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Short Communication

BMI, eating habits and sleep in relation to salivary counts of mutans streptococci in children – the IDEFICS Sweden study

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Abstract
Objective: The objective of the present study was to investigate the association between salivary counts of mutans streptococci (MS) and children's weight status, while considering associated covariates. MS ferments carbohydrates from the diet and contributes to caries by lowering the pH in dental plaque. In adults, high counts of MS in saliva have been associated with overweight, but this has not been shown in children.
Design: Cross-sectional study investigating salivary counts of MS, BMI *Z*-score, waist circumference, meal frequency, sugar propensity and sleep duration, in children.
Setting: West Sweden.
Subjects: Children (*n* 271) aged 4–11 years.
Results: Medium–high counts of MS were positively associated with higher BMI *Z*-score (OR = 1·6; 95 % CI 1·1, 2·3). Positive associations were also found between medium–high counts of MS and more frequent meals per day (OR = 1·5; 95 % CI 1·1, 2·2), greater percentage of sugar-rich foods consumed (OR = 1·1; 95 % CI 1·0, 1·3) and female sex (OR = 2·4; 95 % CI 1·1, 5·4). A negative association was found between medium–high counts of MS and longer sleep duration (OR = 0·5; 95 % CI 0·3, 1·0).
Conclusions: BMI *Z*-score was associated with counts of MS. Promoting adequate sleep duration and limiting the intake frequency of sugar-rich foods and beverages could provide multiple benefits in public health interventions aimed at reducing dental caries and childhood overweight.

Keywords
BMI Z-score
IDEFICS
Mutans streptococci
Meal frequency
Sugar propensity
Sleep duration

During the last decades levels of dental caries seem to be increasing worldwide[1] reflecting the well-known development of childhood obesity. This has led to recent interest in the relationship between caries and obesity as they may have common aetiologies that lend themselves to common solutions[2,3]. For instance, intake of sugar-sweetened beverages has been positively associated with BMI in children[4]. Sugar, especially in large quantity and when consumed frequently, is also known to contribute to the development of caries[5–7]. Further, shorter sleep duration and nocturnal eating have been associated with overweight and obesity[8–10] as well as with dental disease[11,12].

The cariogenic micro-organism mutans streptococci (MS), an important factor in the development of caries, ferments carbohydrates from the diet and contributes to caries by lowering the pH in dental plaque, resulting in tooth demineralization[5,13]. High counts of MS in saliva can be used as a biomarker for intake of fermentable carbohydrates[14], and positive associations between MS and BMI have been identified in adults[15–17] but not in children[18] or adolescents[17]. Given the obesity epidemic, the link between overweight and risk of dental caries is of interest. The objective of present study was to investigate the potential relationship between counts of MS and weight status in children while considering important covariates.

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Methods

Participants

The present study included 294 children, aged 4–11 years, from the Swedish cohort of the IDEFICS study (Identification and prevention of Dietary- and lifestyle-induced health Effects In Children and infantS). IDEFICS is a prospective cohort study on child health with an embedded community-based intervention, including eight centres in Europe. The diet part of the intervention aimed to improve dietary habits by increasing daily consumption of water, fruits and vegetables[19] and thereby decreasing intake of added sugars. Ethics approval was obtained from the central ethical review board in Gothenburg. Parents provided written informed consent, and children gave oral consent for examinations and sample collections. Further information about the IDEFICS study can be obtained from previous reports[19,20].

Data on anthropometrics, diet and sleep were collected during 2009 to 2010 when 1511 (84 %) children from the Swedish cohort returned for a follow-up. Saliva was collected in connection with a dietary sub-study, preceded by a strategic sampling to represent both the control (Alingsås and Mölndal) and intervention (Partille) areas in Sweden, including 728 children, of whom 40 % provided a saliva sample. Reasons for not providing saliva were lack of time, child refused or parents did not want their child to chew the paraffin. After excluding twenty-four children because of inconclusive data on saliva (n 3), missing diet information (n 18) and incomplete anthropometric data (n 3), 271 children are included in the present study.

Cariogenic bacteria in saliva

Paraffin-stimulated saliva was collected during a fasting morning examination and sent to the Department of Cariology at the Dental School in Gothenburg, where it was processed within 24 h. The saliva was shaken on a Whirlimixer, diluted in tenfold steps in 0·05-M phosphate buffer and plated on Mitis Salivarius-Bacitracin agar. The agar plates were incubated anaerobically at 37°C for 2 d. Colony-forming units (CFU) of MS were counted and identified by their colony morphology[21] and divided into two groups for categorical analysis: medium–high counts ($>10^5$ CFU/ml), also referred to as 'higher', and low counts ($\leq 10^5$ CFU/ml). These thresholds are commonly used in dental research and known to predict high or low risk of caries[22].

Meal frequency, sugar propensity and sleep

Meal frequency and sleep duration were calculated from SACINA (Self-Administered Children and Infant Nutrition Assessment), a 24 h diet recall (24-HDR) program[23]. The parents or other caregivers, assisted by a registered dietitian, reported what the child had been eating and drinking the previous 24 h, as well as wake-up time and bed time.

Due to the high day-to-day variation in diet[24,25], usual sugar intake was estimated using the sugar propensity ratio derived from a reproducible[26] and validated[27] FFQ. The sugar propensity ratio is defined as the sum of sugar-rich foods and beverages divided by the sum of all foods reported. A more detailed description of the sugar propensity ratio is presented elsewhere[28,29].

Anthropometrics

Weight of the children was measured to the nearest 0·1 kg by a Tanita BC 420 SMA scale and height was measured to the nearest 0·1 cm by a SECA 225 stadiometer. Measurements were done in the morning, with the children fasting and wearing only underwear. Waist circumference was measured according to a standard protocol[30]. Age- and gender-specific BMI and BMI Z-scores for children and adolescents developed by the International Obesity Task Force[31] were calculated.

Parental education

Data on education level was based on the International Standard Classification of Education (ISCED) for cross-country comparability[32] and used to determine the maximum highest level of the parents' education, a proxy for socio-economic status. Levels 1–3 represent upper secondary school and are classified as low education level while levels 4–6 represent post-secondary education and are classified as high education level.

Statistics

Descriptive statistics were used to define basic characteristics, i.e. mean and standard deviation for continuous variables, and number and percentage for binary variables. For comparison by MS (medium–high v. low) the Student t test was used for continuous variables (age, sugar propensity ratio, sleep duration, meal frequency, BMI Z-score, waist circumference) and Pearson's χ^2 test to compare categorical variables (sex, education level, intervention exposure). Age-adjusted univariate logistic regression was used to investigate the association of potential covariates with medium–high counts of MS. The final model was obtained by multiple logistic regression with stepwise forward selection among the whole set of covariates. Area under the receiver-operating characteristic curve was used to estimate how well the final model predicted the outcome[33]. To examine the possible effect of dietary under-reporting, and age respectively, sensitivity analyses were performed by excluding participants with only one meal reported (n 4) in the 24-HDR, and by forcing age into the multivariable model. For calculation of the area under the receiver-operating characteristic curve, the statistical software package SAS version 9·3 was used; otherwise data were analysed using IBM SPSS Statistics Version 20. The significance level was set to 0·05.

Results

Descriptive properties and comparison by MS status are presented in Table 1. Medium–high counts of MS were found among 18 % of the children. Low counts were found in 82 % (including children with counts below the detection limit). No differences were found between the low and medium–high counts group regarding the proportion of females (44 *v.* 52 %, $P = 0.30$), of high parental education level (86 *v.* 80 %, $P = 0.40$) or of being in the intervention group (59 *v.* 58 %, $P = 0.9$). Children with higher counts were older ($P = 0.01$), reported greater sugar propensity ratio ($P = 0.02$), less sleep ($P = 0.00$) and more frequent meals ($P = 0.03$). Additionally, they had larger waist circumference ($P = 0.03$) and marginally significantly greater BMI *Z*-score ($P = 0.05$) compared with those with low counts of MS.

Table 1 Distribution of variables by bacterial status (low *v.* medium–high counts of mutans streptococci) among children (*n* 271) aged 4–11 years, the IDEFICS Sweden study

	Low counts* ($\leq 10^5$ CFU/ml, *n* 223)		Medium–high counts ($>10^5$ CFU/ml, *n* 48)		
Variable	Mean	SD	Mean	SD	*P* value†
Age (years)	8·2	1·9	8·9	1·6	0·01
Sugar propensity ratio (%)	21·9	3·5	23·5	4·6	0·02
Sleep duration (h)	10·1	0·7	9·7	0·6	0·00
Number of meals	5·5	1·2	5·9	1·2	0·03
BMI *Z*-score	0·1	1·3	0·5	1·2	0·05
Waist circumference (cm)	57·7	6·8	60·5	8·4	0·03

IDEFICS, Identification and prevention of Dietary- and lifestyle-induced health Effects In Children and infantS; CFU, colony-forming units; MS, mutans streptococci.
*No. of participants below detection limit of MS (200 CFU/ml) = 183 (67·5 %); median (interquartile range) count of MS among participants with MS above detection limit = 1·3 (0·46–13·8) × 10^5 CFU/ml.
†*P* values from *t* test for continuous variables.

Table 2 Odds ratios for medium–high counts of mutans streptococci among children (*n* 271) aged 4–11 years, the IDEFICS Sweden study*

Variable	OR	95 % CI	*P* value
Univariate regression			
Age (years)	1·3	1·0, 1·6	0·02
Age-adjusted logistic regression			
Sugar propensity ratio (%)	1·1	1·0, 1·2	0·03
Sleep duration (h)	0·5	0·3, 0·9	0·02
Number of meals	1·3	1·0, 1·8	0·08
BMI *Z*-score	1·4	1·0, 2·0	0·03
Waist circumference (cm)	1·0	1·0, 1·1	0·10
Sex (female)	1·6	0·8, 3·4	0·20
Education level (high)	0·8	0·3, 2·1	0·61
Intervention area	1·0	0·5, 1·8	0·89
Final model (multiple logistic regression with forward stepwise selection of variables)			
Sugar propensity ratio (%)	1·1	1·0, 1·3	0·03
Sleep duration (h)	0·5	0·3, 1·0	0·04
Number of meals	1·5	1·1, 2·2	0·01
BMI *Z*-score	1·6	1·1, 2·3	0·01
Sex (female)	2·4	1·1, 5·4	0·03

IDEFICS, Identification and prevention of Dietary- and lifestyle-induced health Effects In Children and infants.
*No. of participants included in both analyses = 233.

Our main results are presented in Table 2. Using age-adjusted logistic regression, we found that higher counts of MS were associated with sugar propensity ratio, sleep duration and BMI *Z*-score. The final multiple logistic regression analysis with stepwise forward selection identified five variables which independently explained the MS categorization of these children. More frequent meals, sugar propensity ratio, BMI *Z*-score and female sex were all positively associated with medium–high counts, while a negative association was found for longer sleep duration. The area under the receiver-operating characteristic curve was given by 0·78 (95 % CI 0·70, 0·85), indicating good discrimination properties of the final model. The exclusion of children with only one meal reported (*n* 4) in the 24-HDR did not change the results. A separate sensitivity analysis where age was added to the full model chosen by stepwise procedures confirmed the result of lack of association between MS count and age of the child. However, adding age to the final model attenuated the effect of sex to marginal significance while the other estimates remained unchanged (data not shown). No stratifications were made for age or sex due to the small sample size.

Discussion

Our finding that higher BMI *Z*-score was positively associated with higher counts of MS in children is novel although in line with earlier findings in adult populations[15–17]. In the age-adjusted model higher BMI *Z*-score was positively associated with higher counts of MS and the association was strengthened in the multivariable model. The fact that the association between higher BMI *Z*-score and counts of MS remained after mutual adjustments suggests that some other common denominator, not accounted for in present study, may be driving the association. Other micro-organisms like e.g. lactobacilli have also been identified as a risk factor for dental caries in children[5,13]; however, no associations between salivary counts of lactobacilli and higher BMI *Z*-score were found in the present sample (data not shown).

The proportion of sugar-rich foods and beverages reported during a typical week was greater in children with higher counts compared with children with low counts, in agreement with other studies[14,34]. Children with higher counts of MS also reported more frequent meals compared with children with low counts. Higher meal frequency implies snacking and less time for oral clearance, which could lead to increased availability of carbohydrates, promoting colonization of MS. Meal frequency[35] and taste preference for sugar-rich foods[28] have been associated with overweight, and both diet patterns also increase colonization of cariogenic micro-organisms in children[5,7,36]. Therefore, it was surprising that the association between higher BMI *Z*-score and

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medium–high counts of MS observed here could not be accounted for by these factors. Reporting errors in parental estimates of usual sugar intake and meal frequency may explain this result.

Children's food preferences are formed at an early age and are relatively stable during pre-school years[37], although there is an increasing soft drink consumption[38] and a higher preference for fruits and foods rich in fat and sugar in school-aged children[39]. Therefore it was important to consider the age range by adding age to the final model. However, the estimates remained unchanged suggesting no effect of age on the association between propensity for consuming sugar, meal frequency and counts of MS in the present study. Considering the young age groups included (age span 4–11 years) one can speculate that eating habits are still highly moderated by the parents. Furthermore, in Sweden pre-school and school food environments can be expected to be similar for all age groups, which could explain the lack of an age effect on the associations in the present study.

The recent findings of a negative association between longer sleep duration and the development of overweight[8,9] was the reason for investigating the effect of sleep duration on counts of MS. Sleep duration was inversely associated with counts of MS. Night eating has been associated with less sleep, overweight and dental disease in earlier studies[10–12], but since data are not available for the times of sugar consumption, we can only speculate about the possible role of night eating in this context.

Our finding that counts of MS were significantly higher in females has not been reported earlier and could be related to the fact that permanent teeth eruption occurs earlier in girls[40,41], implying a longer period of MS colonization in girls than boys of the same age. This is further supported by the fact that the association between MS and sex was only marginally significant when age was added to the final model.

Despite our unique findings the present study is not without limitations. First, we cannot establish causality from our study. Second, meal frequency and sleep are based on a single 24-HDR which is unlikely to accurately reflect usual habits. However, our finding of meal frequency being positively associated with counts of MS is in line with earlier studies[5,7,36]. Usual sugar intake was assessed by an FFQ, which is considered superior to a single 24-HDR for assessing usual sugar exposure. As with all methods of measuring dietary intake related to obesogenic foods, it is likely that that our study suffers from biased parental reports of usual intake. In contrast to parental reported dietary measures, anthropometric variables and counts of MS were measured objectively and BMI *Z*-score was analysed as a continuous variable, all of which strengthens the study.

Conclusions

In this sample of 4–11-year-olds BMI *Z*-score was associated with higher counts of MS. Meal frequency, propensity to consume sugar, sleep duration and female sex were also independently associated with higher counts of MS. Therefore, public health efforts aimed at reducing dental caries and overweight could provide multiple benefits, as these problems might both be resolved in the same fashion. Important targets for joint interventions should include limiting intake frequency of sugar-rich foods and beverages and promoting an adequate amount of sleep.

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