Microbiota in the Oral Subgingival Biofilm Is Associated With Obesity in Adolescence

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To test the hypothesis whether microbiota in oral biofilm is linked with obesity in adolescents we designed this cross-sectional study. Obese adolescents (n = 29) with a mean age of 14.7 years and normal weight subjects (n = 58) matched by age and gender were examined with respect to visible plaque index (VPI%) and gingival inflammation (bleeding on probing (BOP%)). Stimulated saliva was collected. They answered a questionnaire concerning medical history, medication, oral hygiene habits, smoking habits, and sociodemographic background. Microbiological samples taken from the gingival crevice was analyzed by checkerboard DNA–DNA hybridization technique. The sum of bacterial cells in subgingival biofilm was significantly associated with obesity (P < 0.001). The link between sum of bacterial cells and obesity was not confounded by any of the studied variables (chronic disease, medication, VPI%, BOP%, flow rate of whole saliva, or meal frequency). Totally 23 bacterial species were present in approximately threefold higher amounts, on average, in obese subjects compared with normal weight controls. Of the Proteobacteria phylum, *Campylobacter rectus* and *Neisseria mucosa* were present in sixfold higher amounts among obese subjects. The association between obesity and sum of bacterial cells in oral subgingival biofilm indicates a possible link between oral microbiota and obesity in adolescents.

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INTRODUCTION

Obesity is a large health problem (1) and is associated with increased risk of diabetes type II, cardiovascular disease (2), as well as implicated in a wide range of gastrointestinal disorders such as Crohn's disease and subclinical bowel inflammation (3). Obesity is also associated with enhanced prevalence of chronic periodontitis (4,5). We recently reported that obesity in adolescents was linked to pathological periodontal pockets including enhanced level of proinflammatory cytokines in gingival crevicular fluid (6) as well as with reduced flow rate of whole saliva (7). Adipose tissue represents an endocrine organ from which a high number of proteins and hormones, so called adipokines, are synthesized and secreted (8,9). These adipokines are acting both locally and at distant sites, influencing important biological processes including lipid homeostasis, immune function, insulin sensitivity, control of blood pressure, appetite, and energy balance (10,11).

Circulating adipokines might also influence the immune response at the mucosal level both in the oral cavity and in the gut, thereby affecting the microbial colonization. There are also studies describing an altered microbiological colonization in the gut of obese subjects, data indicating that obese subjects have more Firmicutes and relatively less Bacteroidetes in the gut compared with normal weight control participants (12,13). Whether there is a relationship between obesity and the oral microbiota is so far unclear. In an animal model, however, obesity has been reported to interfere with the ability of the immune system to appropriately respond to infection by the periodontal pathogen *Porphyromonas gingivalis* (14). Furthermore, enhanced colonization of *Tannerella forsythia* in subgingival biofilm was recently reported in obese subjects (15) and *Selenomonas noxia* was detected in saliva from overweight women (16).

Considering these recent findings, the present investigation was undertaken to investigate whether the oral microbiota in the subgingival biofilm is linked to obesity in adolescents. We tested the null-hypothesis that the microbial composition in subgingival biofilm does not differ between obese and normal weight adolescents.

METHODS AND PROCEDURES

In a cross-sectional study design, the present pilot study was conducted on 29 obese subjects and 58 normal weight subjects (**Table 1**). The

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Table 1 Characteristics of the subjects

	Obesity	Controls	
	(*n* = 29)	(*n* = 58)	
Variables	Mean (s.d.)	Mean (s.d.)	*P* value
Male/female	18/11	36/22	1.000[a]
Age (years)	14.7 (1.4)	14.4 (1.8)	0.399[b]
Weight (kg)	109.5 (26.8)	54.3 (11.6)	<0.001[b]
Length (m)	1.70 (0.10)	1.65 (0.13)	0.491[b]
BMI (kg/m^2)	37.4 (6.8)	19.8 (2.3)	<0.001[b]
BMI-SDS[c]	5.7 (1.3)	0.3 (1.1)	<0.001[b]
Country of birth[d]			
Both parents born abroad	13	23	
One parent born in Sweden	5	12	
Both parents born in Sweden	9	22	0.824[a]
Education[e]			
Both parents ≤12 years	11	33	
One parent ≤12 years	10	11	
Both parents >12 years	5	8	0.159[a]
Chronic disease[f]	8/29	6/58	0.061[c]
Asthma	4	4	
Diabetes type 1	0	1	
Diabetes type 2	3	0	
Thyroid dysfunctions	1	0	
Epilepsy	0	1	
Polycystic ovaries	1	0	
Allergy	11/29	17/58	0.417[a]
Medication[g]	11/29	7/58	0.010[a]
Meal frequency	4.7 (1.4)	4.7 (1.2)	0.844[b]
Toothbrushing-morning			
Daily	15	50	
Not daily	14	8	0.002[a]
Toothbrushing-evening			
Daily	14	49	
Not daily	15	9	<0.001[a]
VPI			
0–25%	20	54	
>25%	9	4	0.004[a]
BOP			
0–25%	23	54	
>25%	6	4	0.058[a]
Supragingival calculus	13/29	22/58	0.644[a]
Subgingival calculus	5/29	3/58	0.111[a]
Salivary flow rate (ml/min)	1.3 (0.6)	2.0 (0.9)	0.002[b]

BOP, bleeding on probing; SDS, standard deviation score; VPI, visible plaque index.
[a]χ^2 as statistical method. [b]ANOVA as statistical method. [c]According to Rolland-Cachera. [d]In three cases the father's place of birth was unknown. [e]In nine cases the parent's educational level was unknown. [f]One subject had multiple diagnosis. [g]Three obese subjects and one control used prescribed allergy medication when needed.

obese subjects were newly registered patients at the National Childhood Obesity Center, Karolinska University Hospital, Huddinge, Sweden and consecutively referred to the Division of Pediatric Dentistry for examination of oral conditions. All obesity subjects had a BMI within the obesity range for the age (ISO-BMI >30) (17).

The control subjects (ISO-BMI <25) were recruited from the Division of Pediatric Dentistry, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden and consisted of individuals receiving their regular dental check-up at the department. For each obese adolescent two control subjects were randomly selected and matched with respect to age (±2 months) and gender out of the patient register at the department. Body weight (kg) and height (m) were determined and obesity was expressed as BMI (kg/m^2) as well as by BMI adjusted for age and gender (BMI-SDS) (18). The following exclusion criteria were used for both groups; any antibiotic treatment during the last 3 months and/or ongoing orthodontic treatment. Subjects that admitted a daily smoking habit were also excluded from the present study.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the research. The ethics committee at Karolinska University Hospital approved the study and all subjects gave oral and written consent before participating.

Questionnaire

All adolescents answered a questionnaire that covered topics of their medical condition, medication, meal frequency and oral hygiene habits, smoking habits, as well as their parent's education and country of birth. Parent's country of birth was categorized into "born in Sweden" or "born abroad." The educational level of the parents was stratified according to the years of schooling as (i) ≤9 years, (ii) 10–12 years, and (iii) >12 years.

Clinical examination

The presence of dental plaque on tooth surfaces were recorded when clearly visible and expressed using the visible plaque index (VPI) (19). Gingival inflammation was based on bleeding on probing (BOP) (19) of the gingival sulcus of all teeth (wisdom teeth excluded) at six sites of each tooth. The proportion of surfaces (%) with visible dental plaque and gingival inflammation, respectively, was calculated for each subject.

Incipient alveolar bone loss. In order to determine marginal alveolar bone loss, two bitewing radiographs were taken using standardized technique. The distance between cemento-enamel junction and alveolar bone crest on the radiographs was measured on the mesial and distal surfaces of premolars and first molar by using a Peak scale loupe (sevenfold magnification; Carton Optical, Tokyo, Japan). Incipient marginal alveolar bone loss was classified as positive when the distance from cemento-enamel junction to alveolar bone crest on the radiographs was ≥2 mm (20). The clinical recording was performed by one of the authors (CB).

Calculus. Supragingival calculus was recorded on all teeth as present or absent when clearly visible. Subgingival calculus was recorded as present or absent on proximal surfaces of first molar and premolars on the radiographs taken as well as clinically after probing the gingival sulcus.

Saliva. Stimulated whole saliva was collected by asking the subject to chew on 1 g of paraffin wax for 5 min. The amount of saliva was determined after collection and the saliva secretion rate was expressed as ml/min.

Microbiological sampling

Before sampling, supragingival plaque was eliminated and the gingival margins were wiped dry with a sterile cotton pellet. Samples were taken from one molar in the lower jaw and one incisor site the upper jaw, with a sterile paperpoint. The paperpoint (Size 40; Precise Dental

Internacional, S.A de C.V, Zapopan, Mexico) was inserted into the gingival crevice for 5 s. The paperpoints were stored in Eppendorf tubes at −70 °C until analyzed with respect to microbiology.

Microbiological processing

We analyzed the samples with the checkerboard DNA–DNA hybridization technique. A total of 37 bacterial species and three subspecies were included in the checkerboard panel (**Table 2**). The checkerboard DNA–DNA hybridization method was performed as described by Socransky *et al.* (21). To each vial with a subgingival bacterial sample, 0.15 ml Tris EDTA buffer (10 mmol/l Tris–HCL, 1.0 mmol/l EDTA, pH 7.6) and 0.10 ml of 0.5 mol/l NaOH was added to each RNase DNase, DNA and, pyrogen free sterile 1.5 ml natural flat cap microcentrifuge tubes (Starlab, Ahrensburg, Germany) with oral bacterial samples and then processed.

Bacterial DNA was extracted, concentrated on nylon membranes and fixed by crosslinking using ultraviolet light. The membranes with fixed DNA were placed in a Miniblotter 45 (Roche Diagnostics, Mannheim, Germany). Signals were detected by fluorescence using the Storm FluorImager (Stratalinker 1800; Stratagene, La Jolla, CA) with a setup of 200 µ and 600 V. The digitized information was analyzed by a software program (ImageQuant; Amersham Pharmacia, Piscataway, NJ) allowing comparison of the density of 19 sample-lanes against the two standard-lanes (10^5 or 10^6 cells). Signals were converted to absolute counts by comparisons with these standards (22,23). Detection limit was $\geq 1.0 \times 10^4$ and cell counts below the detection limit was considered as 0. The bacterial phyla, families, and species assayed are identified in **Table 2**.

Statistics

Data analysis was carried out using the statistical software package SPSS, version 18.0 (SPSS, Chicago, IL). For analyzing the data, frequency tables, cross tables, ANOVA, χ^2, and linear regression were used. Bivariate analyses of associations were carried out between the dependent variable obesity and the potential independent variables. To avoid adjusting "sum of bacterial cells" for interacting variables, Pearson's correlation test was used to determine intra correlations between potential confounders. In the multivariate logistic regression with obesity as dependent variable, the variable "sum of bacterial cells" was adjusted for the strongest variable of the intracorrelating potential confounders. The odds ratio (and 95% confidence interval) was calculated and the level of significance was accepted at P values <0.05. In case of the microbiological variables, the Bonferroni test estimated, a P value of <0.01 was required to declare significance to avoid chance of mass significance. In addition, the sensitivity and specificity for bacterial sum as a discriminator of obesity were also determined.

RESULTS

The characteristics of the subjects with respect to age, gender, chronic disease, medication, weight, height, BMI-SDS, VPI%, BOP%, flow rate of whole saliva (ml/min), meal frequency, toothbrushing habits as well as the educational level and birth place of their parents are shown in **Table 1**. In addition to BMI and BMI-SDS, the use of prescribed medication was significantly higher ($P = 0.01$) among the subjects with obesity compared to the controls. The obese subjects also demonstrated significant lower frequency of tooth brushing ($P = 0.002$) as well as significantly higher VPI (>25%) ($P = 0.005$) compared with controls. None of the subjects in the present study exhibited signs of alveolar bone loss (data not shown).

Bacterial cell counts from obese and control subjects

The sum of assessed bacterial cells ($\times 10^5$) as well as the different bacteria species sorted into phyla and family class is demonstrated in **Table 2**. The sum of bacterial cells was significantly higher among subjects in the obesity group ($P < 0.001$) as compared to the controls. On average, approximately threefold higher amounts of bacterial cells were found in the samples from the obesity subjects compared with normal weight controls. Out of six bacterial phyla's determined, five were found at higher counts in the obese subjects and counts of all families under these phyla's were significantly higher ($P < 0.001$) in the obese subjects. Of the Proteobacteria phylum, the bacteria *Campylobacter rectus* and *Neisseria mucosa* were present in sixfold higher amount among obese subjects compared with normal weight group. There was no difference between the groups concerning bacteria in the Spirochaetes phylum.

Out of the totally 40 different bacterial species determined in the bacterial samples, 32 species were present in significantly higher amounts ($P < 0.01$ level) in the obese subjects compared to the normal weight controls. In a bivariate logistic regression analysis with obesity as dependent variable the following variables were significantly associated; VPI% ($P = 0.005$), chronic disease ($P = 0.046$), medication ($P = 0.007$), no daily toothbrushing in the evening ($P < 0.001$) or morning ($P = 0.002$), salivary flow rate ($P = 0.004$), bacterial sum ($P = 0.001$) as well as the following phyla's; Firmicutes, ($P = 0.002$), Bacteroidetes ($P = 0.001$), Actinobacteroides ($P = 0.001$), Proteobacteria ($P = 0.002$), and Fusobacteria ($P = 0.001$) (**Table 3**).

In a multivariate logistic regression analysis, we further tested the association between obesity and the sum of bacterial cells when adjusting for potential confounders. After adjusting for all of these potential confounders there was a significant association between bacterial cell count and the dependent variable obesity (odds ratio = 1.05, $P = 0.006$) (**Table 4**). The sensitivity of the variable "sum of bacterial cells" as a discriminator of obesity was estimated to 0.41 and the specificity was 0.95. After adjusting for potential confounders the sensitivity was 0.75 and the specificity was 0.84.

DISCUSSION

The novel finding in the present study demonstrates an association between oral bacterial cells counts in the subgingival biofilm and obesity in adolescence that was not confounded by VPI%, BOP%, chronic disease, tooth brushing habits, or salivary flow rate (ml/min).

Obesity has become a significant health problem including a potential risk factor of chronic periodontitis (24,25). We recently reported that obesity among adolescents was linked to periodontal risk indicators such as pathological periodontal pockets as well as enhanced levels of proinflammatory cytokines in gingival crevicular fluid (6). This agrees well with the view that obesity is linked to a low-grade inflammatory condition affecting many different organ systems (1,3) including periodontal tissue.

Adolescents as an observation group have several advantages when investigating the link between oral microbiota and obesity as they do not normally suffer from periodontitis and the complexity of potential confounders including interactions with various chronic diseases like diabetes and cardiovascular

Table 2 Bacterial cell counts of phyla, families, and species (×10[5]) in samples taken from the gingival crevice

	Obesity	Controls	
	Mean (s.d.)	Mean (s.d.)	*P* value[a]
Sum of bacterial cells	38.29 (38.38)	11.56 (13.66)	<0.001
Firmicutes	12.83 (13.62)	4.74 (5.66)	<0.001
Staphylococcaceae	0.98 (1.06)	0.44 (0.53)	0.002
Staphylococcus aureus	0.28 (0.31)	0.17 (0.23)	0.063
Subsp. *anaerobius*	0.19 (0.25)	0.07 (0.12)	0.003
Staphylococcus haemolyticus	0.51 (0.57)	0.20 (0.26)	<0.001
Streptococcaceae	5.68 (6.38)	1.78 (2.09)	<0.001
Streptococcus anginosus	0.22 (0.26)	0.11 (0.17)	0.023
Streptococcus constellatus	0.16 (0.19)	0.07 (0.11)	0.009
Streptococcus gordonii	0.36 (0.49)	0.10 (0.11)	<0.001
Streptococcus intermedius	0.27 (0.32)	0.17 (0.30)	0.151
Streptococcus mitis	0.73 (1.05)	0.29 (0.45)	0.007
Streptococcus mutans	0.28 (0.35)	0.13 (0.37)	0.032
Streptococcus oralis	1.46 (1.94)	0.43 (0.63)	<0.001
Streptococcus pneumoniae	1.75 (2.11)	0.40 (0.45)	<0.001
Streptococcus sanguinis	0.46 (0.61)	0.08 (0.09)	<0.001
Clostridiales	0.76 (0.66)	0.36 (0.43)	<0.001
Parvimonas micra	0.76 (0.66)	0.36 (0.43)	<0.001
Lactobacillaceae	0.66 (0.73)	0.21 (0.29)	<0.001
Lactobacillus acidophilus	0.66 (0.73)	0.21 (0.29)	<0.001
Veillonellaceae	0.74 (0.66)	1.95 (3.12)	0.015
Veillonella parvula	0.74 (0.66)	1.95 (3.12)	0.015
Bacteroidetes	6.98 (7.13)	1.94 (3.15)	<0.001
Flavobacteriaceae	5.04 (5.75)	1.15 (2.23)	<0.001
Capnocytophaga gingivalis	1.50 (2.19)	0.32 (0.77)	<0.001
Capnocytophaga ochracea	1.78 (1.90)	0.45 (0.81)	<0.001
Capnocytophaga sputigena	1.76 (2.35)	0.37 (0.93)	<0.001
Porphyromonadaceae	0.87 (1.59)	0.24 (0.62)	0.009
Porphyromonas gingivalis	0.19 (0.26)	0.06 (0.11)	<0.001
Tannerella forsythia	0.68 (1.50)	0.18 (0.58)	0.027
Prevotellaceae	1.07 (1.15)	0.55 (1.04)	0.036
Prevotella intermedia	0.56 (0.70)	0.22 (0.48)	0.009
Prevotella melaninogenica	0.51 (0.61)	0.33 (0.72)	0.255
Actinobacteroides	1.29 (1.44)	0.34 (0.40)	<0.001
Actinomycetaceae	1.91 (1.35)	0.31 (0.36)	<0.001
Actinomyces israelii	0.27 (0.3)	0.08 (0.09)	<0.001
Actinomyces naeslundii	0.69 (0.80)	0.18 (0.23)	<0.001
Actinomyces odontolyticus	0.23 (0.28)	0.06 (0.07)	<0.001
Propionibacteriaceae	0.10 (0.12)	0.03 (0.06)	<0.001
Propionibacterium acnes	0.10 (0.12)	0.03 (0.06)	<0.001
Proteobacteria	12.19 (15.49)	2.90 (3.61)	<0.001
Pasteurellaceae	1.17 (1.87)	0.35 (0.45)	0.002
Aggregatibacter actinomycetemcomitans			
a29523	0.57 (1.11)	0.12 (0.18)	0.004
bY4	0.61 (0.82)	0.23 (0.31)	0.002

Table 2 Continued on nexrt page

Table 2 Continued

	Obesity	Controls	
	Mean (s.d.)	Mean (s.d.)	*P* value[a]
Campylobacteraceae	3.02 (3.54)	1.03 (1.57)	<0.001
Campylobacter gracilis	0.44 (0.56)	0.17 (0.27)	0.003
Campylobacter rectus	0.98 (1.20)	0.15 (0.34)	<0.001
Campylobacter showae	1.59 (2.01)	0.59 (0.89)	0.002
Neisseriaceae	6.69 (9.20)	1.20 (1.87)	<0.001
Neisseria mucosa	4.44 (6.76)	0.71 (1.23)	<0.001
Eikenella corrodens	2.25 (2.66)	0.48 (0.73)	<0.001
Pseudomonadaceae	1.31 (1.92)	0.33 (0.44)	<0.001
Pseudomonas aeruginosa	1.31 (1.92)	0.33 (0.44)	<0.001
Fusobacteria	4.92 (4.89)	1.58 (2.33)	<0.001
Fusobacteriaceae	4.92 (4.89)	1.58 (2.33)	<0.001
Fusobacterium nucleatum			
Naviformevincentii	1.23 (1.27)	0.31 (0.58)	<0.001
Nucleatum	0.96 (0.93)	0.34 (0.51)	<0.001
Polymorphum	1.16 (1.21)	0.34 (0.55)	<0.001
Fusobacterium periodonticum	1.17 (1.22)	0.40 (0.58)	<0.001
Leptotrichia buccalis	0.40 (0.41)	0.20 (0.24)	0.002
Spirochaetes	0.07 (0.07)	0.07 (0.11)	0.716
Treponemataceae	0.07 (0.07)	0.07 (0.11)	0.716
Treponema denticola	0.07 (0.07)	0.07 (0.11)	0.716

[a]ANOVA as statistical method.

diseases are rare. This is the first study demonstrating that obesity is associated with an alteration of the subgingival microbiota in adolescents although increased counts of *T. forsythia* colonizing the periodontal pockets have previously been demonstrated in obese adults (15). We here demonstrate that traditional periodontal pathogens such as *P. gingivalis*, *A. actinomycetemcomitans*, and *P. micra* (21,26), are present at approximately threefold increase in the dental biofilm of obese adolescents compared with the normal weight controls. The periodontopathogen *P. gingivalis*, linked to periodontitis, is known to have a strong impact on the innate immune system. Macrophages are activated to produce a number of proinflammatory mediators including the cytokines interleukin-1 and tumor necrosis-α as well as prostaglandins (27) contributing to a chronic inflammation milieu that may affect the physiopathological mechanisms involved in the development of obesity. This suggestion is well compatible with recent findings that periodontal infection, measured by means of the number of teeth with pathological periodontal pockets (4 mm deep or deeper), was associated with obesity in adults (28). Moreover, it has been reported that human adenovirus-36 is associated with obesity in children (29) indicating the importance of infections in obesity development.

In the multivariate model, obesity was tested as outcome variable although we are fully aware of a potential opposite mechanism whereby obesity directly or indirectly may affect the quantity and/or quality of oral microbiota instead of being an outcome. In the multivariate logistic regression model with obesity as the dependent variable, the sum of bacterial cells of assessed species was significantly associated with obesity even after adjustment of the variables VPI%, BOP%, tooth brushing habits, salivary flow rate, or chronic disease. As medication and chronic disease significantly intercorrelated with each other ($r = 0.8$), so also did toothbrushing in the evening and morning ($r = 0.4$), the variables "medication" and "toothbrushing in the morning" were excluded in the final model. The association between oral microbiota and obesity was not confounded by any of the studied variables. This association between bacterial sum and obesity is interesting and is the first observation that link oral microbiota in dental biofilm to obesity in a population not suffering from chronic periodontitis. Although the odds ratio of "sum of bacterial cells" seems to be low (1.05), one has to consider that an increase from 1×10^5 to 1×10^6 bacteria enhances the risk for obesity by 50%, indicating clinical relevance.

This is a pilot study and its limitation is the cross-sectional design as well as the relatively low number of subjects included. However, the proportion of subjects with obesity in the study group rather well reflects the relation that exists between teenagers with overweight conditions and normal weight subjects in Sweden today.

In agreement with our previous paper, we demonstrate that obese adolescents exhibit a significantly lower flow rate of

Table 3 Bivariate logistic regression analysis with obesity as dependent variable

Variable	β-Coefficient	Wald	df	*P* value	OR	95% CI
VPI >25%	1.86	7.97	1	0.005	6.40	1.76–23.20
BOP >25%	1.26	3.31	1	0.069	3.52	0.91–13.67
Calculus						
Supragingival	0.29	0.38	1	0.537	1.33	0.54–3.28
Subgingival	1.34	3.03	1	0.082	3.82	0.84–17.28
Chronic disease	1.19	3.98	1	0.046	3.30	1.02–10.68
Allergy	0.39	0.66	1	0.418	1.47	0.58–3.77
Medication	1.49	7.22	1	0.007	4.45	1.50–13.23
Tooth brushing						
Morning–not daily	1.69	9.89	1	0.002	5.42	1.89–15.52
Evening–not daily	1.76	11.54	1	0.001	5.83	2.11–16.14
Salivary flow rate (ml/min)	1.56	8.24	1	0.004	0.32	0.15–0.70
Born abroad						
Mother	–0.57	1.50	1	0.220	0.56	0.23–1.41
Father	–0.05	0.01	1	0.920	0.95	0.38–2.40
Education <12 years						
Mother	–0.11	7.63	1	0.836	0.90	0.32–2.50
Father	–0.72	2.04	1	0.152	0.49	0.18–1.31
Sum of bacterial cells	0.05	11.89	1	0.001	1.05	1.02–1.07
Firmicutes	0.10	9.29	1	0.002	1.10	1.03–1.74
Bacteroidetes	0.21	11.86	1	0.001	1.23	1.09–1.38
Actinobacteroides	1.65	11.47	1	0.001	5.22	2.01–13.58
Proteobacteria	0.15	9.37	1	0.002	1.16	1.05–1.27
Fusobacteria	0.27	11.82	1	0.001	1.30	1.12–1.52
Spirochaetes	0.84	0.14	1	0.713	2.32	0.03–03.71

BOP, bleeding on probing; CI, confidence interval; df, degrees of freedom; OR, odds ratio; VPI, visible plaque index.

Table 4 Multiple regression analysis with obesity as dependent variable adjusted for potential confounders

Variable	β-coefficient	Wald	df	*P* value	OR	95% CI
Sum of bacterial cells (unadjusted)	0.045	11.89	1	0.001	1.05	1.02–1.07
Adjusted for						
BOP >25%	0.044	11.33	1	0.001	1.05	1.02–1.07
VPI >25%	0.044	11.04	1	0.001	1.05	1.02–1.07
No daily tooth brushing-evenings	0.039	9.41	1	0.002	1.04	1.01–1.07
Salivary flow rate (ml/min)	0.051	10.71	1	0.001	1.05	1.02–1.08
Chronic disease	0.043	11.24	1	0.001	1.04	1.02–1.07
Sum of bacterial cells (adjusted for all of the above)	0.044	7.44	1	0.006	1.05	1.01–1.08

BOP, bleeding on probing; CI, confidence interval; df, degrees of freedom; OR, odds ratio; VPI, visible plaque index.

stimulated whole saliva (ml/min) than normal weight control subjects (7). In the multivariate model, with obesity as outcome, we also adjusted for flow rate of stimulated whole saliva which did not confound the link between oral microbiota and obesity.

We found approximately threefold higher level of the phylum Firmicutes and approximately fourfold higher level of the phylum Bacteroidetes in the subgingival biofilm of obese adolescents. There was no significant difference of the relative proportion of Firmicutes and Bacteroidetes in the dental biofilm between obesity and controls. In the gut, however, obese subjects had relatively more Firmicutes but less Bacteroidetes compared with lean controls (30). Animal studies support

the hypothesis that gut bacteria influences development of obesity, especially the Firmicutes phylum in mice promotes absorption of monosaccharides that results in enhanced lipogenesis (31).

Approximately 1 g of oral bacteria corresponding to ~10^{11} cells are swallowed daily with the 500–1,500 ml of saliva produced (26). The high amount of oral microbiota ingested may affect energy harvesting in the gut and thereby be involved in the development of obesity. However, it is unknown in what amounts and proportions oral bacteria ingested will survive the defense barriers of the gastrointestinal tract which is an important issue to study.

Recent data suggest that genetic factors affect clustering of bacteria in periodontal pockets (32). Thus it is likely that genetic factors contribute to the difference in bacterial colonization in obese and normal weight adolescent subjects. Future studies of genetic predisposition of obesity and its potential effect on bacterial load and phenotypes in periodontal pockets can presumably provide new information about the link between obesity and oral microbiota.

Oral microbiota plays a major role in the nitrate to nitrite conversion which in its turn is important in regulating blood pressure (33,34). Interestingly, it was reported that the number of nitrate reducing bacteria was greatly reduced in the oral cavity of rats treated with daily application of antiseptic mouthwash (35) indicating a possible role of oral microbiota in regulating blood pressure and thereby in general health. In addition, a recent study reported a relationship between an increased loading of periodontal pathogens in the oral biofilm and hypertension (36) which might be an important link behind the association between oral and cardiovascular comorbidity previously reported (34). Furthermore, it has been demonstrated that there is an association between periodontitis and impaired fasting glucose (35) and that treatment of periodontitis improves glycemic control in subjects with type II diabetes (36). Altogether, these clinical studies indicate that oral condition has an important role on general health. Whether the alteration of the oral microbiota promotes the development of obesity in adolescents or is a consequence of the obese condition or alternatively induced by lifestyle-associated conditions is therefore an essential issue for further research.

In conclusion, the association between obesity and sum of bacterial cells in the oral subgingival biofilm indicates a possible link between oral microbiota and obesity in adolescents.

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DISCLOSURE

The authors declared no conflict of interest.

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