was conducted in both phases of the study, the data from the second exam cannot be correlated with the data from the first due to the lack of systematic calibration of examiners during the second phase (2014). The healthy life study was designed with a focus on the risk factors of T2D in children, and the oral health data were collected as supplementary data. This justifies using binary dental caries severity categories rather than using the decayed missing filled teeth (DMFT) dental caries measure.

Despite collecting the information about beverage consumption as servings per day, we changed it to categories (none, moderate and high) to account for the every day variation in diet. The provided dietary data were collected from children and it was better to use it to provide a general idea about consumption rather than a precise tool to measure consumption.

The dietary questionnaire did not include some important beverages, particularly water, energy drinks, vitamin waters and blended coffee beverages that have gained popularity among children in Kuwait. This is a limitation that precluded studying the impact of more beverages.

Confounders such as oral hygiene practice, parents' education and socioeconomic status were not included in the data collection. Gingival redness and governorate were used as proxies for the oral hygiene level and socioeconomic status, respectively. Data were collected from children, and if it was to be comprehensive and collected from parents, this may have decreased the participation and lead to some incomplete data.

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