# Short Sleep Duration as a Risk Factor for Obesity and Gingivitis in Kuwaiti Children: A Longitudinal Multilevel Analysis

**HARVARD UNIVERSITY**
Harvard School of Dental Medicine

# Short Sleep Duration as a Risk Factor for Obesity and Gingivitis in Kuwaiti Children: A Longitudinal Multilevel Analysis

**A Thesis Presented by**
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# ABSTRACT

*Objectives:* It has been shown that inadequate sleep has negative effect on health including obesity, diabetes, and cardiovascular diseases. Current evidence showed that little sleep impairs immunity, promotes systemic inflammation, and increases the risk of pathological change in many body organs. Aims of this study were to assess the relations between insufficient sleep in developing obesity and gingivitis among a cohort of Kuwaiti children. We also identified the risk behaviors contributed to decreased sleep among those children. This is the first study that has examined the relation between sleep duration and the oral inflammatory condition; gingivitis. In addition, the majority of research related to sleep has been conducted in western countries. Therefore, it seems logical to examine sleep duration as a risk factor for obesity in a population having one of the highest adult prevalence levels of obesity in the world; the Kuwaiti population.

*Methods:* Longitudinal data were collected from 6,316 children 8-14 years old at two time points. Children were approximately equally distributed from 138 elementary schools and representing the 6 governorates of Kuwait. Calibrated examiners in the selected schools conducted oral examinations, sleep evaluation interviews, body weight measurements, nutritional analysis, physical activity assessments, and collected saliva samples. A cross sectional model, and four longitudinal multilevel models were conducted to determine four different outcomes; waist circumference, obesity, gingivitis, and sleep duration at three levels; time, individual, and school. The main independent variables examined were daily sleep hours and salivary glucose levels. Other explanatory variables and confounders assessed were: dental caries, tooth filling, trouble breathing at night, TV and videogame use, and governorate; adjusted for snacking and gender.

*Results:* There was a statistically significant increase in abdominal obesity and gingivitis with shorter sleep duration with significant change over time ($P<0.05$). Children who watched TV or played videogames just before bedtime, and those who used screen activities more than two hours a day, slept significantly less than other children. The magnitude of gingivitis was significantly different between the six governorates of Kuwait ($P<0.05$). There was a statistically significant variation in the observations between schools over time.

*Conclusion:* Longitudinal analysis of Kuwaiti children revealed that obesity and gingivitis increased with shorter sleep duration overtime. Screen based activities including TV and videogame use were major factors contributed to decrease night sleep hours. There was a strong clustering effect within the schools in shaping the three health conditions; obesity, gingivitis, and short sleep duration in Kuwaiti children. Public health intervention programs should target children and their families in schools at higher risk to focus on improving night sleep behavior as well as limit their screen time, in addition to maintaining healthy eating habits, practicing proper oral hygiene measures, and being physically active.

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# INTRODUCTION

Oral health is a fundamental element of general health and wellbeing and the oral cavity can be a reflection of an individual's systemic health. Emerging evidence documents the interconnection between oral and systemic health, and common risk factors, such as socioeconomic status and lifestyle habits, link oral and chronic diseases (1, 2). Hence, a crucial part of advancing oral health is to identify and target common risk factors to sustain optimal oral and general health. In 1948, the WHO defined health as the "complete state of physical, mental, and social well-being and not merely the absence of infirmity"(3). Oral health treatment and promotion has often focused on treating the teeth and gums while ignoring underlying social, behavioral and disease elements. It is essential to change this approach by adopting new perspectives among oral and general health providers, underscoring the social and behavior determinants of total health to achieve optimal physical and social wellbeing quality.

The study described in this work provided a unique opportunity to examine systemic, behavioral, social and oral health variables in a large cohort of Kuwaiti children (n= 8,317). Initiated in 2012, the Kuwait Healthy Lifestyle study was conducted by a joint effort of The Forsyth Institute in Cambridge, MA, USA, and The Dasman Institute in Kuwait. Its goal was to evaluate risk factors in the school-age children of Kuwait for development of type 2 diabetes by using a longitudinal cohort study design. The manuscripts in this thesis are based on the data collected in this large, comprehensive study.

The State of Kuwait is situated at the western head of the Arabian Gulf with an area of 17,820 square km and a population of 3, 548,423 (4). Twenty-six percent of the Kuwaiti population is under 15 years of age; 72% are between 15 and 64 years old, and only 2% of the Kuwaiti population are over 65 years old (4).

Kuwait gained full independence in 1961, and soon after, the oil industry flourished resulting in rapid modernization and urbanization. In parallel to this, there was an abrupt jump in per capita income and exposure to the western lifestyle within the Kuwaiti community. Before the growth of the oil industry, Kuwait was known for its simplicity and austerity. With oil came money and with money came new life habits. Austerity changed to prosperity and simplicity changed to extravagance. Consequently, this wealthier lifestyle contributed to several negative health outcomes such as obesity and other associated adverse health conditions. The widespread adoption of the western diet resulted in added sugar, fast foods, and overabundance of food. Along with the diet came a profound change in lifestyle.

Recent evidence shows that oral and general health share similar risk factors such as diet, tobacco and alcohol use (1, 2). In this analysis, we explored a behavioral risk factor that is recognized as a well-established risk factor for Non-Communicable Diseases (NCDs): short sleep duration (5). The current analysis is the first to investigate the relation between insufficient sleep and one of the most common oral inflammatory conditions in children: gingivitis.

Authorities and stakeholders in Kuwait had previously implemented efforts to address health concerns of the population. In response to poor oral health and lack of adequate treatment and prevention, The Kuwait School Oral Health Program was established in 1982 as a comprehensive school-based and clinical program providing oral health education, prevention and treatment to all Kuwaiti school children. This program is a joint venture between the Ministry of Health, Kuwait and The Forsyth Institute. More recently, the Dasman Diabetes Institute was established in Kuwait in 2010 in response to the epidemic of type 2 diabetes in the country. The mission statement for this Institute is "To prevent, control and mitigate the impact of diabetes and other chronic conditions in Kuwait, through active programs of research, training, education, and health promotion and thereby improve the quality of life in the population" (6).

These two organizations, the Kuwait School Oral Health Program and the Dasman Diabetes Institute, are significant pioneers in initiating health related research in Kuwait. They came together to carry out this study, setting a good example of the collaboration between the oral health and systemic health professions.

The Kuwait Healthy Lifestyle study was a prospective longitudinal cohort study. Data was collected from 6,316 children 8-14 years olds at two-time points (8,371 in 2012-13 and 6,316 in 2014-15). Subjects were approximately equally distributed among 138 elementary schools and represented the six governorates of Kuwait. Calibrated examiners in the selected schools conducted oral examinations, sleep evaluation and dietary interviews, body measurements, physical activity assessments, and collected saliva samples. In the work that follows, we used a portion of these data to address our specific aims. Three multilevel models were conducted to 1) determine the effect of short sleep duration on abdominal obesity and weight gain, 2) investigate the relationship between short sleep and gingival inflammation, and 3) identify risk behaviors contributing to reduced night sleep among Kuwaiti children.

The integration between oral and general health has recently gained a special attention in public health research. It was reported recently by the World Health Organization that

‘Oral health is an essential element of general health and quality of life through an individual's life-course’, yet one that is often neglected in integrated approaches to the promotion of public health. Moreover, recommendations recently released by the FDI World Dental Federation have emphasized the fact that NCDs are a growing global threat. Oral diseases are integral to prevention and control of NCDs. The current momentum for NCDs is a window of opportunity to improve oral health on a global scale by continued advocacy for the integration of oral diseases into action plans for prevention and control of NCDs; with a particular focus on evaluating social and behavioral interventions is needed as well (7).

Emphasizing the perception of the interconnection between oral health and general health is a fundamental concept for identifying appropriate oral health strategies at both individual and community levels. The work presented here highlights the importance of integrating oral health into overall general health by identifying a novel common behavioral risk factor for both obesity and gingivitis, sleep duration.

# MANUSCRIPT 1

## SHORT SLEEP DURATION AS A RISK FACTOR FOR OBESITY IN KUWAITI CHILDREN


## ABSTRACT

Background: The prevalence of obesity in Kuwaiti children is among the highest in the world. Objective: The aims of this study were to assess the role of short sleep duration as a risk factor in increasing abdominal obesity among Kuwaiti children, and to identify life style habits that contribute to sleep and obesity in this population.

Methods: Waist circumference (WC) measurements, sleep evaluation interviews, and life style habits were collected from 8,371 children (10 y) from 185 schools representing all of the six governorates of Kuwait. Multivariate linear regression models were constructed to determine two different continuous outcomes; WC and daily sleep hours. Independent variables included TV and videogame use, difficulty breathing at night, participating in sports programs, and diabetic parents, adjusting for snacking, gender, and governorate.

Results: Short sleep duration was significantly associated with increased WC in Kuwaiti children ($P<0.001$). No significant association was detected between obesity and difficulty breathing at night ($P=0.4$). Watching TV or playing videogames before bedtime, as well as having TV in the bedroom were significant factors related to decreased sleep duration among Kuwaiti children ($P<0.001$).

Conclusion: Short sleep duration is a significant risk factor that increased abdominal obesity among Kuwaiti children. Additionally, it was found that TV and videogame use were major risk factors contributing to decreased sleep duration and increased obesity among this population.

# INTRODUCTION

Obesity in children is a growing global problem. Nowhere is this felt to a greater extent than in Kuwait where, according to the International Diabetes Foundation every fourth adult has type 2 diabetes (8) and by our data every fourth 10-year old child is obese. Given the high prevalence of obesity in Kuwaiti children, there are serious health implications for these children as they reach adulthood, given the fact that overweight in children is a predictor of obesity in adulthood (9). Obesity is associated with an increased risk of costly chronic diseases, particularly cardiovascular disease and diabetes (10-13).

Studies report a remarkable decline in night sleep duration in several different populations, with a parallel substantial raise in the prevalence in obesity in these populations (14). It has recently been suggested that inadequate sleep is a well-established risk factor associated with the development of obesity and metabolic syndrome(15-19). One aim of this analysis was to determine the association between inadequate sleep as a risk factor for increased abdominal obesity. The majority of research related to sleep and obesity has been conducted in western countries, and shows that children who sleep less at night have significantly higher risk of becoming obese than children who sleep longer (16, 20). For these reasons, it seems logical to examine sleep duration as a risk factor for obesity in a population having the highest prevalence of adult obesity in the world; the Kuwaiti population (21). There is a strong body of evidence showing that screen based activity has significantly contributed to decreased sleep time and increased obesity (22-24). For this purpose we evaluated life style habits that might contribute to obesity in Kuwaiti children; such as TV and videogame factors (screen based activity).

# METHODS

**Study Approval**

This cross-sectional study was approved by the Forsyth Institutional Review Board in Cambridge MA, U.S.A. and the Dasman Institute Human Ethical Review Committee in Kuwait (RA/065/2011 and RA/005/2011). Arabic language informed consent was provided to parents (or guardians) and assent provided to the children. Assent was obtained on the day of the study visit prior to participation in the study. Written consent forms were collected and securely stored at the School Oral Health Program of Kuwait.

**Study population**

Between October 2011 and May 2012, cross sectional data were collected from 8,317 children representing 185 public elementary schools in Kuwait. The Kuwaiti participants were in the 4th or 5th grades and their ages ranged between 8-11 years old. There were approximately equal distributions of participants across the six governorates of Kuwait in the sample. Students enrolled in these schools represent all social levels and ethnic groups of Kuwaiti children but not expatriate children. All volunteers were accepted and selection into the cohort was not randomized across the population.

**Data collection**

Subject identification, body weight analysis, sleep evaluation interviews, nutrition analysis interviews, and life style habits information were collected by two calibrated teams. Information on these variables was recorded into a programmed iPad (Apple, Cupertino, CA) system for internet transfer.

***Outcome measures:***

Two outcomes were assessed in this cross sectional study:

1) Waist circumference: WC was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest at minimal respiration for each participant/subject. Each measurement was conducted using a paper anthropometry tape and the resulting circumference was rounded to the nearest centimeter.

2) Sleep duration: Each child was asked: 1) during the past week, at what times did you go to sleep? 2) During the past week, at what times did you wake up? On the weekend, what time do you normally go to sleep? 4) On the weekend, what time do you normally wake up? Based on the responses, the average number of sleep hours per day was calculated for each child.

***Explanatory covariates***

Predictors and potential confounding variables related to abdominal obesity and insufficient sleep tested in this study were: TV and videogame related factors, difficulties breathing at night, trouble falling to sleep, calorie intake, participating in sport programs, snacking, obesity, having diabetic mother or father, gender, and governorate of residence. These variables were obtained from one on one interview with each child by calibrated interviewers. An accounting of these covariates are summarized in the descriptive summary tables (Table 1a, 1b, 2, and 3).

Two separate multivariate linear regression analyses were conducted, one with WC, and another with sleep duration as continuous outcomes. Association parameters were estimated using the ordinary least squares method and subsequently assessed using Wald significance tests. The final models were determined using stepwise selection. All models were estimated in Stata 12 statistical software using the regress function. Two multivariate linear regression models were performed to identify the factors that contributed to abdominal obesity and short sleep duration.

The first model was conducted using WC as the outcome to represent abdominal obesity. Recent evidence shows that increased WC compared with Body Mass Index (BMI) measurements has a stronger correlation with diabetes, cardiovascular diseases, and some cancers (25). We first examined the univariate relationship between WC and daily sleep hours, and then we included other obesity-related independent variables such as TV and videogame variables simultaneously using stepwise selection. Model selection was based on clinical relevance and a predetermined significance level of $\alpha=0.05$.We kept all significant variables in the final model as well as the potential confounders (Table 4).

The second model evaluated the relationship between daily sleep hours as the outcome with life-style risk factor variables related to sleep curtailment as the explanatory variables. The stepwise selection method was used to determine a parsimonious model. The explanatory variables considered for selection were TV and videogame factors, having diabetic parents, and adjusting for obesity, snacking, gender and governorate (Table 5).

# RESULTS

The population descriptive statistics for the analysis are summarized in table 1a. There were more girls in the study than boys. The age of children was tightly clustered around 10 years old. The mean WC for boys was 68.9 cm and 68.6 cm for girls. On average, both boys and girls slept 8.8 hours/day.

BMI was slightly higher in boys ($p=0.03$) and the mean systolic blood pressure was significantly higher in boys ($p<0.001$).

Table 1b summarizes the distribution of body weight categories by BMI percentile and by WC according WHO guidelines (26). BMI percentile identified 3.4% more obese children than WC. By BMI percentile there was a significantly higher percentage of obese boys ($p<0.001$). By WC, there was a significantly higher percentage of obese girls ($p=0.004$). The Pearson correlation between categorization of obesity by the two methods was 0.7 ($p<0.001$).

Kuwait is divided into six governorates. As indicated in Table 2, the percentage of children classified as obese by BMI percentile and by WC are compared for each governorate. By both methods, the lowest percentage obese children were in the Bedouin communities of Ahmadi and Jahra. The largest percentage obese by BMI percentile was Capital whereas by WC it was Mubarak Alkabeer. These communities are populated by mixtures of Saudi and Persian descent as are the governorates of Hawali and Farwania. It is likely that the significance of governorates in regression models is related to this genetic segregation.

The results from the first multivariate linear regression model with WC as the outcome are shown in Table 4. The model shows that short sleep duration significantly predicts increased abdominal obesity among the Kuwaiti children ($P<0.002$), with significant variation between governorates. After adjusting for gender and snacking, children residing in Jahra and Ahmadi governorates had a lower estimate of abdominal obesity than other governorates whereas children living in Hawalli, Farwania and Mubarak Alkabeer had a higher estimate of obesity. These observations are consistent with those of Table 2.

Children who had TV in their bedrooms tended to have a larger WC than children who did not ($P<0.003$). There was no significant association between obesity and trouble breathing during sleep that could be a proxy for obstructive sleep apnea ($P=0.4$). Frequency of snacking (binary yes or no) had no significant effect on the model which is not surprising since 98.3% of the children reported "yes" (Table 3).

Results from the second multivariate regression model with daily sleep hours as the outcome variable (Table 5), revealed that the main factors that predict short sleep duration in children were screen based activity factors; we found that watching TV or playing videogames before bedtime significantly reduced daily sleep hours ($p<0.001$). Moreover, using the bedroom for watching TV and playing videogames significantly predicted shorter sleep duration ($p <0.001$). Playing videogames or watching TV more than 2 hours a day also significantly reduced daily sleep hours ($p<0.01$), as well as eating in front of the TV ($p<0.001$). Additionally, we found that that children with a diabetic parent slept significantly less than children of non-diabetic parents ($p<0.001$). This model was adjusted for obesity, snacking, gender, and governorate.

## DISCUSSION

In the present study, the mean WC for Kuwaiti children ages 8-11 years old was 68.6±0.2 cm with no significant difference between boys and girls. Comparing this with WC in other countries such as USA, Australia, UK, Turkey, Japan, Spain, and UK; Kuwait has the highest mean WC worldwide (25, 27). We used WC as the main outcome to represent abdominal obesity because WC has been strongly correlated with cardiovascular diseases, diabetes, and some cancers; The correlation between WC and these diseases is stronger with WC than with BMI percentile (25). In this study, short sleep duration was significantly associated with increased WC ($P<0.002$). The magnitude and the direction of the relationship between obesity and sleep duration is consistent with other studies of children in different populations (14).

### Relation between short sleep duration and obesity

It has been observed that inadequate sleep in healthy subjects is associated with significant hormonal imbalance resulting in increased food intake and snacking (28); this may affect appetite regulation and glucose metabolism and eventually leads to the development of diabetes and cardiovascular disease (18, 28). According to the Centers of Disease Control and Prevention (CDC) and the pediatric clinical guide for sleep (29), 10 - 11 hours of sleep are considered a healthy average sleep duration for 8-11 year-old children. Our data showed that the mean sleep duration was 8.8±1.6 hours, which is less than the healthy average recommended hours. These results suggest that inadequate sleep may be an obesity risk factor that is prevalent in this population. It has been suggested that children and adolescents may be more vulnerable to the effects of inadequate sleep duration than adults (14, 17). The association between obesity and short sleep may be a bidirectional due to the possibility of preexisting Obstructive Sleep Apnea (OSA)(30). It has been suggested that obese individuals are more likely to have inadequate sleep because of the OSA. However, the relation between OSA and obesity in adolescent might be negative in some populations (31). In this study, we asked the children if they have trouble breathing at night as an indicator for OSA and to examine its relation with abdominal obesity. There was no significant association between having trouble breathing during sleep, suggesting that OSA indicator was not a significant factor related to obesity among Kuwaiti children.

There is evidence that sleep deprivation and disrupted circadian rhythm in shift workers are associated with obesity and type2 diabetes (32). Moreover, individuals who have sufficient high-quality sleep have higher levels of melatonin; a hormone secreted by the pineal gland during sleep at nighttime (19). Melatonin plays a crucial role in the maintenance of circadian rhythm and regulates nighttime sleep (33). In addition, melatonin has been reported to play an important role in carbohydrate metabolism and insulin regulation(18). It has been shown that light-at-night-induced suppression of melatonin secretion resulting in reductions in sleep duration (19, 33). Consequently, persons who have insufficient sleep are at risk of cardio-metabolic impairments caused by melatonin disruption that include obesity and other associated metabolic syndromes (34). Thus, if life-style changes were implemented by improving sleep quality, metabolic hormonal balance could improve and susceptibility for obesity would decrease.

**Short sleep duration and the frequency of use TV and videogames**

In this study, we found that children who frequently watch TV or play videogames just before bedtime sleep significantly less than children who rarely or never watch TV or play videogames before bedtime (P<0.001). Similarly, children who mostly use their beds for watching TV and playing videogames significantly slept less than children who rarely or never used their beds for the screen-based activity (P<0.001). In the present analysis, screen based activities factors were the most significant factors that contributed to reduce night sleep duration among Kuwaiti children. We found that children who watch TV or play videogames more than two hours most weekdays and those who eat in front of the TV most weekdays sleep significantly less than other children (P<0.001). In contrast, children who participated in sport programs outside of school tend to sleep more hours (P=0.06), suggesting that being physically active and minimizing screen based activity might contribute to improve sleep duration.

In this study, 40.3% of the Kuwaiti children have TVs in their bedrooms (Table 3), and 86% of the children watch TV or play videogames just before going to bed. Additionally, 82% of the children are watching TV and playing videogames for more than two hours on most weekdays, while only 21.8% participate in sports programs. These are alarming findings that may explain the general reduction in the sleep duration among these children.

It has been shown that short sleep duration and use of electronic devices are associated with excess body weight (35). Using technology for long periods can suppress melatonin secretion (36). Moreover, it has been suggested that short wavelength light from electronic devices significantly inhibits melatonin secretion resulting in delayed sleep and diminished quality of sleep(35). A randomized study found that individuals who used electronic books before bed time have decreased evening sleepless and have lower level of melatonin secretion than individuals used printed books(37). As mentioned earlier, melatonin has an important role in carbohydrate metabolism and insulin regulation (33). In the present study there was a positive significant association between screen based activity factors, insufficient sleep, and obesity. This supports the hypothesis that disturbance of melatonin secretions by long exposure to electronics devices and inadequate sleep can promote the inflammation in the body through hormonal disruption and potentially can lead to abnormal weight gain, independent of the amount of caloric intake. Additionally, we found that children of diabetic parents significantly sleep less (Table 5) than children who have non-diabetic children ($P<0.001$). Giving the fact that short sleep duration is related to diabetes and metabolic diseases (15, 25, 29), this might be attributed to the family life style factor; potentially, parents who don't value sufficient night sleep may let their children stay up late and these parents are more susceptible to be diabetic.

**Obesity and the frequency of TV and videogames use**

In the present study, we found that 65.6% of the children reported eating in front of the TV on most weekdays (Table 3). Children who have TV in their bedrooms (Table 4) significantly have more abdominal obesity than children who don't have TVs in their bedroom ($p=0.003$). Screen based activity has been considered a major public health issue that is associated with unexpected health conditions (23). Independent of physical activity, adolescents who spend excessive time in screen-based activities are more prone to obesity (36, 38). Strong association has been consistently observed between the presence of the TV in the bedroom and weight gain, mediated by TV viewing time(36, 39). Moreover, screen-based activities have been shown to divert children's attention away from the control of food intake, and eventually distracting them from signals of satiety (40-42). It has been found that the desire to eat fast foods may be increased by TV viewing due to the pairing mechanism between eating fast food and TV viewing overtime(42, 43). This conditioning may also be related to the child's exposure to the unhealthy TV food advertisements (42). Other studies that explain the relation between TV and overeating found that TV has a negative effect on memory; participants were less able to accurately estimate their food intake while watching TV (42-44).

**Environmental factors**

Within Kuwait there are clusters of inhabitants with differing social class, ethnicity, and family origin. There are three identified ethnic groups in Kuwait based on the family origin and tribal background; Bedouin, Saudi, and Persian (45). Each ethnic group is known by certain characteristics and specific life habits that eventually could shape health behaviors and outcomes in this population. A recent genetic study in Kuwait has shown that there is distinct genetic difference among the three ethnic groups and they recommended considering ethnic assessment in public health practice to target risk groups for health intervention programs (45). In this study we found differences in the proportion and magnitude of obesity between the six governorates of Kuwait (Table 2 and table 4). Ahmadi and Jahra governorates are considered Bedouin governorates. These two Bedouin governorates have lower obesity percentage compared to other governorates who have mixed of Saudi and Persian community (Table 2 and Table 4). From our knowledge about Kuwaiti population, Bedouins communities likely have a lower socioeconomic status with less accessibility to restaurants and technology and appear to have become less obese than Saudi and Persian populations within Kuwait.

The hot climate in Kuwait clearly contributes to promotion of a sedentary life style among the Kuwaiti population. The weather is extremely hot during most of the year days with a temperature exceeding 50º Celsius. The lack of awareness of the negative effect of the electronic devises with the scarcity of the indoor sport programs can also substantially contribute to the extreme use of screen base activity as an available alternative to indoor entertainment in this hot environment.

## CONCLUSION

Short sleep duration is a significant risk factor associated with increased abdominal obesity among Kuwaiti children. Screen based activities is a major issue contributing to reduced sleep time and increased obesity. Public health interventions should target children and their families to improve their life style habits by having sufficient night sleep as well as limit screen time in addition to maintaining healthy eating and being physically active.

## TABLES

Table No.1a: Population descriptive statistics (mean ± s.d.). P values computed by two-sample t-test with sex as the grouping variable.

| Variable | Boys (n=3,219) | Girls (n=5,098) | P value |
|---|---|---|---|
| **Age (y)** | 9.9 ± 0.67 | 9.9 ± 0.67 | 0.5 |
| **Waist circumference (cm)** | 68.9 ± 16.3 | 68.6 ± 16.3 | 0.6 |
| **Daily sleep (h)** | 8.8 ± 1.65 | 8.8 ± 1.61 | 0.7 |
| **Weight (kg)** | 40.3 ± 13.8 | 40.8 ± 32.5 | 0.4 |
| **Height (cm)** | 137.1 ± 9.4 | 137.6 ± 9.4 | 0.03 |
| **Body Mass Index (kg/m$^2$)** | 21.0 ± 5.4 | 20.8 ± 5.2 | 0.3 |
| **Systolic blood pressure (mmHg)** | 110.3 ± 17.5 | 108.9 ± 15.4 | <0.001 |
| **Diastolic blood pressure (mmHg)** | 74.6 ± 13.9 | 74.1 ± 24.1 | 0.2 |

Table No.1b: Summary of body weight distribution by BMI percentile and waist circumference[19]. Percent differences between sexes were all highly significant by Pearson chi-square analysis (p<0.001).

| Overweight and obesity by BMI percentile | Total (proportion) | Boys | Girls |
|---|---|---|---|
| **Underweight (<5th)** | 193 (2.3%) | 63 (2.0%) | 130 (2.6%) |
| **Normal Healthy weight (≥5th <85th)** | 4,129 (49.6%) | 1,528 (47.5%) | 2,601 (51.0%) |
| **Overweight (≥85th to <95th)** | 1,791 (21.5%) | 621 (19.3%) | 1,170 (23.0%) |
| **Obese (≥95th)** | 2,204 (26.5%) | 1,007 (31.3%) | 1,197 (23.5%) |
| **Obese by waist circumference** | 1,914 (23.1%) | 688 (21.4%) | 1,226 (24.1%) |

Table No.2: Obesity proportion based on governorates and family origin.

| Governorate | Obesity by BMI percentile (WHO) | Obesity by WC |
|---|---|---|
| **Ahmadi (Bedouin)** | 20.8% | 17.5% |
| **Jahra (Bedouin)** | 23.6% | 21.2% |
| **Mubarak Alkabeer (Mostly Bedouin)** | 24.8% | 28.4% |
| **Farwania (Mostly Bedouin)** | 26.2% | 27.2% |
| **Hawali (Mix Saudi and Persian)** | 30.0% | 24.0% |
| **Capital (Mix Saudi and Persian)** | 33.4% | 24.6% |

Table 3: Summary descriptions of life style habits. P values apply to differences between boys and girls by chi square analysis. (VG=video games).

| Life style habit variables | Total percentage | Boys | Girls | P value |
|---|---|---|---|---|
| | | Number (percentage) | | |
| **Have TV in bedroom** | 40.3% | 1,634(51.2%) | 1,721(33.6%) | <0.001 |
| **Watch TV and play VG before bedtime** ** | 86% | 2,841(89%) | 4,326(84%) | <0.001 |
| **Watch TV or play VG more than two hours 4 days /week or more** * | 82% | 2,580(80.8%) | 4,238(82.5%) | 0.07 |
| **Eat in front of TV 4 days a week or more*** | 65.6% | 2,092(65.6%) | 3.370(65.7%) | 0.09 |
| **Use bed for watch TV and play VG** ** | 37% | 1,052(33%) | 2,132(39.5) | <0.001 |
| **Participate in sport program outside school** | 21.8% | 1,071(33.9%) | 730(14.4%) | <0.001 |
| **Trouble breathing at night**** | 11% | 350(10%) | 567(11%) | 0.9 |
| **Trouble falling to sleep**** | 19.8% | 647(20%) | 1,001(19.5%) | 0.3 |
| **Have diabetic mother** | 8% | 257(8%) | 404(7.8%) | 0.3 |
| **Have diabetic father** | 14% | 451(14.3%) | 714(13.8%) | 0.01 |
| **Snacking** | 98.3% | 3,110(98%) | 5,029(98.5%) | 0.2 |

* Ordinal variable for use during weekdays.
** Ordinal variable (never, rarely, sometimes, often). The result presented for the sometimes and often vs. never and rarely

Table 4: First multivariate linear regression model with waist circumference as the outcome.

| Covariate | Estimate (se) | P value | Confidence Interval |
|---|---|---|---|
| **Daily sleep hours (continuous)** | - 0.4(0.13) | 0.002 | -0.66, -0.14 |
| **Having TV in bedroom*** | 1.3(0.44) | 0.003 | -2.16, -0.42 |
| **Trouble breathing at night **** | 0.54(0.71) | 0.4 | -0.86, 1.95 |
| **Trouble falling to sleep **** | 1.8(0.56) | 0.001 | 0.7, 2.93 |
| **Frequency of snacking*** | 0.6(1.7) | 0.7 | -3.95, 2.71 |
| **Gender *** | 0.7(0.46) | 0.1 | -0.14, 1.63 |
| **Governorate (Reference: Capital)** | | | |
| **Ahmadi** | -2.3(0.6) | 0.001 | -3.6, -1.005 |
| **Jahra** | -0.9(0.6) | 0.1 | 1.0, 3.6 |
| **Hawalli** | 0.4(0.7) | 0.5 | -0.06, 1.9 |
| **Farwania** | 1.4(0.7) | 0.03 | 0.07, 2.8 |
| **Mubarak Alkabeer** | 3.7(0.87) | <0.0001 | 2.0, 5.4 |

* Binary variables
** Ordinal variable of 4 categories (no, rarely, sometimes, often) was used as a continuous variable.

Table 5: Second multivariate linear regression model with daily sleep hours as the outcome variable.

| Factor | Estimate (se) | P value | Confidence interval |
|---|---|---|---|
| **Watching TV or play videogames before bedtime *** | -0.26(0.05) | <0.001 | -0.36, -0.16 |
| **Using bed for watching TV or playing videogames *** | -0.13(0.03) | <0.001 | -0.2, -0.06 |
| **Watching TV and playing videogames more than 2 hours 4 days a week or more**** | -0.15(0.002) | 0.001 | -0.24, -0.05 |
| **Eating in front of the TV every day more than 2 hours 4 days a week or more**** | -0.22 (0.03) | <0.001 | -0.3, -0.15 |
| **Participating in a sport program outside the school (yes vs. no)** | 0.08 (0.04) | 0.06 | -0.17, -0.005 |
| **Have diabetic father** | - 0.16 (0.05) | 0.001 | -0.06, -0.26 |
| **Have diabetic mother (yes vs. no)** | - 0.28 (0.06) | <0.001 | -0.14, -0.4 |
| **Obese (binary variable by WHO percentile)** | 0.14(0.04) | <0.001 | -0.22, -0.06 |
| **Snacking (yes vs. no)** | 0.02(0.1) | 0.8 | -0.25, 0.3 |
| **Sex (boys vs. girls)** | 0.0003(0.03) | 0.9 | -0.074, -0.075 |
| **Governorate (Reference: Capital)** | | | |
| **Ahmadi** | -0.04(0.05) | 0.4 | -0.15, 0.06 |
| **Jahra** | -0.26(0.05) | <0.001 | -0.37, -0.1 |
| **Hawalli** | 0.02(0.06) | 0.6 | -0.09, 0.15 |
| **Farwania** | -0.1(0.06) | 0.06 | -0.2, 0.007 |
| **Mubarak Alkabeer** | 0.18 (0.07) | 0.01 | 0.03, 0.32 |

* Ordinal variable for use during weekdays.
** Ordinal variable (never, rarely, sometimes, often). The result presented for the sometimes and often vs. never and rarely

# MANUSCRIPT 2

## A MULTILEVEL LONGITUDINAL ANALYSIS OF SHORT SLEEP DURATION AND OBESITY/OVERWEIGHT IN KUWAITI CHILDREN


## ABSTRACT

It has been shown that inadequate sleep has negative effect on health including obesity, diabetes, and cardiovascular diseases. Current evidence showed that short sleep duration impairs endocrine system and disrupt metabolism resulting in abnormal weight gain, independent of the amount of calories intake. The aim of this study was to investigate the longitudinal relation between short sleep duration in increasing abdominal obesity among Kuwaiti children.

Longitudinal data were collected from 6,316 children 8-14 years old at two-time points: 2012 and 2014. Children were approximately equally distributed among 138 elementary schools representing the six governorates of Kuwait. Calibrated examiners conducted body weight measurements, sleep evaluation interviews, saliva samples collections, and nutritional analysis.

Two multilevel random intercept and slope models were conducted to determine the relationship between sleep duration and abdominal obesity on three levels: within schools, amongst children, and over time. The primary independent variables were the number of daily sleep hours and salivary glucose level. Other explanatory variables and confounders assessed were: governorate, trouble breathing at night, snacking frequency, and gender.

There was a significant increase of abdominal obesity with shorter sleep duration over time ($p<0.05$). No significant association was detected between abdominal obesity and high glucose level. There were statistically significant variations between schools and children over time. Longitudinal analysis of Kuwaiti children revealed that shorter sleep duration predicts abdominal obesity in Kuwaiti children with strong clustering effect within schools over time.

## INTRODUCTION

It has been recently recognized that healthy sleep patterns play a fundamental role in improving overall health. Current evidence shows that children who sleep less at night have a significantly higher risk of becoming obese than children who sleep longer (16, 20). Similarly, little and disrupted sleep is related to obesity and metabolic syndrome (15, 17-19). Consistent delayed night sleep has been shown to increase the risk and the severity of infectious diseases, cancers, respiratory diseases, and depression (5).

A recent meta-analysis of longitudinal studies, composed of 24,821 subjects, reported that individuals who sleep for shorter durations at night are twice as likely to be at risk of gaining weight and becoming obese compared with individuals who sleep for longer durations (46). All of these studies were conducted in western countries. The aim of the present analysis was to ascertain the longitudinal association between short sleep duration and increased abdominal obesity amongst a population having the highest prevalence of obesity in the world - the Kuwaiti population (21). Previous work has determined a significant relationship between insufficient sleep and obesity amongst Kuwaiti children

(47). However, it is important to keep in mind that this previous analysis used the cross-sectional design, which has its inherent limitations.

In the present study, we used prospective longitudinal multilevel analysis, which has the advantage of allowing accounting for the unmeasured confounders to precisely determine the longitudinal relationship between the predictors and the outcome. Moreover, this multilevel modeling system aids in accounting for the clustering effect between the schools and the variation between children for more precise and accurate inferences (48).

## METHODS

### Study Approval

This prospective cohort study was approved by the Forsyth Institutional Review Board in Cambridge MA, U.S.A. and the Dasman Institute Human Ethical Review Committee in Kuwait (RA/065/2011 and RA/005/2011). Informed consent in the Arabic language was provided to parents (or guardians) and assent provided to the children. Assent was obtained on the day of the study visit before participation in the study. Written consent forms were collected and securely stored at the School Oral Health Program in Kuwait.

### Study population

In the first phase of the study, data was collected from 8,317 children representing the six governorates in Kuwait between October 2011 and May 2012. The Kuwaiti participants were in the 4th and 5th grades, and their ages ranged between 8-11-years-old in 2012 (visit1) for this initial screening. The final data collection occurred between October 2014 and May 2015 (visit2), where the same data were collected from the same cohort of children (n=6,316) representing 138 public schools in Kuwait. The response rate for the second round was very high (95%). There were 2,001 children from visit1 who were unable to follow-up by the second examination visit due to the approaching end of the academic year. We included only the 6,316 subjects who had complete measurements at both visits. The distribution of participants across the six governorates of Kuwait was approximately equal. The students enrolled in these schools represent a wide variety of social class and ethnic groups among the Kuwaiti population, but do not include expatriate children. All volunteer participants were accepted, and selection into the cohort was not randomized across the population.

**Data collection**

Subject identification, body weight measurements, sleep evaluation interviews, saliva samples, nutrition analysis interviews, and lifestyle information was collected by two calibrated teams at each visit. These information variables were recorded into a programmed system on an iPad (Apple, Cupertino, CA) for Internet transfer.

***Outcome measure***

Each of the following tow outcomes was analyzed using two separate multilevel models:

1) Waist Circumference (WC): WC, a continuous variable, was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest at minimal respiration for each participant. Each measurement was conducted using a paper anthropometry tape, and the resulting circumference was rounded to the nearest centimeter.

2) Obesity by WC: A binary variable was created to represent children with abdominal obesity versus children without abdominal obesity. Abdominal obesity was defined as waist circumference ≥ 90th percentile using WHO guidelines (49).

Recent evidence shows that increased WC, compared with Body Mass Index (BMI) measurements, has a strong correlation with diabetes, cardiovascular diseases, and some cancers in (25). Therefore, we used WC over BMI to represent abdominal obesity.

***Explanatory covariates***

1) Sleep duration; was assessed and obtained through "one-on-one" interviews with each child by calibrated interviewers. Each child was asked: 1) during the past week, at what time did you go to sleep? 2) During the past week, at what time did you wake up? Based on the responses, the average number of sleep hours per day was calculated for each child.
2) Salivary glucose level; a single saliva sample was collected at school early in the morning before breakfast at 8:00 AM. Children were instructed not to eat anything before coming to school. The glucose level in the saliva was measured, and a binary variable was created. Values greater or equal to 1.13 mg/dL were defined as high (50), and values less than 1.13 mg/dL were defined as low glucose levels.
Detailed information on the collection of saliva samples was mentioned in another study (51).
Additional independent variables included in our analyses were: snacking, trouble breathing at night, governorates, gender, and quality of sleep information. These variables were assessed and obtained from "one-on-one" interviews with each child by calibrated interviewers. These variables are described in the appendix and summarized in table 5.

**Statistical Analysis**

Our data had a three-level hierarchical structure comprised of the following: time (at the first level), nested within children (at the second level), and nested within schools (at the third level). Level-3 represented the contextual effect of the school environment on the outcome. In order to account for cluster sampling design, estimate associations between sleep and WC/obesity outcomes, we specified three-level two multilevel models.

The first model was a longitudinal linear regression model with WC as a continuous outcome. The second model was a logistic model with obesity, defined by WC, as a binary outcome. The same independent variables were used in both the linear and the logistic models to accurately determine the relationship between abdominal weight gain/obesity and sleep duration, and the relationship between weight gain/obesity and higher salivary glucose level over a period of two years. We also included pre-specified, individual level variables that are known predictors of abdominal obesity and could be confounders of the associations being investigated.

For both models, we performed three-level fixed- and random-effects multilevel longitudinal analyses to assess the influence of school context on the outcome (tested over a period of two years of time), and to consider the variance between schools and between individuals over time. All statistical analysis modeling was conducted in MLwin. MLwiN is a statistical software package for fitting multilevel models (Centre for multilevel modeling-University of Bristol). The steps of model building are described in the appendix.

Variable selection was based on clinical relevance and potential confounding. Beta coefficients for the linear model, odd ratios for the logistic model, and their 95% confidence intervals were estimated at a predetermined significance level of $\alpha=0.05$.

## RESULTS

The population descriptive statistics for the analysis are summarized in Tables 1, 2, 4 and 5. There were more girls (61.4%) in the study than boys (38.5%). The age of children was tightly clustered around 10 years old in visit 1, and around 12 years old in visit 2 (Table 1). On average, both boys and girls slept 8.8 hours/day in visit 1, and 9.9 hours/day in visit 2, with an average bedtime occurring at 10:00PM (Table 1). Table 2 summarizes the distribution of body weight categories by BMI percentile and by WC, according WHO guidelines (26). A big increase in the incidence of childhood obesity over time was observed (obesity by WC in visit 1= 22.6% and in visit 2= 38%). Obesity by WC was higher in girls than it was in boys (obesity by WC in boys in visit 1 = 20.4% and in visit 2=35.4%; obesity by WC in girls in visit 1= 23.4% and in visit 2 =40.2%). Even though the prevalence of obesity has significantly increased over time throughout the six governorates of Kuwait, we showed that children residing in the Capital governorate had the highest prevalence of obesity (42%). On the other hand, children residing in Ahmadi and Jahra governorates had the lowest prevalence of obesity (28.5%, 33.4%, respectively). A small percentage of the children reported having trouble breathing at night (12.4%), which was more common in boys (13.6%) than in girls (12.4%) (Table 5).

Table 6 shows the outcomes from the final two multilevel models for WC and obesity. These two models show statistically significant associations between increases in WC and obesity with shorter sleep duration over time ($P<0.05$). Results from the logistic model shows that one hour more of sleep per night is associated with a 3% decrease in the odds of developing abdominal obesity with a statistically significant rate of the increase of obesity over time (Table 6). Higher glucose levels did not show a significant relation with WC neither with obesity. The logistic model revealed that girls were at higher risk of gaining weight and developing obesity than boys ($P<0.05$). After adjusting for the examined variables, the capital governorate had a significantly higher magnitude of increase in obesity and weight gain compared to the other six governorates of Kuwait (Table 6). In this analysis, a significant relationship was detected between obesity and trouble breathing at night only in the logistic model, but not in the linear model ($OR=1.11$, $P<0.05$); this could be a proxy for obstructive sleep apnea. Frequency of snacking had no significant effect on the linear model, which is not surprising considering that more than 95% of the children reported snacking during the day (Table 5).

In the two models, even after accounting for important individual predictors of abdominal obesity and sleep duration, there were statistically significant time variations for both school- and children- levels at the two time points (Table 7).

# DISCUSSION

Our data showed that 46.6% of Kuwaiti children go to sleep at 10:00PM or after. Most of the children reported that they wake up around 6:00AM. The mean sleep duration amongst those children was less than the minimum required hours for this age group in both visits (Table 1). According to the Centers of Disease Control and Prevention (CDC) and the pediatric clinical guide for sleep (29), 10 - 11 hours of sleep are considered a healthy average sleep duration for 8-12 year-old children. In the present analysis, we showed that one hour more of night sleep is associated with a 3% decrease in the odds of developing abdominal obesity, with significant rate of change in obesity over time (Table 6). This indicates that longer night sleep is a protective factor against obesity in Kuwaiti children. Similarly, the linear model revealed that short sleep duration is significantly associated with increased WC, with significant rise over time (Table 6). Using this multilevel longitudinal modeling increases the precision of the analysis by accounting for the unmeasured confounders, variation between children, clustering within schools, and clustering of the observations between the two time points (48). These findings from the present prospective cohorts ascertain that inadequate sleep may be an obesity risk factor that is prevalent in the child population of Kuwait.

In comparing the WC of Kuwaiti children with the WC of children in the same age groups in other countries, such as the USA (52), Australia, the UK, Turkey, Japan, Spain, and Cuba, we found that Kuwait has the highest mean WC worldwide (25, 27). Additionally, Kuwiti children had a higher mean BMI percentile and larger proportion of obese children than American children among the same age groups (Table 4) (53).

We used WC as the main outcome in the present analysis to represent abdominal obesity because WC has been strongly correlated with cardiovascular diseases, diabetes, and some cancers, and the correlation between WC and these diseases is stronger with WC than with BMI percentile (25).

Short sleep duration plays an important role in the risk of obesity by creating hormonal imbalance (18, 28), potentially affecting appetite regulation and glucose metabolism, and eventually leading to the development of cardiometabolic diseases such as diabetes and cardiovascular disease (18, 28). It has been reported that the association between sleep duration and weight gain is strongest between the ages of 2-18 years old (14, 17), suggesting that children and adolescents may be more vulnerable to the effects of inadequate sleep duration.

The association between obesity and inadequate sleep may be bidirectional due to the possibility of preexisting Obstructive Sleep Apnea (OSA)(30). It has been suggested that obese individuals are more likely to have inadequate sleep because of OSA. However, the relationship between OSA and obesity in adolescents might be negative in some populations (31). In this study, we asked the participating children if they have trouble breathing at night as an indicator for OSA, and to examine its relationship with abdominal obesity. We found that obese children are 11% more odds to experience trouble breathing at night (Table 6), suggesting that OSA indicators might be related to obesity amongst Kuwaiti children. However, the relationship between trouble breathing during sleep and obesity/increased WC was found to be significant in the logistic model, but was not significant in the linear model (Table 6). We haven’t detected any significant associations between increased salivary glucose levels with susceptibility of obesity.

It was also found that individuals who experience sufficient high-quality sleep have higher levels of melatonin, a hormone secreted by the pineal gland during sleep at nighttime (19). Melatonin plays a crucial role in the maintenance of the circadian rhythm and regulating nighttime sleep (33), and is reportedly significant in carbohydrate metabolism and insulin regulation (18). Consequently, persons who experience insufficient sleep are at risk of metabolic impairments caused by melatonin disruption, such as obesity and type2 diabetes (34). Hence, if lifestyle changes were implemented by improving sleep quality, metabolic hormonal balance could improve and susceptibility for obesity would decrease. Moreover, genetic studies suggest that improving the quality of one's sleep at night could decrease the individuals' susceptibility to obesity through maintaining and balancing the metabolic activities (54).

**School and neighborhood effects, and multilevel analysis**

The school environment has recently gained special attention in public health research. Several studies indicate that the surrounding environment strongly influences a child's health (55). School plays a fundamental role in structuring the social context of a child's behavior. Children with similar personal demographics and socioeconomic characteristics tend to live close and share same health conditions (48) because they are exposed to the same surrounding atmosphere, and tend to influence each other, which eventually would shape a child's health behaviors and outcomes (55) .

From statistical perspective, the clustering effect of observations within schools creates group dependency (48) . Group dependency reflects the similarities of children within the context they are nested in. The best analysis to account for the within group dependency is the multilevel analysis (56). Within group correlation violates the regular statistical analysis such as linear regression resulting in incorrect inference (48) . Multilevel analysis has been widely used to investigate the effect of social context on the health outcomes (55). In this longitudinal prospective study, we were able to detect a statistically significant variability between schools across time (Table 7), suggesting that school environment has strong influence in shaping the obesity outcome amongst Kuwaiti children.

In the present analysis, we accounted for the geographical setting and the neighborhood effect on the outcome. We considered the fixed effect to examine the difference between the six governorates of Kuwait on developing obesity. In this study, we found differences in the proportion and magnitude of abdominal obesity between the six governorates of Kuwait (Table 3 and table 6). Future studies should identify health determinants of schools and neighborhoods that promote the progression of some health conditions for potential intervention and prevention. School health intervention programs should consider behavioral sleep improvement by educating children and parents about the importance of sleep on overall health. It has been shown that behavioral sleep improvement is effective and has the potential to support immunity and relieve symptoms of chronic diseases (5).

**Limitation of the study**

The self-reported habitual sleep duration might be susceptible to reporting bias. There were no contextual school level variables measured in the analysis that might explain the big variation between schools.

## CONCLUSION

This prospective cohort showed that short sleep duration is a significant risk factor associated with increased abdominal obesity among Kuwaiti children. Public health intervention programs should target children and their families to focus on improving night sleep behavior as well as maintaining healthy eating habits and being physically active. Future research should identify healthy school determinants, to help designing future cost-effective school intervention and prevention programs for healthy generation.

## TABLES AND APPENDIX

Table 1: Population descriptive statistics (mean ± sd) stratified by sex and visit.

| Variable | Visit 1: n (%) | | | Visit 2: n (%) | | |
|---|---|---|---|---|---|---|
| | Boys | Girls | Total | Boys | Girls | Total |
| **Age (y)** | 9.9 ± 0.01 | 9.9 ± 0.01 | 9,9±0.008 | 12 ± 0.01 | 12± 0.01 | 12±0.008 |
| **Waist circumference (cm)** | 68 ± 0.2 | 68.2 ± 0.2 | 68.1±0.1 | 80 ± 0.3 | 80.5 ±0.2 | 80.5±0.1 |
| **Daily sleep (h)** | 8.9 ± 0.03 | 8.9 ± 0.02 | 8.9 ±0.02 | 9.8 ± 0.02 | 9.9 ± 0.02 | 9.9±0.01 |
| **Weight (kg)** | 40.3 ± 0.2 | 40 ± 0.2 | 44±0.1 | 52.4 ±0.3 | 53.1 ± 0.2 | 52.8±0.2 |
| **Height (cm)** | 137.1 ± 0.9 | 137.6 ± 0.1 | 137.5±0.1 | 150 ±0.3 | 151.4 ± 0.1 | 151±0.1 |
| **Body Mass Index ($kg/m^2$)** | 20.8 ± 0.1 | 22.8 ± 0.08 | 20.8±0.06 | 22.8 ± 0.1 | 23 ± 0.09 | 22.8±0.07 |
| **Systolic blood pressure (mmHg)** | 109.6 ± 0.3 | 108.8 ±0.2 | 109±0.2 | 118 ±0.3 | 116± 0.2 | 117±0.2 |
| **Diastolic blood pressure (mmHg)** | 74.3 ± 0.3 | 73.8 ± 0.2 | 74±0.2 | 79 ± 0.3 | 78.8 ±0.2 | 79.2±0.2 |

Table 2: Summary of body weight distribution showing the proportions of BMI percentile and waist circumference (26) stratified by sex and visit.

| Variable | Visit 1: n (%) | | | Visit 2: n (%) | | |
|---|---|---|---|---|---|---|
| **Overweight and obesity by BMI percentile** | **Boys** | **Girls** | **Total** | **Boys** | **Girls** | **Total** |
| Underweight (<5th) | 17 (0.8%) | 40 (1%) | 57 (1%) | 52 (2%) | 62 (1.8%) | 114 (2%) |
| Normal weight (≥5th <85th) | 847 (40%) | 1,448 (43%) | 2,295 (42%) | 772 (36.7%) | 1,372 (41%) | 2,144 (39.3%) |
| Overweight (≥85th to <95th) | 824 (20%) | 829 (24.7%) | 1,253 (23%) | 436 (20.7%) | 855 (25.4%) | 1,291 (23.6%) |
| Obese (≥95th) | 815 (38.7%) | 1,036 (30.8%) | 1,851 (33.9%) | 843 (40%) | 1,064 (31.7%) | 1,907 (35%) |
| **Obese by waist circumference** | 438 (20.8%) | 786 (23.4%) | 1,224 (22.4%) | 746 (35.4%) | 1,350 (40.2%) | 2,096 (38.4%) |

Table 3: Overweight and obesity proportions by BMI stratified by governorate and visit.

| Governorate | Overweight by BMI N (%) | | Obesity by BMI N (%) | |
|---|---|---|---|---|
| | **Visit 1** | **Visit 2** | **Visit 1** | **Visit 2** |
| **Ahmadi** | 274 (23.8%) | 176 (24%) | 310 (27%) | 327 (28.5%) |
| **Jahra** | 250 (21.5%) | 277 (24%) | 349 (30.1%) | 387 (33.4%) |
| **Mubarak Alkabeer** | 86 (21%) | 95 (23.2) | 129 (31.4%) | 144 (35.3%) |
| **Farwania** | 202 (24%) | 193 (23%) | 292 (34.8%) | 304 (36.4%) |
| **Hawali** | 137 (25.5%) | 139 (25%) | 201 (37.5%) | 199 (35.8%) |
| **Capital** | 304 (22.2%) | 311 (23%) | 570 (41.7%) | 546 (40.3%) |

Table 4: comparing Kuwaiti children to the American Children in BMI and Obesity (52, 53)

| Variable | Kuwaiti children | | American children | |
|---|---|---|---|---|
| Age | 10 years old | 12 years old | 10 years old | 12 years old |
| Mean BMI ((kg/m²) | 20.8 | 22.8 | 18 | 20 |
| Obesity by BMI (%) | 33.9 | 35 | 17.7 | 20.5 |
| Mean waist circumference (cm) | 68 | 80.5 | 65 | 70 |

Table 5: Summary descriptions of variables stratified by sex and visit (*: binary variable, **: Ordinal variable; never, rarely, sometimes, often, the result presented for the sometimes and often vs. never and rarely)

| Variables | Visit 1: n (%) | | | Visit 2: n (%) | | |
|---|---|---|---|---|---|---|
| | Boys | Girls | Total | Boys | Girls | Total |
| Glucose* | 50 (2.3%) | 36 (1%) | 86 (1.5%) | 296 (14%) | 522 (15.6%) | 818 (15%) |
| Snacking* | 2,051 (97%) | 3,279 (97.7%) | 5,330 (97.6%) | 2,006 (95.3%) | 3,207 (95.6%) | 5,213 (95.5%) |
| Participate in sport program* | 716 (34%) | 470 (14%) | 1,186 (21.7%) | 1,088 (51.7%) | 864 (25.7%) | 1,952 (35.7%) |
| Trouble breathing at night** | 230 (10%) | 335 (9%) | 567 (10%) | 304 (13.6%) | 421 (12.4%) | 725 (12.4%) |
| Trouble falling to sleep** | 432 (19.7%) | 625 (17%) | 1,057 (18.8%) | 367 (17%) | 631 (18.8%) | 1,166 (18.2%) |
| Sleep during the day* | 719 (34%) | 1,253 (19.3%) | 1,972 (36%) | 795 (45%) | 1,957 (58.2%) | 2,932 (53.6%) |
| Problem with sleepiness** | 194 (9%) | 276 (8.2%) | 470 (8.5%) | 299 (13.3%) | 685 (19.7%) | 984 (18%) |
| Falling asleep while watching TV** | 1,058 (50.4%) | 1,623 (48.5%) | 2,681 (49.2%) | 1,066 (50.6) | 1,701 (50.6%) | 2,767 (50.6%) |
| Fall asleep during class** | 537 (25.3%) | 947 (28%) | 1,484 (27.2%) | 768 (36.4%) | 1,366 (40.7%) | 2,134 (39%) |
| Fall asleep during homework** | 443 (20%) | 773 (22.7%) | 1,216 (22.2%) | 530 (25%) | 1,036 (30.4%) | 1,566 (28.6%) |
| Trouble getting out of bed in the morning** | 676 (32%) | 1,055 (31.4%) | 1,731 (31.7%) | 1,116 (53%) | 1,782 (53%) | 2,898 (53%) |
| Fall back to sleep after waking up** | 859 (40%) | 1,199 (35.7%) | 2,058 (37.6%) | 1,117 (53%) | 1,746 (52%) | 2,863 (52.4%) |

Table 6: Two multilevel linear and logistic regression models (CI= Confidence interval, OR: Odd Ratio, (S): significant value of 95% confidence intervals; this value estimated at a predetermined significance level of α=0.05, *: Beta coefficient + beta coefficient of the interaction term with time; to show the longitudinal effect over time.

| Covariate (Fixed effect) | Linear regression Waist circumference | | Logistic regression Obesity by waist circumference | |
|---|---|---|---|---|
| | Estimate | 95%CI | OR | 95%CI |
| **Daily sleep hours** | -0.28 | 0.01, -0.57 | 0.9 (S) | 0.83, 0.97 |
| **Daily sleep hours+ time*** | -0.1 (S) | 0.39, 0.002 | 0.97 (S) | 1.001, 1.12 |
| **Time** | 10 | 12.1, 8 | 1.12 | 0.76, 1.66 |
| **Salivary glucose (High vs. normal)** | -1.5 | 1.19, -4.29 | 0.89 | 0.33, 2.38 |
| **Salivary glucose + time (interaction term)** | -0.8 | 0.62, -2.28 | 1.07 | 0.72, 1.58 |
| **Trouble breathing at night** | 0.12 | 0.29, -0.05 | 1.11 (S) | 1.07, 1.16 |
| **Snacking (No vs. Yes)** | -0.32 | 0.3, -0.94 | 0.81 (S) | 0.67, 0.99 |
| **Gender (Boys vs. girls)** | -0.44 | 0.34, -1.22 | 0.81 (S) | 0.72, 0.91 |
| **Governorate (Ahmadi is the reference)** | | | | |
| **Farwania** | 1.2 | 2.51, -0.11 | 1.15 | 0.96, 1.37 |
| **Jahra** | -0.53 | 0.74, -1.8 | 1.1 | 0.92, 1.3 |
| **Hawalli** | 0.6 | 1.97, -0.77 | 1.07 | 0.86, 1.33 |
| **Capital** | 1.3 (S) | 2.51, 0.08 | 1.22 (S) | 1.02, 1.45 |
| **Mubarak Alkabeer** | 0.75 | 2.31, -0.81 | 1.03 | 0.81, 1.3 |

Table 7: Random effect estimates of the previous linear and logistic regression multilevel modls showing the random effect estimates (ICC: Intraclass correlation, *: significant value of 95% confidence intervals; this value estimated at a predetermined significance level of α=0.05).

| Level | Linear regression model | | | Logistic regression model | |
|---|---|---|---|---|---|
| | Visit 1 | Visit 2 | ICC | Visit 1 | Visit 2 |
| | Variation (sd) | Variation (sd) | | Variation (sd) | Variation (sd) |
| **Level 3 (School)** | 3.7 (0.8)* | 5.46 (1.4)* | 3.1% | 1.07 (0.02)* | 1.06 (0.03)* |
| **Level 2 (Children)** | 133.8 (2.5)* | 173 (0.9)* | 96.9% | - | - |
| **Total** | 137.3 | 188.46 | 100% | 1.07 | 1.06 |

# APPENDIX

## Appendix 1: Description of additional independent variables

The following are descriptions of the additional independent variables included in our analyses. These variables were assessed and obtained from "one-on-one" interviews with each child by calibrated interviewers:

The following questions about sleep quality were answered using ordinal responses (never, rarely, sometimes, often). These variables were considered continuous variables in the multilevel models (Table 6) and binary variables in the summary descriptive analysis (Table 5). In the summary table, answers of "sometimes" and "often" were combined and answers of "never" and "rarely" were combined. The results presented in the table compare "sometimes and often" versus "never and rarely".

These variables are:

- Trouble breathing at night: Each child was asked 'Do you have trouble breathing at night?'
- Trouble falling to sleep: Each child was asked 'Do you have trouble falling sleep at night?' The previous two variables were used as a proxy for mouth breathing.
- Problem with sleepiness: Each child was asked: 'How often do you have a problem with sleepiness during the day?'
- Falling asleep while watching TV: Each child was asked: 'How often do you fall asleep while watching television?'
- Fall asleep during class: Each child was asked: 'How often do you fall asleep or get drowsy during class periods?'

- Fall asleep during homework: Each child was asked: 'How often do you get sleepy or drowsy while doing your homework?'
- Trouble getting out of bed in the morning: Each child was asked: 'How often do you have trouble getting out of bed in the morning?'
- Falling back to sleep after being woken up: Each child was asked: 'How often do you fall back to sleep after being woken up in the morning?'

Following are the binary variables used in the analysis:

- Snacking: Each child was asked 'Do you eat snacks during the day?' The response was binary: "yes" if the child ate snacks and "no" if the child did not.
- Participation in sports programs: Each child was asked 'Are you participating in any sports programs outside the school?'
- Gender: A binary variable consisting of "male" and "female".
- Governorates was a categorical variable. There are six governorates of Kuwait (Alahmadi - which was used as the reference - Jahra, Farwania, Capital, Hawalli, and Mubarak Alkabeer).

## Appendix 2: Model building

The multilevel analyses for the two models were performed in four steps. First, a three-level null random intercept model was estimated with only time as a binary, fixed variable to partition the total variance by the three levels of analysis (the two-time points were nested within children, and the children were grouped within schools). Second, the time variable was allowed to vary regarding the children and school levels to account for the differences in the change of outcomes across schools and individuals over time (i.e., random slopes model). Third, to determine the longitudinal effect of the main exposure on the outcome, we added our main predictor variables. We generated the interaction terms between the main variables and time to assess the significance of the rate of change over time. Finally, individual level variables and confounders were inserted into the model. Variable selection was based on clinical relevance and potential confounding.

As is often done with multilevel models, we computed the intraclass correlations (ICCs) at each level. ICCs represent the amount of variability in the outcome attributed to each level, after accounting for other variables in the regression models.

Shown below are the final three models:

Final model for waist circumference as a continuous outcome (linear model, table 6):

$$\mathbf{Y_{ijk} = B0 + B1_{time} + B2_{sleep} + B3_{sleep*time} + B4_{glucose} + B5_{glucose*time} + B6_{sex} + B7_{trouble\_breath} + B8_{snack} + B9_{governorate} + U_{0jk} + U_{1jk} + V_{0k} + V_{1k} + e_{0ijk}}$$

$$U0jk \sim N(0,1)$$

$$\mathrm{V0k} \sim N\ (0,1)$$

Final model for waist circumference as a binary outcome (logistic model, table 6):

$$\mathbf{Logit\ (Y_{ijk}) = B0 + B1_{time} + B2_{sleep} + B3_{sleep*time} + B4_{glucose} + B5_{glucose*time} + B6_{sex} + B7_{trouble\_breath} + B8_{snack} + B9_{governorate} + U_{0jk} + U_{1jk} + V_{0k} + V_{1k}}$$

$$U0jk \sim N(0,1)$$

$$\mathrm{V0k} \sim N\ (0,1)$$

# MANUSCRIPT 3

## SHORT SLEEP DURATION AND SCREEN BASED ACTIVITIES: ALONGITUDINAL MULTILEVEL ANALYSIS


## ABSTRACT

Objective: The aim of this study was to identify lifestyle habits that contribute to night sleep reduction in Kuwaiti population.

Methods: Longitudinal data were collected from 6,316 children 8-14 years old at two-time points: 2012 and 2014. Children were approximately equally distributed among 138 elementary schools representing the six governorates of Kuwait. Calibrated examiners conducted sleep evaluation interviews, lifestyle habits interviews, and body weight measurements. A multilevel random intercept and slope model was conducted to determine the effect of screen-based activities on the daily night sleep hours at three levels: within schools, amongst children, and over time. The primary dependent variable was the number of daily sleep hours. Independent variables assessed were: screen-based activities variables including TV and videogame use, trouble breathing at night, lifestyle habits, and gender.

Results: Screen-based activities were significant factors that reduced daily sleep hours ($p<0.05$). Boys had less daily sleep hours than girls. Additionally, there were statistically significant variations between schools and children over time.

Conclusion: Longitudinal analysis of Kuwaiti children revealed that TV and videogame use were major risk factors contributing to decreased sleep duration with strong clustering effect of the observations within schools across time.

## INTRODUCTION

It has been recently recognized that healthy sleep patterns play a fundamental role in improving overall health. Current evidence shows that children who sleep less at night have a significantly higher risk of becoming obese than children who sleep longer (16, 20). Consistent delayed night sleep has been shown to increase the risk and the severity of infectious diseases, cancers, respiratory diseases, and depression (5). Similarly, little and disrupted sleep is related to costly metabolic diseases (15, 17-19). Metabolic diseases in Kuwaiti children is a growing problem; where, according to the International Diabetes Foundation every fourth adult has type 2 diabetes (57) and by our data every fourth 10-year old child is obese. Given the high prevalence of obesity in Kuwaiti children, there are serious health implications for these children as they reach adulthood, given the fact that overweight in children is a predictor of obesity in adulthood (9). Kuwiti children had a larger proportion of obese children than American children among the same age groups (25, 27, 47, 52).

The aim of the present analysis was to identify the risk behaviors related to delayed night sleep amongst a population having the highest prevalence of obesity in the world - the Kuwaiti population (21). Previous work has determined a significant relationship between insufficient sleep and obesity amongst Kuwaiti children (47). This study also demonstrated that screen-based activities (TV and videogame use) are major risk factors contributing to diminish sleep time (47). However, it is important to keep in mind that this previous analysis used the cross-sectional design, which has its inherent limitations. In the present study, we used a prospective longitudinal multilevel analysis, which has the advantage of allowing one to precisely account for the unmeasured confounders, and to determine the causal relationship between the predictors and the outcome. Moreover, this multilevel modeling system aids in accounting for the clustering effect between the schools and the variation between children for more precise and correct inferences (48).

## METHODS

### Study Approval

This prospective cohort study was approved by the Forsyth Institutional Review Board in Cambridge MA, U.S.A. and the Dasman Institute Human Ethical Review Committee in Kuwait (RA/065/2011 and RA/005/2011). Informed consent in the Arabic language was provided to parents (or guardians) and assent provided to the children. Assent was obtained on the day of the study visit before participation in the study. Written consent forms were collected and securely stored at the School Oral Health Program in Kuwait.

### Study population

In the first phase of the study, data was collected from 8,317 children representing the six governorates in Kuwait between October 2011 and May 2012. The Kuwaiti participants were in the 4th and 5th grades, and their ages ranged between 8-11-years-old in 2012 (visit1) for this initial screening. The final data collection occurred between October

2014 and May 2015, where the same data were collected from the same cohort of children (n=6,316) representing 138 public schools in Kuwait (visit2). The response rate for the second round was very high (95%). There were 2,001 children from visit1 who were unable to follow-up by the second examination visit due to the approaching end of the academic year. We included only the 6,316 subjects who had complete measurements at both visits. The distribution of participants across the six governorates of Kuwait was approximately equal. The students enrolled in these schools represent a wide variety of socioeconomic levels and ethnic groups among the Kuwaiti population, but do not include expatriate children. All volunteer participants were accepted, and selection into the cohort was not randomized across the population.

**Data collection**

Subject identification, sleep evaluation interviews, lifestyle information, and body weight measurements, were collected by two calibrated teams at each visit. These information variables were recorded into a programmed system on an iPad (Apple, Cupertino, CA) for Internet transfer.

The primary outcome is daily sleep hours. Sleep duration tested in this analysis was assessed and obtained through "one-on-one" interviews with each child by calibrated interviewers. Each child was asked: 1) during the past week, at what time did you go to sleep? 2) During the past week, at what time did you wake up? Based on the responses, the average number of sleep hours per day was calculated for each child.

The following are descriptions of the additional independent variables included in our analyses. These variables were assessed and obtained from “one-on-one” interviews with each child by calibrated interviewers:

The following questions about sleep quality were answered using ordinal responses (never, rarely, sometimes, often). In the summary table (Table 1), answers of “sometimes” and “often” were combined and answers of “never” and “rarely” were combined. The results presented in the table compare “sometimes and often” versus “never and rarely”. These variables are:

- Trouble breathing at night: Each child was asked ’Do you have trouble breathing at night?’
- Trouble falling to sleep: Each child was asked ’Do you have trouble falling sleep at night?’ The previous two variables were used as a proxy for mouth breathing.
- TV & (videogames) VG before bedtime: Each child was asked ‘Just before bedtime do you play video games or watch TV?’
- Bed use for TV and VG: Each child was asked: ‘How often do you use your bed for playing, watching TV, or video games?’
- Problem with sleepiness: Each child was asked: ‘How often do you have a problem with sleepiness during the day?’
- Falling asleep while watching TV: Each child was asked: ‘How often do you fall asleep while watching television?’
- Fall asleep during class: Each child was asked: ‘How often do you fall asleep or get drowsy during class periods?’

- Fall asleep during homework: Each child was asked: ‘How often do you get sleepy or drowsy while doing your homework?’
- Trouble getting out of bed in the morning: Each child was asked: ‘How often do you have trouble getting out of bed in the morning?’
- Falling back to sleep after being woken up: Each child was asked: ‘How often do you fall back to sleep after being woken up in the morning?’

The following variables were considered continuous variables (ranging from 0 to 7) in the multilevel modeling. Answers choices were: never, one day a week, two days a week, three days a week, four days a week, five days a week, six days a week, or every day. These variables were:

- Exercise: Each child was asked: ‘How many days a week do you exercise (sports, dance, PE class), bike, skate or play outside?’
- Watching TV or playing videogames for more than two hours: Each child was asked ‘How many days a week do you watch TV, videos or play computer/video games for more than 2 hours? (Playstation, X-box, Gameboy)?’
- Eating in front of the TV: Each child was asked ‘How many days a week do you eat in front of the TV?’

Following are the binary variables used in the analysis:

- TV in the bedrooms: Each child was asked ‘Is there a television set in the room where you sleep?’ The response was binary: “yes” if the child had a TV in the bedroom and “no” if the child did not have a TV in the bedroom.
- Participation in sports programs: Each child was asked ‘Are you participating in any sports programs outside the school?’

- Snacking: Each child was asked ‘Do you eat snacks during the day?’ The response was binary: “yes” if the child ate snacks and “no” if the child did not.
- Gender: A binary variable consisting of “male” and “female”.

**Statistical Analysis:**

Our data had a three-level hierarchical structure comprised of the following: time (at first level), nested within children (at the second level), and nested within schools (at third level) (Figure 1). In order to account for cluster sampling design, and to identify risk behavior factors that are significantly associated with diminishing sleep time amongst children, we specified three-level multilevel model.

We performed three-level fixed- and random-effects multilevel longitudinal analyses to accurately assess the influence of school context on the outcome (tested over a period of two years of time), and to consider the variance between schools and between individuals over time. All statistical analysis modeling was conducted in MLwin. MLwiN is a statistical software package for fitting multilevel models (Centre for multilevel modeling-University of Bristol).

The multilevel analysis was performed in four steps. First, a three-level null random intercept model was estimated with only time as a binary, fixed variable to partition the total variance by the three levels of analysis (the two-time points were nested within children, and the children were grouped within schools). Second, the time variable was allowed to vary regarding the children and school levels to account for the differences in the change of outcomes across schools and individuals over time (i.e., random slopes model). Third, to determine the longitudinal effect of the main exposure on the outcome, we added our main predictor variables. We generated the interaction terms between the main variables and time to assess the significance of the rate of change over time. Finally, individual level variables and confounders were inserted into the model. Variable selection was based on clinical relevance and potential confounding. We used the stepwise model building strategy. We kept all significant variables in the final model. Beta coefficients for the linear model and their 95% confidence intervals were estimated at a predetermined significance level of $\alpha$=0.05.

As is often done with multilevel models, we computed the intraclass correlations (ICCs) at each level. ICCs represent the amount of variability in the outcome attributed to each level, after accounting for other variables in the regression models.

Shown below is the final model:

$$\mathbf{Y_{ijk} = B0 + B1_{time} + B2_{TV\_before\_bed} + B3_{TV\_before\_bed*time} + B4_{use\_bed\_TV} + B5_{use\_bed\_TV*time} + B6_{TV>2h} + B7_{TV>2h*time} + B8_{trouble\_breath} + B9_{trouble\_breath*time} + B10_{sex} + U_{0jk} + U_{1jk} + V_{0k} + V_{1k} + e_{0ijk}}$$

$$U0jk \sim N(0,1)$$

$$\mathrm{V0k} \sim N(0,1)$$

# RESULTS

There were more girls (61.4%) in the study than boys (38.5%). The age of children was tightly clustered around 10±0.008 years old in visit 1, and around 12±0.008 years old in visit 2. On average, both boys and girls slept 8.8 hours/day with an average bedtime occurring at 10:00PM, and an average wake up around 6:00AM.

The population descriptive statistics for the lifestyle habits are summarized in Tables 1. We noticed a considerable increase in the use of screen-based activities over time in this population. 43.4% of Kuwaiti children in this study have a TV in their bedrooms, with boys more likely to have a TV (56.2%) than girls (35.3%). Most Kuwaiti children (86.7%) watch TV and play videogames just before bedtime; once again, this is more commonly found to be a habit of boys (88.7%) than girls (85.5%). Most children (59.5%) watch TV and play videogames for more than two hours a day (four days a week or more), more commonly for boys (62.2%) than girls (59.6%). Most children (58.4%) eat in front of the TV (four days a week or more), with more girls exhibiting this behavior (63.2%) than boys (57.7%). The use of the bed for watching TV and playing video games was reported by 50% of the children, with girls (53.5%) more likely to do so than boys (46.3%). Only 30% of Kuwaiti children reported that they practice exercises four days a week or more, with the higher proportion amongst boys (37%) than girls (24.6%). A small percentage of the children reported having trouble breathing at night (12.4%), which was more common in boys (13.6%) than in girls (12.4%).

At visit 1, 7.7% of the children reported that they had a diabetic mother, and 13.8% reported having a diabetic father. At visit 2, the same children reported that 9.4% had a diabetic mother, and 16.8% had a diabetic father. This indicated a 4.7% increase in the prevalence of diabetes in parents over the two years. These findings confirm the estimated 24% of diabetic adults in Kuwait reported by the International Diabetes Federation (8). Almost all children (95.5%) acknowledged snacking during the day (Table 1). According WHO guidelines (26). A big increase in the incidence of childhood obesity over time was observed (obesity by WC in visit 1= 22.6% and in visit 2= 38%).

Results from the multilevel longitudinal linear model showed that the main factors predicting short night sleep duration in children were screen-based activities (Table 2): watching TV or playing videogames before bedtime significantly reduced daily sleep hours, with significant change over time ($P<0.05$). Moreover, using the bed for watching TV and playing videogames predicted significantly shorter sleep duration over time ($P<0.05$) (Table 2). Playing videogames or watching TV more than 2 hours a day also significantly reduced daily sleep hours, with significant rate of increase over time ($P<0.05$) (Table 2).

For this models, there were statistically significant time variations for both school- and children- levels, found also in the rate of change in sleep duration across children and schools (Table 3). In another word, there was a strong clustering effect of the observations within the schools in the two time points.

## DISCUSSION

Our data showed that the mean sleep duration amongst Kuwaiti children (8.8 hours/day) was less than the minimum required hours for this age group. According to the Centers of Disease Control and Prevention (CDC) and the pediatric clinical guide for sleep (29), 10 - 11 hours of sleep are considered a healthy average sleep duration for 8-12 year-old children. This indicates that there is an issue prevalent in Kuwaiti children in terms of short sleep duration.

In the present analysis, we showed that children who frequently watch TV or play videogames just before bedtime sleep significantly less than children who rarely or never watch TV or play videogames before bedtime, with a significant dose-response relationship over time (P<0.05) (Table 2). Similarly, children who mostly use their beds for watching TV and playing videogames sleep significantly less than children who rarely or never used their beds for these screen-based activities, with a significant dose-response in the relationship over time (P<0.05). We also found that children who watch TV or play videogames for more than two hours most weekdays tend to sleep less at during the weekdays than children who spend less time on screen-based activities, with significant dose-response relationship over time (P<0.05) (Table 2). There is a strong body of evidence showing that screen-based activities have significantly contributed to decreased night sleep time . Most importantly, using the prospective longitudinal design in this analysis, we can infer the causal relationship between screen-based activities and little night sleep among those children. In addition, using this longitudinal multilevel modeling increases the precision of the analysis by accounting for the unmeasured confounders as well as accounting for the clustering within schools, and clustering of the observations between the two time points (48). These findings from the present prospective cohorts ascertain that TV and videogames use may be a short sleep duration risk factor that is prevalent in the child population of Kuwait.

Current evidence reported a remarkable decline in night sleep duration in several different populations, with a substantial parallel rise in the prevalence of metabolic diseases in these populations (14).

In this study, a substantial rise in the time spent on screen-based activities has been observed over the two years. We show that 43.4% of the Kuwaiti children in the study have TVs in their bedrooms (Table 1), and 86.7% watch TV or play videogames just before going to bed. In addition, 50% of the children are using their beds for watching TV and playing videogames, while only 30% engage in physical exercise most of the weekdays (Table 1). These are alarming findings that may explain the general reduction in the sleep duration amongst these children. using electronic devices for long periods can suppress melatonin secretion (36). It has been suggested that short wavelength light released from electronic devices significantly inhibits melatonin secretion, resulting in both delayed sleep and a diminished quality of the night sleep (35). A randomized study comparing a group of people who used electronic books with a group that used printed books before bedtime found that individuals who used electronic books sleep fewer night hours, and have lower levels of melatonin secretion than the group who used the printed book before bedtime (37). Melatonin plays an important role in carbohydrate metabolism and insulin regulation (18). Morover, it has been shown that short sleep duration and the use of electronic devices are associated with excess body weight (35).

In the present study, screen-based activities were the most significant factors contributing to reduced night sleep duration among Kuwaiti children. Screen based activity has been considered a major public health issue, associated with unexpected health conditions such obesity (23). We showed in our analysis that 65.6% of the children reported eating in front of the TV on most weekdays (Table 1). Independent of physical activity, adolescents who spend excessive time on screen-based activities are more prone to obesity (36, 38). Strong associations have been consistently observed between the presence of the TV in the bedroom and weight gain, mediated by TV viewing time(36, 39). It has been reported that screen based activities have the ability to divert children's attention away from the control of food intake, eventually distracting them from signals of satiety (41, 42) suggesting that screen activities could mediate the relation between sleep and obesity. A previous cross-sectional study using the same data showed that Kuwaiti children of diabetic parents sleep significantly less than children who have non-diabetic parents. Giving the fact that short sleep duration is related to diabetes and metabolic diseases (34, 35, 39, 58) this could be attributed to the family lifestyle factor: it is possible that parents who don't value sufficient night sleep may let their children stay up late, and these parents are more susceptible to be diabetic.

Another important findings presented in this study, is that schools that children attend make a difference in the examined outcome, the sleep duration. The strong clustering effect within schools in reducing the sleep time over time indicates that the school context has a substantial influence in shaping children's behaviors and attitude in the Kuwaiti children (Table 3).

Children spend a considerable amount of their time at school (59). Fifty percent of a child's waking time is spent in schools; therefore, school is considered a unique social entity that could configure children's health attitudes and behaviors that eventually shape their health outcomes (59, 60). Studies show that health outcomes of children with high genetic susceptibility to certain health conditions could be improved if surrounded with a healthy protective environment (55). Therefore, most common health intervention programs are school based, because the school can provide the social contextual framework that would support any selected intervention and enhance targeting risk factors for several health conditions (59).

It has been shown that modifying sleep behavior and improving the quality of sleep is efficient and has the potential to support immunity and relief symptoms of chronic diseases (5). Therefore, school health intervention programs should consider behavioral sleep improvement by educating children and parents about the importance of sleep quality on overall health. Children's behaviors may be more easily influenced than adults resulting in a greater potential to accept and adopt new behaviors if they are exposed to healthy practices. Therefore, targeting children's beliefs and attitudes is an essential step to building healthy behavior that can enhance overall health and prevent chronic diseases. A recent review explored school determinants for optimal health and included WHO and CDC guidelines. This review reported that school health programs could predict children's health outcomes (60-63). Strong evidence shows that school health programs can effectively change children's health behaviors and decrease symptoms of chronic diseases (59). Therefore, schools can act as an influential instrument to improve child health outcomes. Research in the US and other developed countries has successfully identified primary school health determinants (60). Based on this, school health policies and guidelines have been introduced and implemented to ameliorate chronic disease such as obesity (64). This improvement has evolved from the substantial effort to integrate scientific research into practice.

Findings from our prospective longitudinal study might be the first step for future research and policy implementation to advance total health for Kuwaiti children and others who face similar health challenges. The following are recommendations for future work, which evolved from our study: 1) introduce school health intervention and awareness programs to educate children and their families about the importance of sleep health and how to improve night sleep quality and limit the screen-based activities, and 2) promote public health research to identify healthy school determinants, to help designing future cost-effective school intervention and prevention programs.

**Study limitation**

The different teams who performed the one-on-one interviews at each visit might affect the objectivity of the children responses. The self-reported habitual sleep duration and other lifestyle habits might be susceptible to reporting bias. There were no contextual school level variables measured in the analysis that might explain the big variation between schools.

# CONCLUSION

This prospective cohort showed that screen-based activities are a major issue contributing to reduced sleep time, with a significant dose in response relationship over time. Public health intervention programs should target schools and educate children and their families to focus on improving night sleep behavior as well as limit their screen time.

## FIGURES AND TABLES

**Figure 1.** Schematic representation of the three-level hierarchical structure of the final analytic sample.

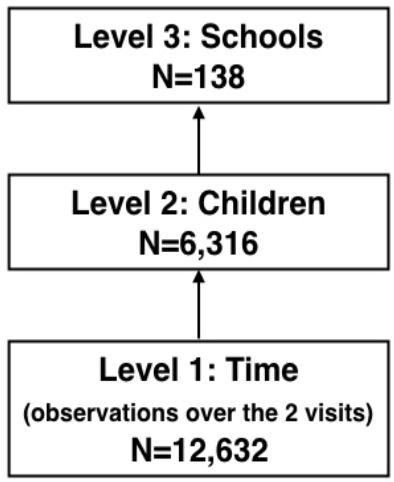

Table 1: Summary descriptions of lifestyle habits stratified by sex and visit (VG=video games, *: Ordinal variable for use during weekdays more than two hours 4 days a week or more, **: Ordinal variable; never, rarely, sometimes, often, the result presented for the sometimes and often vs. never and rarely, #: binary variable).

| Variables | Visit 1: n (%) | | | Visit 2: n (%) | | |
|---|---|---|---|---|---|---|
| | Boys | Girls | Total | Boys | Girls | Total |
| TV in bedroom# | 1,079 (51.3%) | 1,055 (31.4%) | 2,134 (39%) | 1,182 (56.2%) | 1,186 (35.3%) | 2,368 (43.4%) |
| TV &VG before bedtime** | 1,865 (88.6%) | 2,791 (83%) | 4,656 (85.2%) | 1,869 (88.7%) | 2,872 (85.5%) | 4,741 (86.7%) |
| Watch TV or play VG >2h* | 1,691 (80.2%) | 2.733 (79.4%) | 4,424 (80.9%) | 1,323 (62.2%) | 1,904 (59.6%) | 3,360 (59.5%) |
| Eat in front of TV* | 1,360 (64.5%) | 2,160 (64.2%) | 3,520 (64.3%) | 1,219 (57.7%) | 1,986 (63.2%) | 3,205 (58.4%) |
| Use bed for TV & VG** | 673 (32%) | 1,288 (38.4%) | 1,961 (35.9%) | 976 (46.3%) | 1,777 (53%) | 2,753 (50%) |
| Participate in sport program# | 716 (34%) | 470 (14%) | 1,186 (21.7%) | 1,088 (51.7%) | 864 (25.7%) | 1,952 (35.7%) |
| Exercise * | 1,295 (61.5%) | 1,884 (55.7%) | 3,179 (58.3%) | 801 (37%) | 891 (24.6%) | 1,692 (30%) |
| Trouble breathing at night** | 230 (10%) | 335 (9%) | 567 (10%) | 304 (13.6%) | 421 (12.4%) | 725 (12.4%) |
| Trouble falling to sleep** | 432 (19.7%) | 625 (17%) | 1,057 (18.8%) | 367 (17%) | 631 (18.8%) | 1,166 (18.2%) |
| Diabetic mother# | 160 (7%) | 264 (7.8%) | 424 (7.7%) | 205 (9%) | 310 (9.2%) | 515 (9.4%) |
| Diabetic father# | 300 (14.2%) | 454 (13.5%) | 754 (13.8%) | 379 (18%) | 538 (16%) | 917 (16.8%) |
| Snacking# | 2,051 (97%) | 3,279 (97.7%) | 5,330 (97.6%) | 2,006 (95.3%) | 3,207 (95.6%) | 5,213 (95.5%) |
| Sleep during the day# | 719 (34%) | 1,253 (19.3%) | 1,972 (36%) | 795 (45%) | 1,957 (58.2%) | 2,932 (53.6%) |
| Problem with sleepiness** | 194 (9%) | 276 (8.2%) | 470 (8.5%) | 299 (13.3%) | 685 (19.7%) | 984 (18%) |
| Falling asleep while watching TV** | 1,058 (50.4%) | 1,623 (48.5%) | 2,681 (49.2%) | 1,066 (50.6) | 1,701 (50.6%) | 2,767 (50.6%) |
| Fall asleep during class** | 537 (25.3%) | 947 (28%) | 1,484 (27.2%) | 768 (36.4%) | 1,366 (40.7%) | 2,134 (39%) |
| Fall asleep during homework** | 443 (20%) | 773 (22.7%) | 1,216 (22.2%) | 530 (25%) | 1,036 (30.4%) | 1,566 (28.6%) |
| Trouble getting out of bed in the morning** | 676 (32%) | 1,055 (31.4%) | 1,731 (31.7%) | 1,116 (53%) | 1,782 (53%) | 2,898 (53%) |
| Fall back to sleep after waking up** | 859 (40%) | 1,199 (35.7%) | 2,058 (37.6%) | 1,117 (53%) | 1,746 (52%) | 2,863 (52.4%) |

Table 2: Multilevel longitudinal linear regression model with daily sleep hours as the outcome variable, (S): significant value of 95% confidence intervals; this value estimated at a predetermined significance level of α=0.05, *: Ordinal variable for use during weekdays more than two hours 4 days a week or more (presented in the model as a continuous variable), **: Ordinal variable; never, rarely, sometimes, often (presented in the model as a continuous variable), #: Beta coefficient + interaction term with time; to show the effect over time.

| Covariate (fixed effect) | Estimate | 95% Confidence Interval |
|---|---|---|
| **Watch TV/ use videogames before bedtime *** | -0.084 # | -0.06, -0.13 (S) |
| **Use bed for watching TV / play videogames *** | -0.05 # | -0.01, -0.089 (S) |
| **Watching TV/ use videogames >2h/day**** | -0.07 # | -0.05, -0.089 (S) |
| **Trouble breathing at night**** | -0.05 # | -0.03, -0.15 (S) |
| **Sex (boys vs. girls)** | -0.092 | -0.034, -0.15 (S) |

Table 3: Random effect estimates of the previous linear regression multilevel model showing the random effect estimates ((S): Significant value of 95% Confidence Intervals (CI); this value estimated at a predetermined significance level of α=0.05), ICC: Intraclass correlation).

| Level (random effect) | Visit 1 | Visit 2 | ICC |
|---|---|---|---|
| | Variation (CI) | Variation (CI) | |
| **Level 3: School** | 0.05 (0.03, 0.06) (S) | 0.04 (0.06, 0.17) (S) | 2.4% |
| **Level 2: Children** | 2.2 (2.12, 2.27) (S) | 1.6 (4.8, 5.17) (S) | 97.6% |
| **Total** | 2.25 | 1.64 | 100% |

# MANUSCRIPT 4

## A LONGITUDINAL MULTILEVEL ANALYSIS OF SHORT SLEEP DUTARION AND GINGIVITIS IN KUWAITI CHILDREN


## ABSTRACT

*Objective:* It has been shown that insufficient night sleep has negative effects on health by suppressing immunity and promoting systemic inflammation that ultimately triggers tissue damage in different body organs. The aim of this study was to investigate the relationship between sleep duration and gingivitis as an oral inflammatory condition among a cohort of Kuwaiti children.

Methods: Longitudinal data were collected from 6,316 children 8-14 years old at two time points: 2012 and 2014. Children were approximately equally distributed from 138 elementary schools representing the six governorates of Kuwait. Calibrated examiners conducted gingival assessments, sleep evaluation interviews, saliva samples collections, body weight measurements, and nutritional analysis.

A multilevel random intercept and slope analysis was conducted to determine the relationship between sleep duration and gingivitis on three levels: within schools, amongst children, and over time. The outcome was the progression of gingival inflammation in children over time. The main independent variables were the number of daily sleep hours and salivary glucose level. Other explanatory variables and confounders assessed were: governorate, dental caries and fillings, obesity by waist circumference, and trouble breathing at night, adjusted for snacking frequency and gender.

Results: There was a statistically significant increase of gingivitis with shorter sleep duration over time ($p<0.05$). Elevated salivary glucose levels significantly predict gingivitis over time ($p<0.05$). Children who had more carious or filled teeth had more gingivitis ($p<0.05$). No significant association was detected between gingivitis and trouble breathing at night. The magnitude of gingivitis was significantly different between the six governorates of Kuwait ($p<0.05$). Additionally, there was high clustering effect of the observations within schools and between children across time.
Conclusion: Longitudinal analysis of Kuwaiti children revealed that shorter sleep duration and higher glucose level in saliva predict gingivitis with significant variations between schools and children over time.

## INTRODUCTION

A growing body of evidence documents the connection between oral and systemic health (1, 2, 7, 65). Common risk factors link oral and chronic disease such as diet, tobacco use, socioeconomic status, and lifestyle habits (1, 2, 7, 65). Hence, a crucial part of advancing oral health is to identify and target common risk factors to sustain optimal oral and general health. Emerging studies suggest that persistent oral inflammation can enhance chronic diseases such as diabetes, cardiovascular and respiratory disease (2). Apart from the proper oral hygiene required to maintain optimal oral health, other common risk factors of oral and systemic health should be identified to advance our understanding of the relationship between the two. Insufficient sleep is well established risk factor that negatively affects daytime functioning, and contributes to progression of several chronic diseases in children (66) such as obesity, diabetes, cardiovascular, and other cognitive disorders (15-18). Short night sleepers are at greater risk of developing cancer (32) and hypertension (67) as well.

Adequate night sleep is responsible for circadian clock alignment (19). Well-aligned circadian rhythm plays a crucial role in regulating the endocrine system and hormonal secretion (68). Current evidence shows that delayed and disrupted night sleep contributes to circadian clock genes alteration (69), hormonal disorder, and subsequent metabolic deterioration, resulting in several adverse health outcomes (68).

Furthermore, data shows that healthy sleep behavior supports the immune system by regulating the defense mechanism against pathogens (5). Insufficient and disrupted sleep can significantly impair immune cell function and diminish response to infection observed by circulating inflammatory cytokines and chemical mediators that can eventually lead to tissue damages (5).

To our knowledge, this is the first study that has investigated the relationship between sleep duration and oral diseases. In the present analysis, we examined short sleep duration as a predictor for developing gingivitis.

The interconnection between insufficient sleep and systemic inflammation has been reported (5). Short sleep duration is associated with glucose intolerance and insulin resistance due to the hormonal disruption (54). Glucose intolerance is a major risk factor for endothelial inflammation in diabetic and non-diabetic individuals, compromising microvasculature and impairing wound healing (70). On the other hand, if an association between insufficient sleep and metabolic disorder was determined, this relationship might be mediated by behavioral dietary intake (54), since gaining weight and gingival inflammation share common behavioral determinants, such as diet. It is plausible that sleep loss in children might increase their susceptibility to frequent snacking during the night. Evidence shows that later bedtimes are associated with more frequent snacking and sugary intake before bedtime (54). Increased snacking and sugary intake without proper oral hygiene is a causal factor for plaque accumulation and subsequent gingival inflammation (2). Moreover, interrupting the circadian clock due to insufficient sleep enhances one's appetite and increases the desire to consume high calories food by disrupting appetite hormones (54).

There is a strong link between late bedtime, glucose intolerance, and systemic inflammation in non-diabetic patients (70, 71). We hypothesized that short sleep duration could be a risk factor for gingival inflammation, aggravated by increased glucose levels (Figure 1). In this analysis, we investigated the longitudinal relationship between short sleep duration and salivary glucose levels in developing gingival inflammation amongst Kuwaiti children, using multilevel analysis to account for the clustering effect in different contextual levels.

# METHODS

## Study Approval

This longitudinal study was approved by the Forsyth Institutional Review Board in Cambridge MA, U.S.A. and the Dasman Institute Human Ethical Review Committee in Kuwait (RA/065/2011 and RA/005/2011). Informed consent was provided to parents (or guardians) in Arabic, and assent was provided to the children. Assent was obtained on the day of the study visit prior to participation in the study. Written consent forms were collected and securely stored at the School Oral Health Program in Kuwait.

## Study population

Data was collected from 8,317 children representing the six governorates in Kuwait between October 2011 and May 2012. The Kuwaiti participants were in the 4th and 5th grades, and their ages ranged between 8-11 years old in 2012 (visit 1) for this initial screening. Second visit data was collected between October 2014 and May 2015 from the 6,316 cohort of children representing 138 public schools in Kuwait. Response rate for the second round was very high (95%). There were 2,001 children from visit1 who were unable to follow-up by the second examination visit due to the approaching end of the academic year. We included only the 6,316 subjects who had complete measurements at both visits. In the complete data set of 6,316 children, the distribution of participants across the six governorates of Kuwait was approximately equal. Students enrolled in these schools represented all socioeconomic levels and ethnic groups among the Kuwaiti population, but did not include expatriate children. All volunteer participants were accepted and selection into the cohort was not randomized across the population.

**Data collection**

Subject identification, body weight analysis, sleep evaluation interviews, saliva samples, nutrition analysis interviews, and life style information were collected by two calibrated teams. Information on these variables was recorded into a programmed iPad (Apple, Cupertino, CA) system for Internet transfer.

***Outcome measure:***

Gingivitis (red sites) was the main outcome for this analysis; general assessment of gingival inflammation was performed using a mouth mirror, and the results were recorded for each participant in the iPad. Gingival assessments included two values: the average number of gingival margins that were red and swollen, and the total number of teeth in the subjects' mouths. Redness was scored as a binary parameter (1=red or swollen, 0=not red) at 3 sites of the teeth (interproximal, buccal, and lingual) and the sum of each was recorded. For each subject, the total number of permanent teeth and primary teeth were counted. The percent of red sites was the sum of interproximal + buccal + lingual/(3*total number of teeth). This variable was measured as a continuous variable in the multilevel model.

***Explanatory covariates:***

Predictors and potentially confounding variables related to gingivitis and sleep duration tested in this analysis were assessed and obtained through "one-on-one" interviews with each child by calibrated interviewers. Our main predictor for this analysis was sleep duration. Each child was asked the following: 1) during the past week, at what time did you go to sleep? 2) During the past week, at what time did you wake up? Based on the responses, the average number of sleep hours per day was calculated for each child. A single saliva sample was collected at school early in the morning before breakfast at 8:00AM. Children were instructed not to eat anything before coming to school. The glucose level in the saliva was measured and a binary variable was created. Values greater or equal to 1.13 mg/dL were defined as high (50), and values less than 1.13 mg/dL were defined as low glucose levels. Saliva sample collection is described in detail in an earlier study (51).

The following describes the additional independent variables and summarized in Table 1 and 2:

- History of dental caries: A continuous variable was calculated by dividing the number of carious and filled teeth by the total number of existing teeth (including primary and permanent).
- Snacking frequency: Each child was asked ‘Do you eat snacks during the day?’ The response was binary: “yes” if the child ate snacks and “no” if the child did not.
- Waist Circumference (WC): WC was measured at the midpoint between the bottom of the rib cage and the top of the iliac crest at minimal respiration for each participant. Each measurement was conducted using a paper anthropometry tape and the resulting circumference was rounded to the nearest centimeter. A binary variable was created to represent children with abdominal obesity. Abdominal obesity was defined as waist circumference ≥ $90^{th}$ percentile (72).
- Difficulties breathing at night: Each child was asked ’Do you have trouble breathing at night?’ The responses were ordinal (never, rarely, sometimes, often). Answers of “sometimes” and “often” were combined and answers of “never” and

"rarely" were combined. The results presented compare "sometimes and often" versus "never and rarely" together.

- Trouble falling to sleep (sleep trouble): Each child was asked 'Do you have trouble falling sleep at night?' The responses were ordinal (never, rarely, sometimes, often). The results presented compare "sometimes and often" versus "never and rarely" together. The previous two variables were used as a proxy for mouth breathing.
- Gender: A binary variable consisting of "male" and "female"
- Governorates: There are six governorates of Kuwait (Alahmadi is the reference, Jahra, Farwania, Capital, Hawalli, and Mubarak Alkabeer).

***Statistical Analysis:***

Our data had a multilevel structure comprised of time (first level), nested within children (second level), and nested within the schools (third level). Level-3 represented the contextual effect of the school environment on the outcome. We fit the data using multilevel longitudinal linear regression models to determine the linear relationship between gingivitis and sleep duration over a period of two years. We also examined the individual level variables to verify which individual factors were associated with the outcome. We performed three-level fixed and random-effects multilevel longitudinal analysis to assess the influence of school context on the risk of gingivitis over a period of two years, and to consider the variance between schools and individuals over time. All statistical analysis modeling was conducted in MlwiN. MLwiN is a statistical software package for fitting multilevel models (Centre for multilevel modeling-University of Bristol) (73).

The multilevel analysis was performed in five steps. First, a three level null model was estimated with only time as a binary fixed variable to partition the total variance by the three levels of analysis (the two time points were nested within children, and the children were grouped within schools). Second, the time variable was allowed to vary at children and school levels to account for the difference in the change of gingivitis across schools and individuals over time. Third, to determine the longitudinal effect of sleep duration on gingivitis, we added our main predictor variable 'sleep duration'. We then generated an interaction term between sleep duration and time to assess the significance of the rate of change between sleep duration and gingivitis. Forth, individual level variables were inserted into the previous model. Variable selection was based on clinical relevance and potential confounding. Beta coefficients and their confidence intervals were estimated at a predetermined significance level of a confidence interval of 95%. We tested for mediators and moderators for the relation between gingivitis and sleep duration by generating interaction terms and conducting separate multilevel models. We kept all significant variables in the final model as well as the potential confounders (Table 4).

As a sensitivity analysis, we conducted two similar models to the previous final model, stratified by gender to estimate the relationship between sleep duration and susceptibility to gingival inflammation over time for boys and girls separately. As is often done with multilevel models, we computed the intraclass correlations (ICCs) for the outcome variable. ICCs represent the amount of variability in the outcome attributed to each level, after accounting for other variables in the regression models.

Shown below is the final model:

$$Y_{ijk} = B0 + B1_{time} + B2_{sleep} + B3_{sleep*time} + B4_{glucose} + B5_{glucose*time} + B6_{sex} + B7_{trouble\_breath}$$
$$+ B8_{snack} + B9_{governorate} + B10_{obesity} + B11_{decay} + U_{0jk} + U_{1jk} + V_{0k} + V_{1k} + e_{0ijk}$$

$$U0jk \sim N(0,1)$$

$$V0k \sim N(0,1)$$

## RESULTS

The population descriptive statistics for the analysis are summarized in Table 1and 2. There were more girls (62%) in the study than boys (38%). The age of children was tightly clustered around 10 years old in visit 1, and around 12 years old at visit 2. 74% of the students had gingivitis during the first visit data collection period while only 42% of the students had gingivitis during the second data collection visit. On average, both boys and girls slept 8.8 hours/day in visit 1, and 9.9 hours/day in visit 2. This is less than the recommended average of 10-11 hours for this age group, with an average bedtime occurring at 10:00PM. There was substantial raise in the proportion of children with high salivary glucose levels with older children at time 2 (high glucose in visit 1= 1.6%, high glucose in visit 2 = 15%) (Table2). Additionally, a big increase in the incidence of childhood obesity over time was observed (obesity in visit 1= 22.6%, obesity in visit 2= 38%). Over the two years, each child developed an average of three decayed teeth (Table 1). Three governorates demonstrated higher prevalence of gingival inflammation than the other three governorates. Children residing in Jahra, Ahmadi, and Farwania governorates had a higher prevalence of gingivitis (table 3).

Table 4 shows the results from the final multilevel longitudinal linear model with gingivitis as a continuous outcome. This model shows statistically significant increases in gingival inflammation with shorter sleep duration with statistically significant change over time ($P<0.05$). Higher glucose levels appear to be an important predictor of gingivitis with a statistically significant rate of change over time ($P<0.05$). Not surprisingly, gingivitis increased with more decayed and filled teeth. Boys are at higher risk of developing gingivitis than girls ($P<0.05$). There were statistically significant differences between the six governorates of Kuwait. Jahra and Farwania had positive magnitude of gingivitis compared to the other three governorates, Capital, Hawali, and Mubarak, adjusting for other variables. In this analysis, no significant relationship was detected between obesity and gingivitis, nor between trouble night breathing and gingivitis ($P>0.05$).

Table 5 shows the random effect results of the multilevel model. There was a statistically significant time variation for both school- and children- levels. Even after accounting for important individual predictors of gingivitis, there were statistically significant variations in the rate of change in gingivitis across children and schools.

After testing for mediators and moderators, we found that sleep duration can partially mediate the relationship between salivary glucose levels and gingivitis. Sensitivity analysis models for both boys and girls showed consistent results with the final model.

# DISCUSSION

## Gingivitis

Inflammation is a protective mechanism of the host cells in response to infection toward tissue healing and recovery. Inflammatory process should be a normal defense reaction performed by different body organs in order to restore biological function. However, if it persists for long period of time, it might indicate a compromised immune response (2). Gingivitis is an inflammatory reaction of gingival tissues. The clinical signs of gingival inflammation are redness of the gingival margin with swelling, and exudation of gingival fluid (74). This is due to the aggregation and enlargement of blood vessels with migration of chemical mediators towards the subepithelial connective tissue as a part of the defense mechanism to eliminate infection (2, 65, 74). Strong bodies of evidence suggest that the main cause of gingivitis is consuming a sugary diet with poor oral hygiene, eventually fostering plaque biofilm accumulation around gingival margins with subsequent bacterial colonization (2, 74). These bacteria release toxins within the gingival epithelial tissue that provoke inflammatory responses in order to get rid of the bacterial toxins. Severity of the inflammation depends on the balance between the quantity and hostility of the existing bacteria, and the efficiency of the host immune response (75). For example, individuals with poor oral hygiene are more likely to have increased bacteria and are more susceptible to develop gingivitis, whereas individuals with a compromised immune response might be at higher risk of gingival inflammation regardless of oral hygiene status (76). It has been hypothesized that inflammation is enhanced in hyperglycemic diabetic patients indexed by the higher level of inflammatory mediators (2). These mediators reduce the ability of the host cells to kill the bacteria, which explains the lower capacity of tissue healing and repair in patients with glucose intolerance (70). Recent evidence suggests the role of diminished sleep in promoting inflammation and tissue damage through compromising immunity and disturbing metabolism (5).

Unfortunately, because it is not painful, gingivitis might not be considered as a serious condition by dentists and physicians. However, if gingivitis remains untreated, the inflammatory process may expand; breakdown of collagen fibers and bone resorption of the periodontal supporting tissue will take place (75, 77).

Gingivitis is a preventable condition. Because gingivitis usually precedes periodontitis and is reversible, the prevention of gingivitis is the essential early step of preventing periodontitis (77, 78). The process of quantifying gingivitis prevalence, incidence, and its distribution is important in order to identify and prevent the risk factors, and advance oral health among the population (79). Although several gingivitis indices have been proposed with many different methodologies, no one has universal application or acceptance (77, 80). Some indices use ranking scores to grade the severity of gingival inflammation. Others used the "present or absent" dichotomous indices (77). Recording if inflammation is present or not in the gingiva might be a convenient measurement in epidemiological and public health studies. Further studies have demonstrated that an efficient index for public health studies should be simple and easy to conduct, with minimal instrumentation and examiner training, and should be reproducible (81). In the present analysis, we used the ‘"present or absent" index to assess the gingival inflammation state in children. We consolidated the grading scores into binary (inflamed vs. not inflamed) to have discrete dichotomous scores that are readily differentiable from one another, and clearly defined to ensure the assessment precision and to maximize examiner reliability (82) .Other work suggested that this dichotomous index might decrease the subjectivity between examiners, as it was shown that the major reliability issue is the examiner's difficulty in accurately reporting the grading of the severity of gingival inflammation, specifically recording the differences between "mild and moderate" gum inflammation (83).

Guidelines for the choice of "most suitable" index to measure gingivitis were documented: (1) simple with low cost; (2) with clear components and easily understood; (3) the index should be equally sensitive across its range indicating the clinical phases of the disease; (4) the index must be amenable to statistical analysis (81). In this analysis, we made sure that our gingivitis measurement index was consistent with the above criteria. It's important to understand that the choice of index should depend on the purpose of the study, its duration, the type and extent of change expected and most importantly, the index should measure what it supposes to measure for evaluating the outcome of the study (77, 83). For example, for epidemiological surveys and public health assessment, non-invasive partial recording of selected teeth or sites may be sufficient (77, 82). Evidence does not support the assumption that invasive indices are truly objective (77). Conducting non-invasive index in assessing gingivitis in longitudinal studies with visually detected color and architectural changes can substitute the invasive index in public health assessments (80).

Gingivitis is highly prevalent in children (84) and is found to be the second most frequent oral disease in children (85, 86). Children with healthy gingival tissues most likely will grow up with healthy periodontal condition (87). Studies in the Arab region have reported a high prevalence of gingivitis in children (85). Additionally, studies regarding gingivitis amongst young children in several different populations such as the US, England, Australia, Sweden, and UAE showed that gingivitis prevalence greatly varies between 15% up to 85% (79, 85). It isn't clear whether these variations in the prevalence of gingivitis showed real population differences, or if they were subjected to some measurement errors (79). It was reported that gingival inflammation in younger children is substantially lower than in older children and young adults (88). The difference between the young children and adults agrees with the results from earlier studies (89), and is consistent with findings from this study. Variation between different age groups might be attributed to growth, hormonal, and puberty factors. Puberty between different age groups varies and might be considered a normal physiological age factor contributing to changes in the incidence of gingival inflammation with age (88). Other work has suggested that the high prevalence of gingivitis amongst young children is contributed to the eruptive gingivitis during the mixed dentition phase (90). In addition, data suggests differences in the bacteriology and the inflammatory cell response between children and adults (88). It has been found that the prevalence of gingivitis in children around 11 years of age is quite high and might reach 90% (79). From 11 years of age upward, and as children enter their teen years; the prevalence of gingivitis begins to go down consistently, reaching the peak at 17 years (90, 91). In the present study, we found that 74% of the Kuwaiti children had gingivitis at 10 years old; the percentage of gingivitis significantly declined at age 12 years to reach 42% during the second data collection period, which was consistent with previous studies (Table 1).

**Gingivitis and sleep**

In this prospective longitudinal analysis, even when we accounted for the variation between children and the clustering within schools, we showed that children who slept fewer hours were more susceptible to develop gingival inflammation, with statistical significant rate of change over time (Table 4). The longitudinal multilevel design of this study adds precision to the relationship between little sleep and increased gingivitis as opposed to the cross sectional design (56). To our knowledge, this is the first study that examines the relationship between sleep duration and an oral inflammatory condition using multilevel analysis. However, a strong body of evidence shows that insufficient sleep could trigger inflammation in different body organs due to hormonal disruption (92). In addition, experimental trials found that night sleep deprivation suppresses immunity, observed by increased inflammatory markers, with dose response relation (5). Since the immune system's role is to detect pathogens and antigens to prevent cell damage, failure to eliminate these antigens will result in inadequate immune response and potential abnormal tissue damage. Data suggests that immune cell activity is influenced by the circadian process and sleep quality through supporting and maintaining adequate vascular permeability with infiltration of immune cells and chemical mediators required for eliminating pathogens (5). Immune cell function requires metabolic energy, which is found to be maintained during the night sleep (5). Clinical studies on flu and hepatitis vaccines show that insufficient night's sleep explains the impaired poor antibody response to the vaccines by suppressing the immune system (5).

**Gingivitis and glucose intolerance**

Strong body of evidence reports that adequate night sleep regulates the overall hormonal and neural balance through circadian system processes (33, 93). The daily melatonin secretion during sleep is a reliable marker of circadian clock (33, 93). Studies on shift workers demonstrate disrupted circadian rhythm due to night sleep deprivation, which ultimately results in significant delays of melatonin secretion (18, 32). The melatonin hormone is released by the pineal gland essentially during the night sleep and is responsible for regulating insulin secretion (18). Delayed night sleep deteriorates melatonin and insulin secretion, resulting in consequent insulin resistance and glucose intolerance (94).

It has long been recognized that glucose intolerance and insulin resistance are the essential metabolic causal factors contributing to tissue damage and impaired endothelial tissue function in different body organs in diabetic individuals (71, 95).

Gingivitis is a common complication of diabetes disease in adult and children (2, 76). Strong evidence shows that diabetic children and adolescents are at high-risk of developing gingival inflammation independent of oral hygiene status (76). The mechanism of gingival inflammation in diabetic patients is related to the hyperglycemic state that impairs microvasculature and wound healing in endothelial gingival tissue (84). This explains why diabetic individuals who have good glycemic control are less susceptible to gingival inflammation. There is emerging evidence showing that elevated glucose levels in non-diabetic subjects can predict diabetes (96). This suggests that increased glucose levels could cause complications even in non-diabetic individuals. In the present analysis, we demonstrated that children with higher salivary glucose levels (> 1.13 mg/dl) had significantly greater gingivitis with a significant rate of change over time (Table 4).

Current evidence reports the correlation between salivary glucose concentration and blood glucose level, indicating that salivary glucose can be a reliable measure to estimate the level of blood glucose in non-diabetic individuals. It has also been used to detect pre-diabetes, and to assess glycemic levels in diabetic patients (97).

The relationship between higher glucose levels and increased gingivitis has been reported (71). Increased glucose levels, even in non-diabetic individuals, can predict gingivitis as this study showed (Table 4). Additionally, we showed that sleep partially mediated the relationship between increased glucose levels and gingivitis. In other words, children who slept fewer night hours are at risk of developing hyperglycemia and consequent gingival inflammation. A study among healthy subjects showed that intentionally delaying night sleep and disturbing the circadian clock caused hyperglycemia and subsequent insulin resistance (98).

On the other hand, a wealth of evidence indicates that sleep curtailment could significantly affect the appetite hormones such as leptin and ghrelin, leading to an increase in calorie intake and abnormal weight gain. Individuals who reported less night sleep tend to consume more snacks rich in sugar (54). Increased night waking time is associated with more sedentary habits, TV watching, and snacking (99). It was not clear whether abnormal weigh gain in short sleepers is due to metabolic deterioration caused by endogenous circadian disruption, or is mediated by stimulating appetite through insufficient sleep. Nonetheless, there is a strong agreement in the literature that short sleep duration contributes to disturbing the circadian clock process and leads to hormonal disruption (54). In this study, we found that children with a higher number of dental caries had significantly more inflamed gingival surfaces (Table 4). This might be contributed to the fact that children who snack more frequently and consume a more sugary diet are expected to have a greater history of dental caries, plaque accumulation, and eventually more gingivitis. Moreover, the relationship between higher levels of salivary glucose with increased risk of dental caries has been reported (76). This indicates that increased salivary glucose can promote oral infection, including development of dental caries and gingivitis. Evidence shows that individuals with higher HbA1c (average plasma glucose concentration) have increased gum problems and dental caries compared to those with lower HbA1c concentration (76), which supports the findings in the present study. As mentioned earlier, there is a strong correlation between the concentration of glucose in plasma and salivary glucose (97).

A recent cohort suggested that there is a bidirectional relationship between periodontitis and systemic chronic disease that is confirmed by the circulating C-Reactive Protein (CRP), an inflammatory biomarker (2, 100). Persistent oral inflammation might exacerbate cardiovascular disease and diabetes mellitus by oral and systemic endothelium bacterial transmission (1, 65). Sleep disturbance, on the other hand, is associated with increased systemic inflammatory markers through hormonal disruption (101). Consequently, this chronic systemic inflammation could induce a state of glucose intolerance and consequent metabolic disorder (76, 84). It is still not clear whether oral inflammatory conditions promote chronic disease or if it is the chronic disease that is triggering oral inflammatory conditions. Nonetheless, experimental studies show that treating oral diseases such as periodontitis could reduce systemic inflammation, control glycemic level, and enhance endothelial function in different organs (1).

An experimental study shows that obese subjects are at higher risk of developing insulin resistance and subsequent gingivitis (102). Insulin resistance promotes gingivitis through the oxidative stress indexed by inflammatory biomarkers in the gingiva independent of existing bacteria, suggesting that insulin resistance can promote endothelial destruction and progression of gingivitis even in absence of bacterial infection (103).

The links between metabolic syndrome and gingival inflammation have been observed as early as in childhood (104). In this study, however, no significant association between obesity and gingivitis was detected (Table 4). Similarly, other studies have not observed an association between gingival inflammation and obesity (75, 105).

Based on the current evidence and findings from this longitudinal multilevel analysis, we suggest two different pathways that explain this significant relationship between short sleep duration and increased gingival inflammation: 1) circadian misalignment disturbs metabolic hormones and causes glucose intolerance that aggravates gingival inflammation (figure 1), or 2) less sleep stimulates appetite and leads to greater consumption of sugar-rich snacks, resulting in plaque accumulation and gingival inflammation.

**School and neighborhood effects, and multilevel analysis**

The school environment has recently gained special attention in public health research. Several studies indicate that the surrounding environment strongly influences a child's health (59). School plays a fundamental role in structuring the social context of a child's behavior. This environment can shape a child's norms, values, and culture (59). Children with similar personal demographics and socioeconomic characteristics tend to live close and share same health conditions (48) because they are exposed to the same surrounding atmosphere, and tend to influence each other.

From statistical perspective, the clustering effect of observations within schools creates group dependency (48) . Group dependency reflects the similarities of children within the setting they are nested in. The best analysis to account for the within group dependency is the multilevel analysis (56). Within group correlation violates the regular statistical analysis such as linear regression resulting in incorrect inference (77). Multilevel analysis has been widely used to investigate the effect of social context on the health outcomes (59). Ignoring this clustering effect within group might result in biased and imprecise estimates (106). In this longitudinal prospective study, we were able to detect a statistically significant variability in gingivitis between children across time; as well significant variability between schools across time, suggesting that school context is strongly related to gingival disease (Table 5). Additionally, there was substantial change of different level variations over the two visits (Table 5). The significant variations of the time level might be attributed due to the physiological changes of gingivitis attributed to age factor and teeth eruption (88, 90). Through simultaneous examination of the three levels effect with the individual characteristics, we ascertained how children are related to each other within schools, and how gingivitis changed consistently over time in relation to the examined predictors.

In the present analysis, we accounted for the geographical setting and the neighborhood effect on the outcome. We considered the fixed effect to examine the difference between the six governorates of Kuwait on developing this oral condition, gingivitis. Within the six governorates of Kuwait, there are clusters of inhabitants with differing social classes, ethnicities, and family origins. There are three identified ethnic groups in Kuwait based on family origin and tribal background: Bedouin, Saudi, and Persian (45). Each ethnic group is known by certain characteristics and specific life habits that could eventually shape health behaviors and outcomes in this population.  A recent genetic study in Kuwait has shown that there is a distinct genetic difference between the three ethnic groups, and recommended considering ethnic assessment in public health practices to target at-risk groups for health intervention programs (45). In this study, we found differences in the proportion and magnitude of gingivitis between the six governorates of Kuwait (Table 3 and table 4). Ahmadi, Farwania, and Jahra governorates are considered mostly Bedouin governorates. These three governorates have a higher magnitude of gingivitis compared to the other three, which are composed of Saudi and Persian communities (Table 4). From our knowledge about the Kuwaiti population, the majority of Bedouins families is likely on the lower socioeconomic scale, possibly resulting in lower health literacy, and appear to have a greater prevalence of gingival disease than the other governorates of Kuwait. Future studies should identify the features of schools and neighborhoods that promote the progression of some health conditions for potential intervention and prevention. School health intervention programs should consider behavioral sleep improvement by educating children and parents about the importance of sleep on overall health. It has been shown that behavioral sleep improvement is effective and has the potential to support immunity and relieve symptoms of chronic diseases (5).

**Limitation of the study**

The different teams who performed the gingival assessment at each visit might affect the objectivity of the gingival measurements. One potential unmeasured confounder in the relationship between sleep, glucose level, and gingivitis could be the presence of a sugary diet and oral hygiene measures. Another unmeasured variable for developing gingivitis is oral hygiene habits, including teeth brushing and flossing. There were no contextual school level variables measured in the analysis that might explain the big variation between schools.

## CONCLUSION

Findings from this longitudinal multilevel analysis showed that short sleep duration and salivary glucose levels are two strong predictors of gingival inflammation, with significant increases over time in the Kuwaiti children population. Modifying night sleep behavior could maintain normal endocrine function, control normal glucose levels and potentially advance oral and overall health.

# FIGURES AND TABLES

**Figure 1: Hypothesis of the relation between short sleep duration and gingivitis**

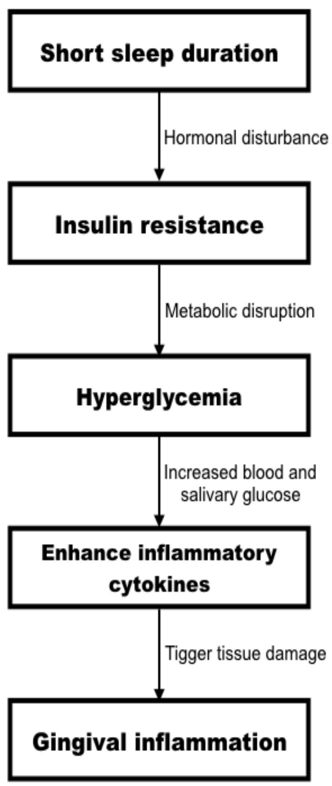

Table 1: descriptive summary statistics for the continuous variables by visit and gender

| Variable | Visit 1 (mean ± sd) | | | Visit 2 (mean ± sd) | | |
|---|---|---|---|---|---|---|
| | Total | Boys | Girls | Total | Boys | Girls |
| Age (y) | 9,9 ± 0.008 | 9.9 ±0.01 | 9.9 ±0.01 | 12 ±0.008 | 12 ±0.01 | 12 ±0.01 |
| Daily sleep (h) | 8.9 ±0.02 | 8.9 ±0.03 | 8.9 ±0.02 | 9.9 ±0.01 | 9.8 ±0.02 | 9.9 ±0.02 |
| Gingivitis (% Red) | 74 ±0.3 | 72.8 ±0.4 | 74.9 ±0.3 | 42.5 ±0.2 | 44 ±0.4 | 41.6 ±0.3 |
| Dental caries (%With fillings and/or decay) | 11 ±0.1 | 11.7 ±0.2 | 10.4 ±0.1 | 14.4 ±0.1 | 14.6 ±0.2 | 14.3 ±0.2 |

Table 2: descriptive summary statistics for the categorical and binary variables by visit and gender (visit 1: first visit in 2012, visit 2: second visit in 2014)

| Variable | Visit 1: n (%) | | | Visit 2: n (%) | | |
|---|---|---|---|---|---|---|
| | Total | Boys | Girls | Total | Boys | Girls |
| **Sex** | 5456 | 2,103 (38.5%) | 3,353 (61.4%) | 5456 | 2,103 (38.5%) | 3,353 (61.4%) |
| **High Salivary Glucose (> 1.13 mg/dl)** | 86 (1.5%) | 50 (2.3%) | 36 (1%) | 818 (15%) | 296 (14%) | 522 (15.6%) |
| **Snacking N (% yes)** | 5,330 (97.6%) | 2,051 (97%) | 3,279 (97.7%) | 5,213 (95.5%) | 2,006 (95.3%) | 3,207 (95.6%) |
| **Obese by Waist Circumference N (% yes)** | 1,224 (22.4%) | 438 (20.8%) | 786 (23.4%) | 2,096 (38.4%) | 746 (35.4%) | 1,350 (40.2%) |
| **Trouble breathing at night N (% yes)** | 567 (10%) | 230 (10%) | 335 (9%) | 725 (12.4%) | 304 (13.6%) | 421 (12.4%) |

Table 3: summary of prevalence of gingivitis by governorates and visit

| Governorate | Mean (se)<br>Visit 1 | Mean (se)<br>Visit 2 | Total number |
|---|---|---|---|
| **Ahmadi** | 86% (12.7) | 41.2% (18.4) | 2,454 |
| **Farwania** | 81.8% (17.3) | 45% (20.3) | 1,806 |
| **Hawali** | 71% (19.5) | 41.4% (20.08) | 1,192 |
| **Jahra** | 82.3% (18.08) | 45.1% (17.6) | 2,639 |
| **Capital** | 59.8% (18.9) | 38.7% (13.4) | 2,915 |
| **Mubarak** | 52.9% (25.7) | 48% (21.08) | 856 |

Table 4: Linear regression multilevel model with gingivitis as the outcome. (CI: Confidence interval, *: The estimate for the beta coefficient + the interaction term with time; to show the rate of change over time, [S/NS]: Significant based on 95% confidence intervals; estimated at a predetermined significance level of α=0.05.

| Covariate (fixed effect) | Estimate | 95% CI [S/NS] |
|---|---|---|
| **Sleep duration** | -0.9 | -0.31, -1.48 [S] |
| **Sleep +interaction with time*** | -0.4 | 0.8, 0.1 [S] |
| **Glucose level (high vs. low)** | 8.7 | 1.44, 15.95 [S] |
| **Glucose + interaction with time*** | 4.7 | -0.27, -7.72 [S] |
| **Time** | -37.06 | -32.5, -41.5 [S] |
| **Dental caries and filling** | 0.2 | 0.17, 0.22 [S] |
| **Obesity by waist circumference (obese vs. non obese)** | -0.3 | -1.08, 0.48 [NS] |
| **Trouble breathing during sleep** | 0.1 | -0.29, 0.49 (NS) |
| **Gender (boys vs. girls)** | 2.2 | 1.41, 2.98 [S] |
| **Snacking (yes vs. no)** | 1.5 | -0.06, 3.06 [NS] |
| **Governorate (Ahmadi is the reference)** | | |
| **Jahra** | 0.35 | -1.21, 1.91 [NS] |
| **Farwania** | 0.18 | -1.19, 1.55 [NS] |
| **Hawalli** | -6.2 | -4.43, -7.96 [S] |
| **Capital** | -13.2 | -11.82, -14.57 [S] |
| **Mubarak Alkabeer** | -11.5 | -9.4, -13.59 [S] |

Table 5: Random effect estimates of the multilevel linear regression. (ICC: Intraclass correlation, [S/NS]: Significant based on 95% confidence intervals; estimated at a predetermined significance level of α=0.05.

| Level | Visit 1 Estimate (sd)[S/NS] | Visit 2 Estimate (sd)[S/NS] | ICC |
|---|---|---|---|
| **Level 3: School** | 87.6 (6.8)[S] | 42.3 (13.3)[S] | 12.6% |
| **Level 2: Children** | 271.4 (6)[S] | 292.6 (9.3)[S] | 87.3% |
| **Total** | 359 | 334.9 | 100% |

# DISCUSSION AND SUMMARY

Health outcomes are important indicators of a country's development; a healthy educated population is more active and productive. In contrast, societies with a high prevalence of diseases require substantial investments of money to overcome the health problems that affect the productivity and growth of a nation. Unhealthy populations are unable to work efficiently, and become a burden on the economy of their country (107). Therefore, it is important to assess the health status of communities to create effective health prevention programs and develop strategies to overcome future health problems.

After observing the same children over a period of two years in our study, we were able to demonstrate that the prevalence of abdominal obesity increased significantly from 22.4% at10-years of age to 38.4% for the same children at 12-yearchildren's of age. Given the high prevalence and incidence of obesity in Kuwaiti children, there are serious health implications for these children as they reach adulthood (9). Obesity is associated with an increased risk of costly chronic diseases, particularly cardiovascular disease and diabetes (10-13).

By conducting different modeling analyses presented in this study, we demonstrated consistent findings that short sleep duration is significantly associated with increased waist circumference. Our findings also showed that the additional hours of night sleep was associated with a significant decrease of abdominal obesity, which indicates that longer night sleep is a protective factor against obesity in Kuwaiti children. We also found that children who slept fewer hours at night were more susceptible to developing gingival inflammation. In other words, children who slept fewer hours at nighttime were more susceptible to becoming obese and developing more gingival inflammation. This suggests that Kuwaiti children are vulnerable to the effect of sleep on their oral and general health. In addition, by examining various reported behavioral risk factors, screen-based activities (TV and videogame use) were the primary causal factors that dramatically reduced night sleep duration among those children. An interesting finding was that higher glucose levels in saliva predicted gingival inflammation. However, we did not detect any association between high salivary glucose levels and abdominal obesity. Current data show that short sleep duration is associated with glucose intolerance and insulin resistance due to the hormonal disruption (54). Glucose intolerance is a major risk factor for endothelial inflammation, compromising microvasculature and impairing wound healing resulting in gingival tissue damages (70). It has also been reported that insufficient and disrupted sleep can significantly impair immune cell function and diminish response to infection by circulating inflammatory cytokines and chemical mediators that can eventually lead to gingival tissue damages (5).

The relationship between short sleep and obesity has been well documented in the literature. It has been observed that inadequate sleep in healthy subjects is associated with significant hormonal imbalance resulting in increased food intake and snacking (28). Moreover, it was found that persons who have insufficient sleep are at risk of metabolic impairments caused by melatonin and insulin disruption resulting in obesity and other symptoms of metabolic diseases (34).

Using the longitudinal design in this analysis, we can infer the causal relationship between short sleep and increased abdominal obesity and gingivitis, as well as between screen-based activities and short sleep duration. Multilevel modeling increases the precision of the analysis by accounting for the unmeasured confounders, accounting for the variation between children, clustering within schools, and clustering of the observations between the two time points (48).

Another important findings presented in this thesis, is that schools that children attend made a difference in the three examined health outcomes, obesity, gingivitis, and sleep. The strong clustering effect within the schools in reducing the sleep time and eventually developing obesity and gingivitis over time indicates that the school context has a substantial influence in shaping children's behaviors in the Kuwaiti population. Children spend a considerable amount of their time at school (59). Fifty percent of a child's waking time is spent in schools; therefore, school is considered a unique social setting that could configure children's health attitudes and behaviors that eventually shape their health outcomes (59, 60). Studies show that health outcomes of children with high genetic susceptibility to certain health conditions can be improved if surrounded by a healthy protective environment (55). Therefore, most common health intervention programs are school based, because the school can provide the social contextual framework that would support any selected intervention and enhance reduction the risk of factors for disease conditions (59).

It has been shown that modifying sleep behavior and improving the quality of sleep is efficient and has the potential to support immunity and relief symptoms of chronic diseases (5). Therefore, school health intervention programs should consider behavioral sleep improvement by educating children and parents about the importance of sleep quality on overall health. Children's behaviors may be more easily influenced than adults resulting in a greater potential to accept and adopt new behaviors if they are exposed to healthy practices. Therefore, targeting children's beliefs and attitudes is an essential step to building practices that can enhance overall health and prevent chronic diseases. A recent review explored school determinants for optimal health and included WHO and CDC guidelines. This review reported that school health programs could predict children's health outcomes (60-63). According to this report, school health intervention and prevention programs that target nutrition and physical activities can significantly promote children's health (60). Several studies have documented that poor physical and structural school environments are associated with poor children's health (108). Strong evidence shows that school health programs, based on nutrition and healthy eating can effectively change children's health behaviors and decrease weight and obesity (59). Therefore, schools can act as an influential instrument to improve child health outcomes. Research in the US and other developed countries has successfully identified primary school health determinants (60). Based on this, school health policies and guidelines have been introduced and implemented to ameliorate chronic disease such as obesity(64). This improvement has evolved from the substantial effort to integrate scientific research into practice.

Findings from our prospective longitudinal study might be the first step for future research and policy implementation to advance total health for Kuwaiti children and others who face similar health challenges. The following are some recommendations for future work, which evolved from our study: 1) introduce school health intervention and awareness programs to educate children and their families about the importance of sleep health and how to improve night sleep quality and limit the screen-based activities, 2) implement health regulations in schools regarding healthy food, increased physical activities, and oral hygiene measures, 3) promote public health research to identify healthy school determinants, to help designing future cost-effective school intervention and prevention programs, and 4) dentists and physicians should work together to integrate oral and systemic risk factors, and discuss how to provide prevention strategies for optimal oral and general health.

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