Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 301–307 doi: 10.1111/j.1600-051X.2009.01374.x

Adiposity and gingival crevicular fluid tumour necrosis factor-α levels in children

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Khosravi R, Tran SD, Lambert M, O'Loughlin J, Kâ K, Feine JS, Caron C, Tremblay A, Nicolau BF. Adiposity and gingival crevicular fluid TNF-α levels in children. J Clin Periodontol 2009; 36: 301–307. doi: 10.1111/j.1600-051X.2009.01374.x.

Abstract

Aim: To investigate whether adiposity is associated with gingival crevicular fluid (GCF) tumour necrosis factor-α (TNF-α) levels in children. We also examined whether this relationship is mediated through plasma fasting insulin.

Materials and Methods: This preliminary study used cross-sectional data from the baseline-visit of the Quebec Adipose and Lifestyle InvesTigation in Youth cohort, which is an ongoing longitudinal study investigating the natural history of obesity in Quebec children. Study participants (76 girls and 102 boys) include children aged 8–10 years and their families, living in the Montreal and Quebec City areas. TNF-α level was measured in pooled samples ($N = 4$) for each child by enzyme-linked immunosorbant assay. Height and weight were measured. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Sex/age-specific BMI was categorized into normal (<85th percentile), overweight (85th–95th percentile) and obese (⩾95th percentile) defined by the 2000 US-CDC growth charts. Insulin resistance was measured using fasting plasma insulin in children. Data analysis involved descriptive and multiple linear regression analyses.

Results: Our results suggest that obesity in boys was associated with a 37% increase of GCF-TNF-α level. However, when accounting for insulin resistance this association was reduced and disappeared while the model's goodness of fit improved.

Conclusions: These findings provide support for the link between adiposity in children and GCF-TNF-α level, which appears to be mediated by insulin resistance.

Key words: plasma insulin; obesity; periodontal disease; TNF-α; children

Accepted for publication 6 December 2008

Obesity has reached an epidemic level (James 2008). 1.1 billion adults and 10% of children are diagnosed as overweight or obese worldwide (Haslam & James 2005). Cardiovascular diseases (CVD), type 2 diabetes mellitus (DM2), atherosclerosis, and destructive periodontal diseases are among the reported adverse consequences of obesity (Must et al. 1999, Saito & Shimazaki 2007). Findings over a decade ago on the physiological function of adipose tissue reveal that adipocytes express proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and IL-6, collectively called adipokines. In obesity, overexpression of these proinflammatory mediators induce chronic inflammation (Hotamisligil 2006). Some studies suggest that this low-grade chronic inflammation is an early event in the development of obesity-associated co-morbidities such as CVD and DM2 (Mohamed-Ali et al. 1997).

The link between obesity and destructive periodontal disease was first reported in Zucker rats in 1977 (Perlstein & Bissada 1977). Almost two decades later, Saito et al. (1998) demonstrated the association between obesity and destructive periodontal disease in human for the first time. Afterward, several epidemiological studies in adults reported obesity as a risk factor for destructive periodontal disease (Al-Zahrani et al. 2003, Wood et al. 2003, Saito et al. 2005, Linden et al. 2007). Although the mechanism behind this association remains unclear, the potential biological

Conflict of interest and sources of funding statement

This study was supported by Fonds de la recherche en santé du Québec (FRSQ) and Canadian Institutes of Health Research (CIHR). Roozbeh Khosravi is the recipient of a MSc fellowship from the Armand-Frappier Foundation. Belinda Nicolau holds a New Investigator Award from the CIHR.

explanation might be that obesity-associated chronic inflammation predisposes individuals to an exaggerated inflammatory response to periodontal pathogens residing in the biofilm. In addition, overexpression of adipokines in obesity (particularly TNF-α) influences the metabolism of insulin resistance, which in turn, may further augment the systemic inflammatory response (Genco et al. 2005).

Several epidemiological studies in adults have reported a positive association between adiposity and destructive periodontal disease in adults; however, there is no study, to our knowledge, which has investigated the effect of childhood adiposity on periodontal tissue. With an ever-increasing prevalence of childhood overweight/obesity, it is of high importance to understand the early changes in the periodontal tissues of these children.

The Quebec Adipose and Lifestyle InvesTigation in Youth (QUALITY) cohort study, which began in 2005, is a large longitudinal investigation of the natural history of obesity in Quebec children, in Canada. It focuses on biological, genetic, lifestyle, and environmental factors as determinants of obesity and attempts to understand the natural history of obesity that may be useful for designing preventive strategies for this condition. Here we present the preliminary cross-sectional results from the baseline visit. We aim to investigate the association between body mass index (BMI) (a measure for adiposity) and the level of gingival crevicular fluid (GCF)-TNF-α (a potential precursor of destructive periodontal disease). We hypothesized that obese children will have higher level of GCF-TNF-α. In addition, we hypothesized that this association is partly mediated through fasting insulin levels.

Materials and Methods

Study design and population

The QUALITY cohort study is an ongoing longitudinal investigation of the natural course of obesity in Quebec children. Participants (children and their parents) were systematically recruited through schools located within 75 km of Montreal and Quebec City, Canada. Eligibility criteria included that: (i) the participants were aged 8–10 years; (ii) both biological parents were available for the study; (iii) at least one biological parent was obese (i.e., BMI of mother and/or father $\geqslant 30$, mother's waist circumference >88 cm, or father's waist circumference >102 cm); (iv) the participants were of Caucasian origin to avoid heterogeneity (i.e., population stratification bias) for genetic analyses. Children were excluded if they had a chronic disease (e.g., diabetes, cystic fibrosis, and inflammatory bowel disease, etc.) and/or a psychological problem that, in the opinion of the investigators would interfere with the conduct of the study. The families, which fulfilled the aforementioned criteria, were invited to participate in the study. During a 1-day hospital visit, participants were interviewed to collect data on socio-demographic, behavioural, and psychological factors; children provided fasting blood samples; weight, height, blood pressure, and Tanner sexual maturity stages using standardized procedures. All participants received a standardized dental examination. The Ethics Review Board of CHU Sainte-Justine approved this study. Written informed consent was obtained from the participants.

This nested cross-sectional exploratory analysis focuses on 178 of the first 300 children enrolled in QUALITY cohort, for whom baseline dental examination data were available.

Dental clinical examination

Children were examined lying on a dental chair with a standardized source of artificial light. Dental examinations included clinical and an inflammatory indicator of periodontal health. The presence or absence of visible plaque, bleeding on probing, and calculus was measured on the buccal and lingual surfaces of six community periodontal index (CPI) teeth (World Health Organization report 1997). The proportion of positive sites was then calculated and categorized into *low* and *high* severity of dental plaque, dental calculus, or gingival bleeding using the median as a cut-off point. GCF samples were collected using Periopaper strips (ProFlow Inc., Amityville, NY, USA) inserted into the mesio-buccal sites of teeth #1-1, #1-6, #3-1, and #3-6 and held for 30 s. Periotron units were recorded by a Periotron 6000 machine (ProFlow Inc.) and converted to the actual volume (μl) according to the standard curve. The four strips obtained from each child were stored in a cryotube at -80°C until laboratory analysis.

TNF-α and insulin measurements

Thawed strips were placed in a 96-well microplate containing 200 μl of distilled water in each well, and GCF was eluted from the strips on a platform shaker for 30 min. GCF-TNF-α levels were measured using an enzyme-linked immunosorbant assay (ELISA) according to the manufacturer instructions (R&D Systems, Minneapolis, MN, USA). The minimum volume of GCF that was considered adequate for ELISA analysis was 0.2 μl. The inter-assay coefficients of variation for GCF-TNF-α levels were 16% at 9.02 pg/μl. Fasting plasma insulin was measured with the ultra-sensitive Access® immunoassay system (Beckman Coulter Inc., Fullerton, CA, USA) which has no cross-reactivity with proinsulin or C-peptide. The inter-assay coefficients of variation were 6.4% at 62.7 pg/μl and 5.5% at 390.8 pg/μl.

Anthropometric measures

Height was measured with a stadiometer as participants stood straight against a wall keeping their heads in the Frankfort horizontal plane and looking ahead. It was recorded to the nearest millimeter (0.1 cm) during maximal inspiration. Weight was measured to the nearest 0.1 kg using a spring scale that was calibrated daily. All measurements were taken twice, with participants wearing lightweight indoor clothing without shoes or sweaters. If the measurements differed by more than 0.2 cm for height, or 0.2 kg for weight, a third measurement was taken, and the average of the two closest measures was used in the statistical analysis. BMI was computed as weight/height^2 (kg/m^2) and to assess the effect of overweight and obesity in our data analysis it was categorized into normal (BMI $<$ 85th percentile), overweight (BMI $\geqslant$ 85th percentile $<$ 95th percentile) or obese BMI ($\geqslant$95th percentile) using the age- and sex-specific percentiles of the US Centers for Disease Control and Prevention (CDC) 2000 growth charts. Plasma insulin level (pmol/l), a proxy measurement for insulin resistance, was measured in fasting blood samples. Insulin levels were analysed with the ultra-sensitive insulin kit using the access® immunoassay system machine (Beckman Coulter Inc.).

Other variables

Data on socio-economic position (SEP) and behavioural factors were collected in questionnaires completed by the parents. Family income, an indicator of SEP, was measured in an ordinal scale containing 12 categories. Due to relatively small numbers in some of the categories, this variable was categorized into *low* (<$80,000) and *high* income (≥$80,000) using the median as cut-off point. Last dental visit and tooth brushing habits were categorized as visits *within the last year*/visits *more than 1 year ago* and *two or more times per day/1 or less time per day*, respectively. Pubertal development stage was assessed by the Marshall and Tanner method (Marshall & Tanner 1969, 1970) wherein pubic hair pattern in both sexes, breast development in girls, and genitalia development in boys were assessed on a scale of 5 (1 = pre-adolescent, 5 = mature).

Data and statistical analysis

All analyses were carried out using SPSS version 15 (SPSS Inc., Chicago, IL, USA) and the level of significance was set at 5%. Our initial data analysis examined univariate relations between BMI and GCF-TNF-α levels using a non-parametric test (the Kruskal–Wallis test). Because GCF-TNF-α levels were not normally (Gaussian) distributed, we $\log_e$ transformed this variable. The regression coefficients for the 100 $\log_e$-transformed TNF-α levels represent the percentage change in GCF-TNF-α per unit change in the independent variable (Cole 2000). To facilitate the interpretation of the results, we computed age- and sex-specific *z*-score for fasting plasma insulin levels. The distance between the upper quartile (75th percentile) and the lower quartile (25th percentile), defined as the inter-quartile range (IQR), was calculated for GCF-TNF-α levels. To examine the correlation between GCF-TNF-α and plasma fasting insulin levels, Spearman's rank bivariate analysis was performed (Table 2). The associations between BMI and plasma insulin levels with GCF-TNF-α levels were tested in multiple linear regression. BMI was modelled as binary dummy variables and its association with GCF-TNF-α levels was assessed before and after adjustment for potential confounders including age in months and a SEP indicator (family income). To determine whether fasting plasma insulin mediates the effect of BMI, we included both BMI and fasting plasma insulin in the model and compared the parameters obtained with those of the main effect model. Sex interactions terms for BMI and fasting plasma insulin were investigated in the model for GCF-TNF-α levels. Because the sex interaction terms were statistically significant for BMI ($p = 0.039$) and fasting plasma insulin ($p = 0.023$), all analyses were conducted separately according to sex. All models were also tested using BMI as a continuous variable. However, in order to facilitate the interpretation of the results, we decided to present the results using the variable as categorical. As this is an exploratory study, our data analysis involved multiple testing methods. Ideally, we would have to correct for multiple comparisons. However, due to the nature of the data analysis, this correction procedure was not performed. Therefore, we cannot completely disregard the possibility that our observed difference might be due to chance.

Results

Of 300 first families recruited in the baseline wave of the Quality Cohort, clinical dental data were available for 178 participants (102 boys and 76 girls). Out of the 122 (41% of the total sample) subjects excluded from the analysis, 70% (86 subjects) had no dental exams or no GCF-TNF-α data (no Periotron machine available) and the remaining 30% ($n = 36$) had the GCF- TNF-α level measured with a less sensitive ELISA kit. We decided to exclude the 36 subjects in order to avoid any bias in the result. Table 1 compares selected characteristics of the 178 participants included in the analysis with those of the 122 participants not included. The included and not included subjects presented similar characteristics including age, fasting plasma insulin level, BMI, tooth brushing habit, last dental visit, and the stage of puberty; nonetheless, family income was the only distinctive trait.

The mean age of boys and girls was 9.6 (SD 0.8) and 9.5 (SD 0.9) years, respectively (Table 1). More than half of participants were from a household with a high SEP. Approximately 40% of boys and girls were overweight or obese. The median of GCF-TNF-α levels was higher in boys (38 pg/μl IQR = 72) than in girls (32 pg/μl IQR = 55). Almost 80% of the children visited their dentists in the previous year and brushed their teeth more than once a day.

While overweight or obese boys had higher mean values for GCF-TNF-α levels than those who had normal BMI, there was little or no difference on GCF-TNF-α levels among girls (Table 2). In addition, plasma insulin levels were positively correlated with GCF-TNF-α levels only among boys (Spearman $r = 0.210$; $p = 0.035$). Pubertal development stage and oral health-related behaviour and clinical indicators of periodontal status (bleeding on probing, plaque scores) were not associated with GCF-TNF-α levels (Table 2).

The results of the multiple linear regression models examining the effects of adiposity and GCF-TNF-α levels are presented in Table 3. Because low-grade systemic inflammation produced by the excess of adipose tissue may affect insulin resistance and this in turn may mediate the association between adiposity and GCF-TNF-α levels, we examined these associations using three models. Because BMI and fasting plasma insulin are correlated (Kruskal–Wallis test; $p < 0.0001$), we assessed their independent contributions to variations in GCF-TNF-α levels. In the first model, after controlling for all covariates, BMI remained positively associated with GCF-TNF-α level. Obese boys were associated with 37% increase of GCF-TNF-α level (Table 3, model 1). However, this relationship was not significant for girls. The second model presents the results of the association between fasting plasma insulin and GCF-TNF-α levels after adjusting for family income and age. An increase of 1 SD in fasting plasma insulin was associated with 16% increase in GCF-TNF-α level (Table 3, model 2). The third model displays the results of the independent contributions of BMI and plasma insulin to variations on GCF-TNF-α levels. The positive association between BMI and GCF-TNF-α levels was attenuated after adjustment for fasting plasma insulin. In addition, goodness of fit improved when these two variables were included in the model (Table 3, model 3).

Discussion

The purpose of this study was to investigate the effect of adiposity measured by BMI and its potential metabolic consequences in relation to children's periodontal health status. We used GCF-

Table 1. Selected characteristics of participants included in the analysis compared with those not included

Characteristics	Boys			Girls		
	included ($N = 102$)	not included ($N = 68$)	*p*-value	included ($N = 76$)	not included ($N = 54$)	*p*-value
Age (yrs), mean (SD)	9.6 ± 0.8	9.8 ± 0.8	0.804	9.5 ± 0.9	9.6 ± 0.9	0.279
Percentile logN GCF-TNF-α (pg/μl), mean (SD)	38 (72)	N/A	–	32 (55)	N/A	–
Fasting plasma insulin (pmol/l), mean (SD)	30 ± 17	31 ± 16	0.737	35 ± 22	38 ± 26	0.560
Body mass index, *n* (%)						
Normal weight	61 (60)	34 (50)	0.449	46 (61)	28 (52)	0.272
Overweight	21 (20)	17 (25)		10 (13)	13 (24)	
Obese	20 (20)	17 (25)		20 (26)	13 (24)	
Family income, *n* (%)						
Up to $80,000	44 (43)	42 (62)	0.013	33 (43)	33 (61)	0.035
More than $80,000	58 (57)	26 (38)		43 (57)	21 (39)	
Proportion of gingival bleeding, *n* (%)						
Low	74 (73)	N/A	–	55 (72)	N/A	–
High	28 (27)			21 (28)		
Proportion of dental plaque, *n* (%)						
Low	31 (30)	N/A	–	32 (42)	N/A	–
High	71 (70)			44 (58)		
Tooth brushing habit, *n* (%)						
Twice a day or more	70 (69)	48 (71)	0.461	57 (75)	42 (78)	0.440
Once in a day or less	32 (31)	20 (29)		19 (25)	12 (22)	
Last dental visit, *n* (%)						
With in the last year	74 (73)	50 (73)	0.516	60 (79)	45 (83)	0.348
More than 1 year	28 (27)	18 (27)		16 (21)	9 (17)	
Tanner stage (puberty), *n* (%)						
Stage I	92 (90)	63 (93)	0.397	44 (58)	34 (63)	0.107
Stage II	10 (10)	5 (7)		26 (34)	20 (37)	
Stage III	–	–		6 (8)	–	

GCF, gingival crevicular fluid; IQR, inter-quartile range; SD, standard deviation; TNF-α, tumour necrosis factor-α.

Table 2. Bivariate correlation analysis between GCF-TNF-α levels and several variables, by sex

Characteristics	Boys ($N = 102$)		Girls ($N = 76$)	
	median (IQR)	*p-value*	median (IQR)	*p-value*
Body mass index (BMI)				
Normal	22 (62)	0.016	30 (50)	0.986
Overweight	48 (74)		34 (69)	
Obese	67 (100)		32 (74)	
Family income				
Low (<80,000)	20 (80)	0.386	26 (69)	0.358
High (⩾80,000)	45 (69)		38 (48)	
Levels of dental plaque				
Low	45 (96)	0.198	36 (50)	0.490
High	34 (64)		24 (69)	
Levels of gingival bleeding				
Low	36 (68)	0.313	26 (45)	0.317
High	42 (77)		32 (72)	
Dental visit				
With in the year	43 (71)	0.497	36 (62)	0.015
More than 1 year	27 (79)		12 (40)	
Tooth brushing habit				
Twice a day or more	43 (69)	0.865	28 (50)	0.380
Once in a day or less	28 (76)		33 (112)	
Puberty				
Tanner stage I	41 (72)	0.826	35 (65)	0.422
Tanner stage II	19 (98)		17 (43)	
Tanner stage III	–		39 (71)	
	Spearman *r*	*p-value*	Spearman *r*	*p-value*
Fasting plasma insulin	0.210	0.035	0.071	0.540

GCF, gingival crevicular fluid; IQR, inter-quartile range; TNF-α, tumour necrosis factor-α.

TNF-α level as a sub-clinical indicator (precursor) of destructive periodontal disease. The study's main contribution is the demonstration of a positive association between BMI and GCF-TNF-α levels, independent of SEP and age of child. In addition, plasma insulin level, a surrogate measure of insulin resistance, was positively associated with GCF-TNF-α levels. Interestingly, the association between fasting plasma insulin and GCF-TNF-α is not independent of BMI, suggesting that the association between BMI and GCF-TNF-α is partly mediated through insulin resistance. These associations were evident only for boys.

Interpretation of findings

Our results demonstrate that BMI and fasting plasma insulin level are related to GCF-TNF-α levels in boys. To our best of knowledge, this is the first study, which investigates the associations between BMI, fasting plasma insulin, and GCF-TNF-α level in children. Our results are in line with the growing evidence suggesting adiposity is a risk factor for adult destructive periodontal disease. Overweight and obesity has been associated with an increased risk

Table 3. Multiple linear regression analysis of the association between tumour necrosis factor-α (TNF-α) concentration in the gingival crevicular fluid (GCF) in relation to body mass index (BMI) and age- and sex-specific plasma insulin *z*-score*

	Boys ($N = 101$)			Girls ($N = 76$)		
	β (95% CI)†	*p*-value	R^2	β (95% CI)	*p*-value	R^2
Model 1*						
Normal weight	Reference	–	0.085	Reference	–	0.001
Overweight	26.6 (– 0.3–53.6)	0.053		– 0.4 (– 36.7–35.9)	0.981	
Obese	37.1 (9.6–64.5)	0.009		1.5 (– 25.7–28.7)	0.912	
Model 2*						
Plasma Insulin	16.2 (5.6–26.7)	0.003	0.093	5.7 (– 05.9–17.3)	0.330	0.013
Model 3*						
Normal weight	Reference	–	0.124	Reference	–	0.022
Overweight	23.1 (– 4.6–50.9)	0.101		– 6.8 (– 44.3–30.8)	0.721	
Obese	24.3 (– 10.4–59.0)	0.168		– 14.7 (– 52.3–22.9)	0.438	
Plasma Insulin	9.1 (– 4.7–22.9)	0.194		10.1 (– 6.1–26.4)	0.218	

*Model 1 tests for the variation of GCF-TNF-α levels in relation to variation in BMI; Model 2 tests for the variation of GCF-TNF-α levels in relation to variation in plasma insulin; Model 3 tests for the variation of GCF-TNF-α levels in relation to variation of BMI and plasma insulin. All models were adjusted for age and socio-economic position (family income).

†Regression coefficients (β) represent the percentage change in mean TNF-α concentration in the GCF per unit increase in BMI category and in 1 standard deviation for plasma insulin.

SD, standard deviation.

of periodontal diseases in American, Japanese, and Brazilian adult populations (Al-Zahrani et al. 2003, Wood et al. 2003, Saito et al. 2005, Reeves et al. 2006). Similarly, adolescents and young adults who had a high waist circumference and weight were reported to show a 5% and 6% increased risk of destructive periodontal disease measure by loss of attachment, respectively (Reeves et al. 2006).

Our findings show a positive association between BMI and GCF-TNF-α levels support the biological explanation that the chronic inflammation in obesity predisposes individuals to an exaggerated inflammatory response to periodontal pathogens residing in biofilm. In addition, the results suggest that fasting insulin levels may partially mediate this association. Indeed, previous studies have suggested that overexpression of adipokines in obesity (particularly TNF-α) influence the metabolism of insulin, which in turn may further augment the systemic hyper-inflammatory response (Genco et al. 2005).

Our research is, to our knowledge, the first to show that an association between BMI, fasting insulin, and GCF-TNF-α levels in children. These findings provide some support for the growing evidence suggesting that exposures in early life have lasting or lifelong effects on the development of adult chronic diseases including destructive periodontal disease. Indeed, these are chronic diseases and thus by their own nature develop over a relatively long period of time. There are time lags between exposure, disease initiation, and progression and clinical recognition. Molecular and cellular changes involved in initiating disease processes occur before the disease manifesting itself as overt pathology. Thus one may suggest that the positive association between obesity and GCF-TNF-α level found in this study is the first step in the cellular and molecular chain of events, which will lead to development of adult destructive periodontal diseases. Indeed, there is a wealth of evidence based on experimental and clinical studies that TNF-α plays essential role in soft tissues (interleukins and matrix-metalloproteinases activation) and alveolar bone destruction (Stashenko et al. 1991, Vilcek & Lee 1991, Hanemaaijer et al. 1993). For example, inhibition of TNF-α decreased the infiltration of inflammatory cells in a diabetic experimental mouse (db/db) model, when compared with a group of mice with similar amounts of *Porphyromonas gingivalis* (Naguib et al. 2004). All animals had similar quantity of periodontal pathogens and those who had augmented level of TNF-α express more aggressive form of destructive periodontal disease. Therefore, it is plausible that the low-grade inflammation produced by the excess of adipose tissue over a long period of time influence the development of periodontal diseases.

This study found a positive association between obesity and levels of TNF-α reported only among boys. The evidence that destructive periodontal disease is more common among males supports this finding. Indeed, there is evidence suggesting males have worse oral health-related behaviour than females. Boys have lower frequency of tooth brushing, dental visits and higher levels of dental plaque, bleeding, and calculus than girls (Freire et al. 2001, Nicolau et al. 2005, Lopez & Baelum 2006). In this study, although not statistically significant, boys had the high level of dental plaque and gingival bleeding compared to girls (Table 1). High levels of plaque and gingival bleeding may augment the systemic effects produced by the adipose tissue, which in turn, may explain gender difference reported in this study.

Another possible explanation for the gender difference found in this study is related to hormonal levels. It has been suggested that estrogen has anti-inflammatory effects (Weitzmann & Pacifici 2006). Estrogen has higher level in girls in puberty compared with boys; moreover, the age of puberty in girls is earlier than boys. For example, girls aged 8–10 years old, as represented by the sample of this study, have higher level of estrogen than boys with the same age. This anti-inflammatory effect of estrogen might decrease the obesity-associated inflammation below the threshold level to trigger an inflammatory response to bacteria residing in the biofilm. This phenomenon may be even more pronounced in overweight/obese girls because they often experience advanced maturation and their sex hormones levels increase earlier than physiological timeline (De Simone et al. 1995).

Study strengths and limitations

One of the major limitations of the current study is its cross-sectional nature, which undermines us to draw a temporal relationship between adiposity and GCF-TNF-α levels. On the other hand, this is the first epidemiological study conducted in children to show the association between adiposity and GCF-TNF-α levels. Missing information on the oral health component of the QUALITY Cohort, is another limitation of this study. This paper refers to the first 300 families recruited in the study. Out of

these 300 families, we had data in 178 (60%). However, as showed in Table 1, except for family income, there were no differences between those included/non included in our analyses (Table 1). Family income, an indicator of socio-economic position, is associated with oral health mainly through material deprivation, disadvantaged lifestyle, or/ and life course health inequality (Sisson 2007). Accordingly, two explanations would account for the selection bias in the current study. First, the median family income in this study represent higher than Québec median family income ($65,000) thus in this study social inequalities is less likely to have an impact on the recruited children oral health. Second, the exclusion of the subjects was not systematically performed. For this reason we believe that the family income difference between those included in the analysis and those who were not occurred by chance, and would not affect our results.

In this study, we found no association between gingival bleeding and the GCF-TNF-α levels. The reasons for the lack of association between these variables are not clear. A possible explanation is the fact that we measured gingival bleeding as a binary variable (presence and absence of bleeding) rather than severity of gingival bleeding (a scaled variable; such as gingival index system). Thus, GCF-TNF-α level may reflect a more accurate measure of inflammation. In addition, GCF-TNF-α levels were assessed in four sites while gingival bleeding was assessed in 12 sites. This was performed based on the evidence that the four selected sites represent the sites, which are at higher risk of inflammation in the mouth (Kingman & Albandar 2002). In this context, one may postulate that the TNF-α level detected in GCF is merely a reflection of the serum TNF-α level instead of locally produced TNF-α. To answer this question, we will analyse serum TNF-α level in samples which are currently stored in the freezer. Moreover, we used indirect measures of body fat (i.e., BMI) and insulin resistance. BMI is highly correlated to direct measures of adiposity such dual photon absorptiometry (DAX) ($r = 0.8$) (Dietz & Robinson 1998). Plasma insulin is moderately correlated to IR in individuals with normal glucose tolerance (as measured by the euglycemic hyper-insulinemic clamp technique) (Laakso 1993). Finally, another issue of concern is the study's sample selection. Although our sample selection strategy for the QUALITY cohort was comprehensive, involving all schools located within 75 km of Montreal and Quebec cities areas, the study's inclusion criteria (selection of a population at risk of obesity) compromise the representativeness of the study sample in relation to the general population, and limits its external validity (ability to generalize the results to the general population).

Conclusion

Although the implications of this study and public health recommendations need to take into account the potential limitations, our findings contribute to generating a hypothesis that adiposity – a risk factor of chronic destructive periodontal disease in adults – may induce higher level of GCF-TNF-α earlier in life (childhood). This overexpression of TNF-α may be a precursor of chronic destructive periodontal disease because an increased inflammatory response leads alveolar bone destruction (Rossomando et al. 1990, Graves & Cochran 2003, Bostanci et al. 2008).

Our findings may also provide some further support for policies using the common risk approach (Sheiham & Watt 2000) in order to prevent childhood adiposity and its metabolic and vascular consequences. Future results of the Quality Cohort such as the incidence of periodontal disease in obese children will further clarify the findings reported here.

Acknowledgements

The authors acknowledge generous help of Dr. Martin Kim, Mr. Saaed Khalili, Dr. Larissa Vilela, and Dr. Diego Ardenghi in the laboratory analyses and the clinical examinations.

References

Al-Zahrani, M. S., Bissada, N. F. & Borawskit, E. A. (2003) Obesity and periodontal disease in young, middle-aged, and older adults. *Journal of Periodontology* **74**, 610–615.

Bostanci, N., Emingil, G., Afacan, B., Han, B., Ilgenli, T., Atilla, G., Hughes, F. J. & Belibasakis, G. N. (2008) Tumor necrosis factor-alpha-converting enzyme (TACE) levels in periodontal diseases. *Journal of Dental Research* **87**, 273–277.

Cole, T. J. (2000) Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. *Statistics in Medicine* **19**, 3109–3125.

De Simone, M., Farello, G., Palumbo, M., Gentile, T., Ciuffreda, M., Olioso, P., Cinque, M. & De Matteis, F. (1995) Growth charts, growth velocity and bone development in childhood obesity. *International Journal of Obesity Related Metabolic Disorders* **19**, 851–857.

Dietz, W. H. & Robinson, T. N. (1998) Use of the body mass index (BMI) as a measure of overweight in children and adolescents. *Journal of Pediatrics* **132**, 191–193.

Freire, M. C., Sheiham, A. & Hardy, R. (2001) Adolescents' sense of coherence, oral health status, and oral health-related behaviours. *Community Dentistry and Oral Epidemiology* **29**, 204–212.

Genco, R. J., Grossi, S. G., Ho, A., Nishimura, F. & Murayama, Y. (2005) A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *Journal of Periodontology* **76**, 2075–2084.

Graves, D. T. & Cochran, D. (2003) The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *Journal of Periodontology* **74**, 391–401.

Hanemaaijer, R., Koolwijk, P., le Clercq, L., de Vree, W. J. & van Hinsbergh, V. W. (1993) Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. *Biochemical Journal* **296** (Part 3), 803–809.

Haslam, D. W. & James, W. P. (2005) Obesity. *Lancet* **366**, 1197–1209.

Hotamisligil, G. S. (2006) Inflammation and metabolic disorders. *Nature* **444**, 860–867.

James, W. P. (2008) The epidemiology of obesity: the size of the problem. *Journal of Internal Medicine* **263**, 336–352.

Kingman, A. & Albandar, J. M. (2002) Methodological aspects of epidemiological studies of periodontal diseases. *Periodontology 2000* **29**, 11–30.

Laakso, M. (1993) How good a marker is insulin level for insulin resistance? *American Journal of Epidemiology* **137**, 959–965.

Linden, G., Patterson, C., Evans, A. & Kee, F. (2007) Obesity and periodontitis in 60–70-year-old men. *Journal of Clinical Periodontology* **34**, 461–466.

Lopez, R. & Baelum, V. (2006) Gender differences in tooth loss among Chilean adolescents: socio-economic and behavioral correlates. *Acta Odontologica Scandinavica* **64**, 169–176.

Marshall, W. A. & Tanner, J. M. (1969) Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood* **44**, 291–303.

Marshall, W. A. & Tanner, J. M. (1970) Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood* **45**, 13–23.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S. & Coppack, S. W. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. **82**, 4196–4200.

Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G. & Dietz, W. H. (1999) The disease burden associated with overweight and obesity. **282**, 1523–1529.

Naguib, G., Al-Mashat, H., Desta, T. & Graves, D. T. (2004) Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. *Journal of Investigation Dermatology* **123**, 87–92.

Nicolau, B., Marcenes, W., Bartley, M. & Sheiham, A. (2005) Associations between socio-economic circumstances at two stages of life and adolescents' oral health status. *Journal of Public Health Dentistry* **65**, 14–20.

Perlstein, M. I. & Bissada, N. F. (1977) Influence of obesity and hypertension on the severity of periodontitis in rats. *Oral Surgery, Oral Medicine, Oral Pathology* **43**, 707–719.

Reeves, A. F., Rees, J. M., Schiff, M. & Hujoel, P. (2006) Total body weight and waist circumference associated with chronic periodontitis among adolescents in the United States. **160**, 894–899.

Rossomando, E. F., Kennedy, J. E. & Hadjimichael, J. (1990) Tumour necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans. **35**, 431–434.

Saito, T. & Shimazaki, Y. (2007) Metabolic disorders related to obesity and periodontal disease. *Periodontology 2000* **43**, 254–266.

Saito, T., Shimazaki, Y., Kiyohara, Y., Kato, I., Kubo, M., Iida, M. & Yamashita, Y. (2005) Relationship between obesity, glucose tolerance, and periodontal disease in Japanese women: the Hisayama study. *Journal of Periodontal Research* **40**, 346–353.

Saito, T., Shimazaki, Y. & Sakamoto, M. (1998) Obesity and periodontitis. **339**, 482–483.

Sheiham, A. & Watt, R. G. (2000) The common risk factor approach: a rational basis for promoting oral health. *Community Dentistry and Oral Epidemiology* **28**, 399–406.

Sisson, K. L. (2007) Theoretical explanations for social inequalities in oral health. *Community Dentistry and Oral Epidemiology* **35**, 81–88.

Stashenko, P., Jandinski, J. J., Fujiyoshi, P., Rynar, J. & Socransky, S. S. (1991) Tissue levels of bone resorptive cytokines in periodontal disease. *Journal of Periodontology* **62**, 504–509.

Vilcek, J. & Lee, T. H. (1991) Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. **266**, 7313–7316.

Weitzmann, M. N. & Pacifici, R. (2006) Estrogen deficiency and bone loss: an inflammatory tale. **116**, 1186–1194.

Wood, N., Johnson, R. B. & Streckfus, C. F. (2003) Comparison of body composition and periodontal disease using nutritional assessment techniques: third National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Periodontology* **30**, 321–327.

World Health Organization report. (1997) *Oral health surveys: basic methods*. Geneva: World Health Organization.

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Clinical Relevance

Scientific rationale for the study: Several studies have shown that obesity is associated with destructive periodontal disease in adults. However, there is a paucity of information on this association in children. The biological explanation also remains poorly understood. This study investigates whether BMI is associated with an inflammatory marker, namely, TNF-α in the GCF of children.

Principal findings: Obesity in boys was associated with a 37% increase of GCF-TNF-α level. This association was attenuated after including insulin resistance in the model, suggesting a possible mediating role for insulin resistance.

Practical implications: Recognizing adverse consequences of obesity on oral-health, such as periodontal diseases, would allow dentists to monitor or treat their patients, in a timely manner.