The Influence of Childhood Obesity on Oral Inflammation

BY

CHAD M SILVER
B.S., Indiana University, 2015
D.M.D, University of Illinois Chicago School of Dentistry, 2019

THESIS
Submitted as a partial fulfillment of the requirements
for the degree of a Master of Science in Oral Sciences
in the Graduate College of the
University of Illinois Chicago (UIC), 2021

Chicago, Illinois

Thesis Committee:

Christina Nicholas (Chair and Primary Advisor), PhD, Dept. of Orthodontics
Sahar Alrayyes, DDS, MS, Dept. of Pediatric Dentistry
Afsar Naqvi, PhD, Dept. of Periodontics
Christine D. Wu, PhD, Dept. of Pediatric Dentistry

ACKNOWLEDGEMENTS

I would like to thank my one-stop support system, Dr. Nicholas, for her expertise and guidance over the past 18 months since beginning this project. Dr. Nicholas has been helpful every step of the way and I could not have accomplished this without her. To the rest of my committee: Dr. Alrayyes, Dr. Naqvi, and Dr. Wu – thank you for always being quick to offer advice and recommendations. The process of completing this project exemplified a true team effort. Thank you to the American Academy of Orthodontics foundation for funding this project. Thank you to Nish Shivnani, thank you for making the process of recruiting eligible patients so simple in such a short period of time. To my co-residents, thank you for accepting last-minute schedule changes to aid in data collection – your flexibility made the goals of this project attainable.

To the patients who agreed to partake in this study for the betterment of education for all, thank you. The extra time each patient spent with me during appointments did not go unappreciated.

Finally, thank you to my family, friends, and out-of-work support system that support me in all of my endeavors.

Table of Contents

CHAPTER PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

LIST OF ABBREVIATIONS

BMI	Body Mass Index
CCL2	Chemokine Ligand 2
CCL3	Chemokine Ligand 3
CPI	Community Periodontal Index
CRP	C-Reactive Protein
GCF	Gingival Crevicular Fluid
GI	Gingival Index
IL	Interleukin
MPO	Myeloperoxidase
PBS	Phosphate Buffered Saline

SUMMARY

In the United States, approximately one third of all children are overweight or obese. Obesity rates can vary based on demographics such as location, socioeconomic status, and racial/ethnic factors. Obesity rates in children in Chicago are higher than the national average. Obesity is defined as a Body Mass Index (BMI) greater than the 95th percentile for age. Inflammation can be associated with obesity, which may be associated with increased oral inflammatory levels when evaluating plaque scored and gingival bleeding.

The purpose of this prospective clinical study was to evaluate whether there are difference in levels of oral inflammation when comparing obese and non-obese children age's ten to twelve. Fifty-four children were recruited at the University of Illinois Chicago College of Dentistry, Pediatric Dental Department. A subset of twenty subjects was included in additional analysis. For each subject, plaque score and gingival bleeding upon treatment was assessed and recorded. Plaque score and gingival bleeding were used in this study as a proxy for oral inflammation. Gingival crevicular fluid (GCF) was collected to investigate whether there is a difference in cytokine levels between obese and non-obese subjects.

Obese children displayed significantly higher levels of plaque ($p<0.001$) and significantly more bleeding upon treatment ($p<0.05$) compared to non-obese subjects. In further analysis of a subset of ten obese and ten non-obese subjects, obese children displayed significantly higher levels of pro-inflammatory cytokines CCL2 and CCL3 compared to non-obese children. Each of these factors suggest the presence of higher levels of oral inflammation in obese children.

I. BACKGROUND

I.A Childhood Obesity

In the United States, approximately one-third of all children are estimated to be overweight or obese.[1] Specifically, obesity describes a state of excess fat content in the body. The Center for Disease Control defines normal weight range as 5th-85th percentile, overweight as a BMI greater than or equal to 85th-95th percentile for age and sex, obesity as a BMI greater than the 95th percentile for age and sex, and severe obesity as 120% above the 95th percentile for age and sex.[2] The current prevalence of overweight and obesity can be divided into specific age groups (age 2-5, 22.8%; age 6-11, 34.2%; age 12-19, 34.5%).[3] More specifically, obesity rates vary slightly between age groups (age 2-5, 8.4%; age 6-11, 17.7%; age 12-19, 20.5%).[3] While obesity may best be evaluated as a concrete measurement of adiposity in children and adolescents, BMI is currently the preferred measurement.[4]

Childhood obesity rates vary based on geographic, racial/ethnic, and socioeconomic factors.[2] Parental obesity is also a contributing factor that increases the likelihood of childhood obesity.[5] Other factors that contribute to childhood obesity are feeding styles, stress, diet, breast-feeding, and adverse life experiences.[3] Obesity rates are significantly higher in minority groups compared to non-Hispanic whites.[3] Further, obesity rates in non-Hispanic black children and adolescents (19.5%) were higher compared with non-Hispanic white children and adolescents (14.7%).[6] The odds for obesity were found to be lower in non-Hispanic asian children (8.6%) compared to non-Hispanic white children and adolescents.[6] Obesity rates were found to be higher in households where the head of the home had less than a high school degree or a high

school degree compared to households in which the head holds a degree greater than a high school degree, suggesting that education may be an important factor in prevention of childhood obesity.[6] From 1988 to 2014, obesity rates were on an overall gradual incline.[6]

Overweight and obesity rates in Chicago youth are higher than the national average for kindergarten, 6th grade, and 9th grade students.[7] Within the Chicago community, one predominantly white neighborhood displayed significantly lower extremely high obesity rates compared to communities that are majority black and Hispanic.[8] Obesity rates in Chicago are higher in black and Hispanic children, both male and female, compared to white children.[8]

I.B Obesity and Oral Inflammation

Inflammation has been shown to be increased in patients diagnosed with obesity. Blood tests comparing levels of adiponectin, leptin, leukocytes and C-reactive protein (CRP) in normal weight to obese subjects found an significantly increased level of leukocytes, leptin, and CRP in obese individuals.[9] Using bleeding on probing as a proxy for oral inflammation, increased BMI is suggested to be associated with increased oral inflammation and higher likelihood of periodontal disease when assessed via the CPI. [10]

Oral inflammation can be defined as the presence of disease-initiating microorganisms within periodontal tissues, oftentimes with the presence of marginal plaque or bleeding on probing used as a proxy.[10] In the pathologic process of periodontal disease, specific pathogens, including but not limited to *Porphyromonas gingivalis, Prevotella intermedia,* and *Fusobacterium nucleatum,* are known to initiate a host immune response leading to pathologic breakdown of periodontium.[11] The host response that occurs is multifaceted in nature, mediated

by proinflammatory cytokines and metalloproteinases increasing polymorphonuclear leukocyte in periodontal tissue and leading to tissue breakdown.[12] In addition to bacterial initiation of inflammatory processes, current literature suggests that adipose tissue is capable of releasing proinflammatory cytokines in oral tissues.[4]

Gingival inflammation related to plaque levels has been correlated to accelerated dental exfoliation of primary teeth and eruption of permanent teeth.[13] A significant theory related to this expedited eruptive pattern is increased inflammation in periodontal tissues which may alter bone composition and reduce jaw bone quantity and quality.[13] A common method for evaluating gingival health is the Gingival Index (GI). [14] This method identifies and quantifies gingival inflammation and plaque presence on four surfaces (mesial, buccal, distal, and lingual) of each tooth based on the level of inflammation and is scored from 0-3. [14]

I.C Obesity and Dental Maturation

Recent literature has identified an association between childhood BMI and the level of dental development, finding a faster dental development in obese and overweight children relative to normal weight children.[15–18] Obese and overweight children may present with a more advanced dental age relative to chronological age.[15] There also may be genetic predisposition to obesity and advanced dental age.[15] One early study comparing dental development in obese and normal weight children found an average of 18-month advancement in dental development in obese children compared to normal weight children.[16] However, the reasoning for why obese children develop significantly faster than normal weight children remains unknown.

One factor that may contribute to advanced dental development is the presence of oral inflammation. Lal and colleagues (2008) found an association between gingival inflammation and tooth eruption in a sample of children with diabetes mellitus. Further, children with known chronic inflammatory disease, such as juvenile rheumatoid arthritis and diabetes mellitus, display advanced dental maturity.[13,19] With the knowledge that cytokines have a significant role in the recruitment of osteoclasts/osteoblasts during dental eruption and bone turnover, one may hypothesize that there may be an association between increased levels of pro-inflammatory cytokines and obesity.[20]

I.D Childhood Obesity and Oral Health

Significant disparities in oral health may be attributed at least in part to varying obesity rates among different communities.[1] Gingivitis can be defined as an inflammatory disease of the mucosa surrounding the teeth that is of bacterial origin.[21] In a study defining obesity by waist circumference, obese children demonstrated a significantly higher level of erythematous gingival tissue compared to non-obese children.[22] Further, a known collagen breakdown byproduct, hydroxyproline, was found to be increased in the salivary and blood content of obese children with gingivitis. [21] In children, a positive association between overweight/obese insulin-resistant children and gingival inflammation has been noted.[23] Further, a positive correlation between BMI and dental caries has been appreciated in both males and females.[24] Current literature also suggests that BMI and waist circumference can be a successful predictor of Community Periodontal Index (CPI) scores in boys.[25] In adults, there is a known association between obesity and periodontal disease.[26]

Obese children have also been documented to display a significantly higher levels of gingival plaque compared to normal weight children.[27] Obesity in children is consistent with poorer oral hygiene, as well as a higher level of bleeding upon probing during dental examinations.[28] Lifestyle factors, such as sedentary activities like watching TV and playing video games, are known contributors to obesity and have been associated poorer oral hygiene in pre-adolescent children.[29] Oral hygiene habits, including tooth brushing, are reported to be superior in adolescents who exercise more compared to others who exercise significantly less.[30] From an orthodontic standpoint, obese children display poorer compliance with removable orthodontic appliances.[31]

Current research has produced mixed results on whether obese children have more caries[32] or not.[33,34] The relationship between obesity and dental caries in children are likely due to common etiological risk factors including a high sugar diet, lower socioeconomic status, as well as other environment and social factors.[32] Obese children have also been found to engage in a higher fat, lower nutrient diet as well as a high sugar/ cariogenic diet.[35] Other studies have suggested that obese children have a significantly higher DMFT score in the primary dentition compared to normal and underweight children.[36]

I.E Study Objectives

The objectives of this study were:

1) To evaluate whether obese children have poorer oral hygiene by evaluating plaque scores and gingival marginal bleeding upon dental treatment

2) To evaluate whether obese children displayed varying levels of pro- and anti-inflammatory cytokines in gingival crevicular fluid compared to non-obese children

I.F Hypotheses

H_{01}: There is no difference in plaque scores or gingival marginal bleeding between obese and normal weight children

H_{02}: There is no difference in pro- and anti-inflammatory cytokine levels in obese and normal weight children

II. METHODS

II.A Study Approval

This study was approved for exemption by the Institutional Review Board of the University of Illinois Chicago (IRB #2017-0956), Chicago, IL (Appendix A). The study has been funded by the American Association of Orthodontists Foundation Biomedical Research Award (PI: Nicholas).

II.B Study Subjects, Inclusion/Exclusion Criteria

Study Subjects:

Fifty-four pediatric patients aged 10-12 were recruited from the University of Illinois Chicago College of Dentistry's Pediatric Dentistry Clinic for this study. This age is most representative of the late-mixed dentition period of dental development. Height and weight were used to obtain BMI (>95th percentile: Obese; 5th-85th percentile: normal). Plaque indices were recorded and gingival marginal bleeding was recorded. Within the subjects recruited, we included a subsample of twenty patients whose GCF samples were analyzed for levels of pro- and anti-inflammatory cytokines. From here on, these twenty subjects are designated as "Cytokine Subjects" or "Cytokine Sample". The total group of all fifty-four subjects will be designated as "All Subjects" or "Overall Sample".

Inclusion Criteria:

- 10.0-12.9 years of age
- BMI in the "normal" (5th-85th percentile) or "obese" (95th+) range for age/sex/height

- Healthy (with no systemic diseases)
- Patient in the Department of Pediatric Dentistry at the University of Illinois at Chicago

Exclusion Criteria:

- Poor oral health
- Craniofacial anomalies
- Congenitally missing teeth/extra teeth
- Inflammatory diseases
- Taking prescription medications
- Active carious lesions involving the pulp

II.C Methodology

Informed consent for participation was obtained by each participant's legal guardian and assent was obtained by the participant. Body mass index was calculated by dividing weight (Kg) by height (m^2) and was adjusted for age, sex, and ethnicity. A diagnostic panoramic radiograph was taken for each subject. For each patient, periodontal health was evaluated in two ways. First, a plaque score was taken by noting the presence of plaque and/or calculus on the buccal, lingual, mesial, and distal surfaces of two anterior teeth and two posterior teeth (calculation in Appendix C). Second, gingival crevicular fluid (GCF) samples were taken from 4 anterior sites and 4 posterior sites using PerioPaper^{TM}. The quantity of GCF collected was verified using a Periotron score of at least 200µl. Next, gingival marginal bleeding was noted (Y/N) for each patient.

For our power analyses, we set our significance level at 0.05 and power at 0.8. Based upon published literature[37] we were able to calculate power for one of the cytokines examined (MPO). There is no published literature regarding the levels of the other studied cytokines in obese and normal BMI children. The effect sizes of MPO was fairly large (d=2.14) yielding a required sample size of approximately 5 individuals in each group. Gomes Ferraz et al. (2014) was used to calculate power for plaque scores (d=-0.674; n= 36 individuals per group).[28]

The legal guardian of each participant was asked to fill out a brief questionnaire (see Appendices A and B). The questionnaire covers the following topics: racial/ethnic identity; diet; tooth brushing habits; prior orthodontic treatment; age at first menses (for females).

II.D Cytokine Analysis

The sites selected for collection were subjected to washing by the dental unit's air-water syringe. The areas were isolated from salivary contamination with cotton rolls, air-dried. GCF was collected using sterile Periopaper strips (Oraflow, Plainview, NY) by gently inserted into the entrance of the sulcus/periodontal pocket and left in place for 30 seconds.[38] Mechanical irritation was avoided, and the strips contaminated with blood were discarded. The GCF sample volumes were measured with Periotron 8000 (Periotron 8000, Proflow, Inc., Amityville, NY). We collected multiple strips to obtain Periotron reading of ≥200. After the measurement of the volume collected, the paper strips were placed in Eppendorf tubes and kept frozen at −80°C until needed. GCF was eluted in 250 μl phosphate buffered saline (PBS) by incubating at 4°C overnight. Eluted GCF was centrifuged at 10,000 for 10 min, the paper points/strips were removed; both paper points/strips and the supernatants were kept frozen at −20°C until assayed. Gingival crevicular fluid samples were used to measure pro- (IL-1β, IL-6, IL-8, CCL2, CCL3,

MPO and TNF-α) and anti-inflammatory cytokines (IL-10) using multiplex bead assays from Bio-Techne. Bead-based analyte quantification was performed on MAGPIX® System (EMD Millipore Sigma, Burlington, Massachusetts, USA) conducted at the University of Illinois at Chicago's Flow Cytometry Core. In brief, 25 µl of each sample was ran in duplicate as per manufacturer's instructions. Data was acquired on xPONENT® software package and analyzed on MILLIPLEX® Analyst 5.1 software. The mean florescence intensity for each analyte was converted to pg/ml using a standard curve. Data are presented as ±SEM for each group. Due to COVID-19 University of Illinois closure and research protocol restrictions, only 20 out of 54 subjects were included in the cytokine analysis.

II.E Descriptive Statistics

For the overall sample, 43% of this group was obese (BMI >95th percentile; n=23) and 57% was non-obese (BMI = 5th-85th percentile; n=31) (Table 1). For the cytokine subsample, 50% of this group was obese (BMI >95th percentile; n=10) and 50% was non-obese (BMI = 5th-85th percentile; n=10).

Table I: BMI AND ETHNICITY OF STUDY SUBJECTS (All subjects, n=54)

	Normal	Obese	Mean BMI	Median BMI	Standard deviation	% Obese
African-American	6	6	23.46	22.41	6.75	50%
Asian	1	1	21.38	21.38	2.20	50%
Caucasian:	-	-	-	-	-	-
Hispanic	16	14	22.87	22.12	5.76	46.7%
Non-Hispanic	5	2	20.21	20.36	5.85	28.6%
Unknown	3	0	17.63	18.51	1.42	0%
Total	31	23	22.22	21.22	5.98	42.6%

Table II. BMI OF MALE AND FEMALE STUDY SUBJECTS (All subjects, n=54)

	Normal	Obese	Mean BMI	Median BMI	Standard deviation	% Obese
Males	18	13	21.36	22.07	5.02	41.9%
Females	13	10	22.95	19.89	6.76	40.9%
Total	31	23	22.22	21.22	5.98	42.6%

Table III. BMI AND ETHNICITY AMONG CYTOKINE SUBJECTS (n=20)

	Normal	Obese	Mean BMI	Median BMI	Standard deviation	% Obese
African-American	4	2	22.52	18.71	7.79	33%
Asian	0	1	23.58	23.58	0	100%
Caucasian:	-	-	-	-	-	-
Hispanic	4	6	24.06	24.12	6.12	60%
Non-Hispanic	0	1	27.46	27.46	0	100%
Unknown	2	0	17.18	17.18	1.56	0%
Total	10	10	23.06	21.90	6.50	50%

Table IV. BMI OF MALE AND FEMALE CYTOKINE SUBJECTS (n=20)

	Normal	Obese	Mean BMI	Median BMI	Standard deviation	% Obese
Males	6	5	21.23	20.21	4.09	45.5%
Females	4	5	24.36	21.68	8.02	50%
Total	10	10	23.06	21.90	6.50	50%

Table V: DESCRIPTION OF BMI GROUPS (All subjects, n=54)

	Mean BMI score	Median BMI score	Standard Deviation	Range (Min-Max)	Mean BMI Percentile
Normal	18.00	18.59	2.73	11.72-24.12	50.26
Obese	27.91	26.75	4.21	22.18-37.55	97.05

Table VI. DESCRIPTION OF BMI GROUPS (Cytokine Subjects only, n=20)

	Mean BMI score	Median BMI score	Standard Deviation	Range (Min-Max)	Mean BMI Percentile
Normal	17.67	18.07	1.79	14.94-21.21	46.3
Obese	28.45	27.21	4.81	23.58-37.55	97.11

II.F Statistical Analysis

Mean, median, and standard deviation values for plaque score and gingival bleeding were calculated for the obese and non-obese groups using Microsoft Excel. These two oral health outcomes variables were then compared across groups using two-sided T-tests. We also conducted partial correlations analysis to control for the effect of reported frequency of tooth brushing.

The mean differences and standard deviations for levels of pro-inflammatory cytokines were assessed using Microsoft Excel. Cytokine levels were compared across obese and non-obese groups using t-sample T-tests. Partial correlations analysis was conducted to control for the effects of plaque score on cytokine outcomes. All statistical analyses (other than descriptive statistics, which were conducted in Excel) were conducted in R.

III. RESULTS

III.A Study Results

III.A.1 Overall Sample

A total of 54 subjects who met our inclusion criteria were enrolled in this study spanning from November 2018 – December 2020. For the total sample (n=54), 23 subjects had obese BMIs and 31 subjects had normal weight BMIs (Table I). The majority of the sample was Hispanic Caucasian (n=30), followed by Black/African American (n=12), non-Hispanic Caucasian (n=7), unspecified (n=3), and Asian (n=2) (Table I). There were 31 male and 23 female subjects (Table II).

III.A.2 Cytokine Sample

Twenty subjects were included in our cytokine study sample. The majority of the sample was Hispanic Caucasian (n=10), followed by Black/African American (n=6), non-Hispanic Caucasian (n=1), unspecified (n=2), and Asian (n=1) (Table III). There were 11 male and 9 female subjects (Table IV). Ten subjects had obese BMIs and 10 subjects had normal weight BMIs. Raw data can be found in Appendix D and E.

For the obese group in the overall sample, the maximum plaque score was 100% and minimum 50%. The average plaque score was 77% and the standard deviation was 13.7. For the normal weight group in the overall sample, the maximum plaque score was 93% and the minimum was 15%. The average plaque score was 47% and the standard deviation was 20.7 (Table VII). Within the cytokine sample, the maximum plaque score for the obese group was 90% and the minimum was 60%. The average plaque score was 73% and standard deviation was

8.1. The normal weight group within the cytokine data set had a maximum plaque score of 50%, a minimum of 15%, an average plaque score of 33.5% and a standard deviation of 13.4 (Table VIII). For the overall sample and the cytokine subsample, there were statistically significant differences in plaque scores between obese and non-obese subjects ($p<.001$)(Figure I). A partial correlations analysis was run examining the relationship between plaque score and BMI percentile, controlling for reported tooth brushing frequency (Figure I).

Table VII. PLAQUE SCORES OF OBESE AND NORMAL BMI SUBJECTS (n=54)

	Maximum	Minimum	Average	Standard Deviation
Obese	100	50	76.78	13.67
Normal	93	15	46.54	20.78

Table VIII. PLAQUE SCORES OF OBESE AND NORMAL CYTOKINE SUBJECTS (n=20)

	Maximum	Minimum	Average	Standard Deviation
Obese	90	60	73	8.12
Normal	50	15	33.5	13.42

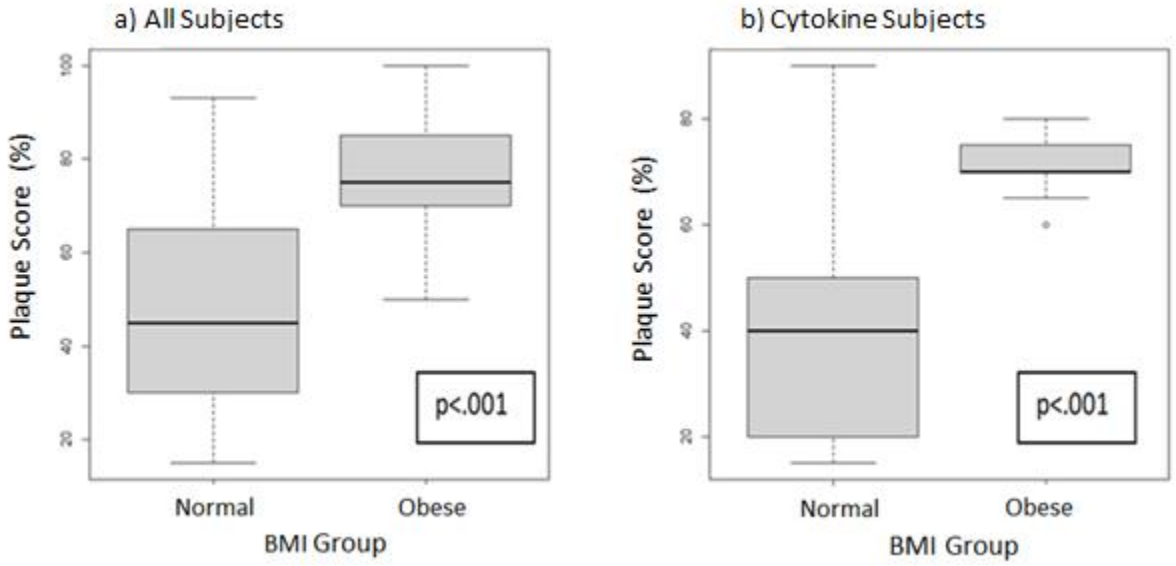


Figure 1. Plaque scores in obese and normal weight children in (a) all subjects and (b) cytokine subjects. In both cases, there were statistically significantly higher plaque scores in the samples of children with obesity.

Statistical distribution between the overall data set and cytokine data set appear similar. The cytokine subset displays a smaller range of plaque scores (30 for obese, 35 for normal) compared to the overall data set (50 for obese, 78 for normal).

Within the overall sample, 78% of the obese BMI group displayed bleeding during prophylactic pediatric dental treatment compared to 29% bleeding during treatment in the non-obese group. Within the cytokine sample, 90% of the obese subjects displayed bleeding upon treatment compared to 40% of the non-obese group (Table IX). There was a statistically significant difference in bleeding upon treatment between BMI groups for the overall sample ($p<.001$) and for the cytokine sample ($p=0.042$).

Table IX. BLEEDING DURING TREATMENT (n=54, n=20)

	Overall Sample		**Cytokine Sample**	
	Obese	**Normal**	**Obese**	**Normal**
Bleeding	78% (18)	29% (9)	90% (9)	40% (4)
No Bleeding	22% (5)	71% (22)	10% (1)	60% (6)
Total	100% (23)	100% (31)	100% (10)	100% (10)

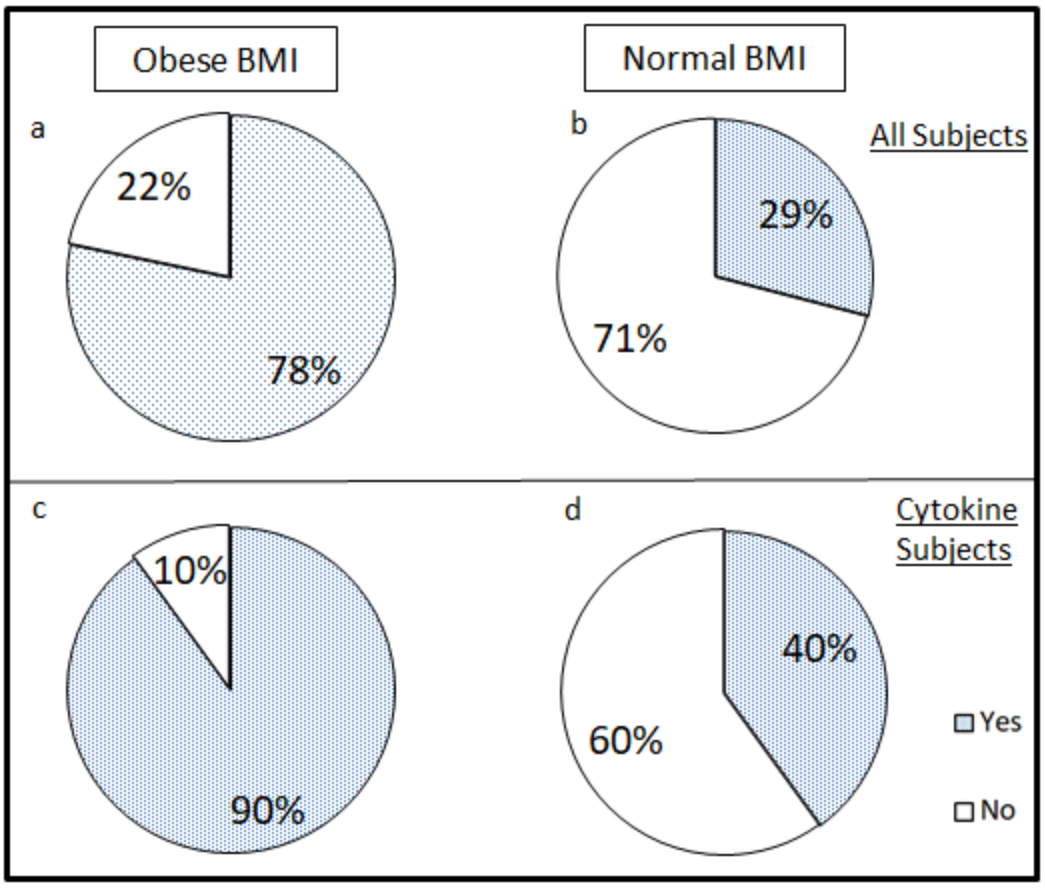


Figure 2. Percent of subjects who exhibited bleeding vs. no bleeding during prophylactic dental treatment. The top row (a & b) are all subjects and the bottom row (c & d) are the cytokine subjects. In both groups, high BMI individuals displayed higher levels of bleeding during treatment (Overall Sample: $p<0.001$; Cytokine Sample: $p=0.042$).

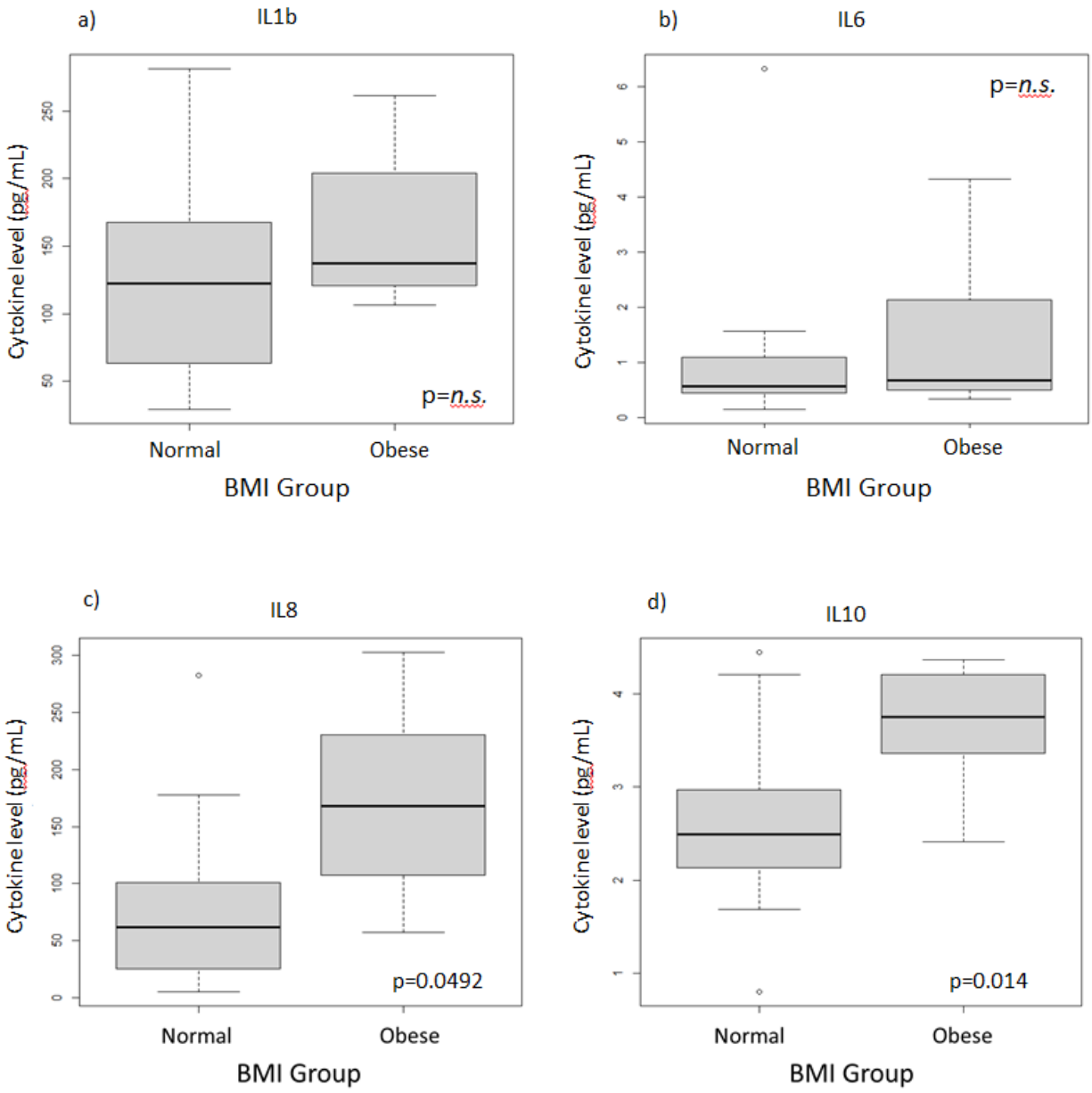
The average level of pro- and anti-inflammatory cytokines were compared across groups (Tables X and XI). When cytokine levels were compared across between obese and normal weight subjects, IL8, IL10, CCL2, and CCL3 were statistically significantly different at $\alpha<0.05$. A Bonferroni correction for multiple testing yielded a significance cut-off of $\alpha<0.006$. After correcting for multiple testing, only CCL2 and CCL3 remain statistically significant. Because plaque is known to be associated with oral inflammation, we ran a series of partial correlations analyses comparing BMI percentile and cytokine levels controlling for plaque score. When plaque score was controlled for, none of the relationships remained statistically significant. However, while a correlation was seen between plaque score and the levels of two cytokines (CCL2 and CCL3 at $\alpha=0.006$), these relationships were also negated when controlling for BMI percentile.

Table X. CYTOKINE LEVELS IN OBESE GROUP, (pg/mL) (n=10)

	Maximum	Minimum	Average	Standard Deviation
IL-10	4.37	2.41	3.54	0.66
CCL2	69.23	47.57	59.21	6.83
IL-1b	261.49	59.34	153.73	62.85
CCL3	277.21	208.2	248.6	23.32
MPO	104745	56111.9	80832.9	14126.1
TNFa	4.59	1.48	2.45	0.93
IL-6	4.33	0.34	1.52	1.49
IL-8	302.79	57.64	171.52	78.23

Table XI. CYTOKINE LEVELS IN NORMAL WEIGHT GROUP (pg/mL)(n=10)

	Maximum	Minimum	Average	Standard Deviation
IL-10	4.45	0.8	2.64	1.04
CCL2	64.18	28	46.2	10.96
IL-1b	281.56	29.13	131.83	68.66
CCL3	268.23	136.79	202.56	38.51
MPO	96248.3	38731.9	70261.7	15264.7
TNFa	7.48	0.85	2.28	1.94
IL-6	6.33	0.15	1.45	2.04
IL-8	282.45	5.15	85.52	82.08


a)
IL1b
Cytokine level (pg/mL)
50
100
150
200
250
p=n.s.
Normal
Obese
BMI Group
b)
IL6
Cytokine level (pg/mL)
0
1
2
3
4
5
6
p=n.s.
Normal
Obese
BMI Group
c)
IL8
Cytokine level (pg/mL)
0
50
100
150
200
250
300
p=0.0492
Normal
Obese
BMI Group
d)
IL10
Cytokine level (pg/mL)
1
2
3
4
p=0.014
Normal
Obese
BMI Group

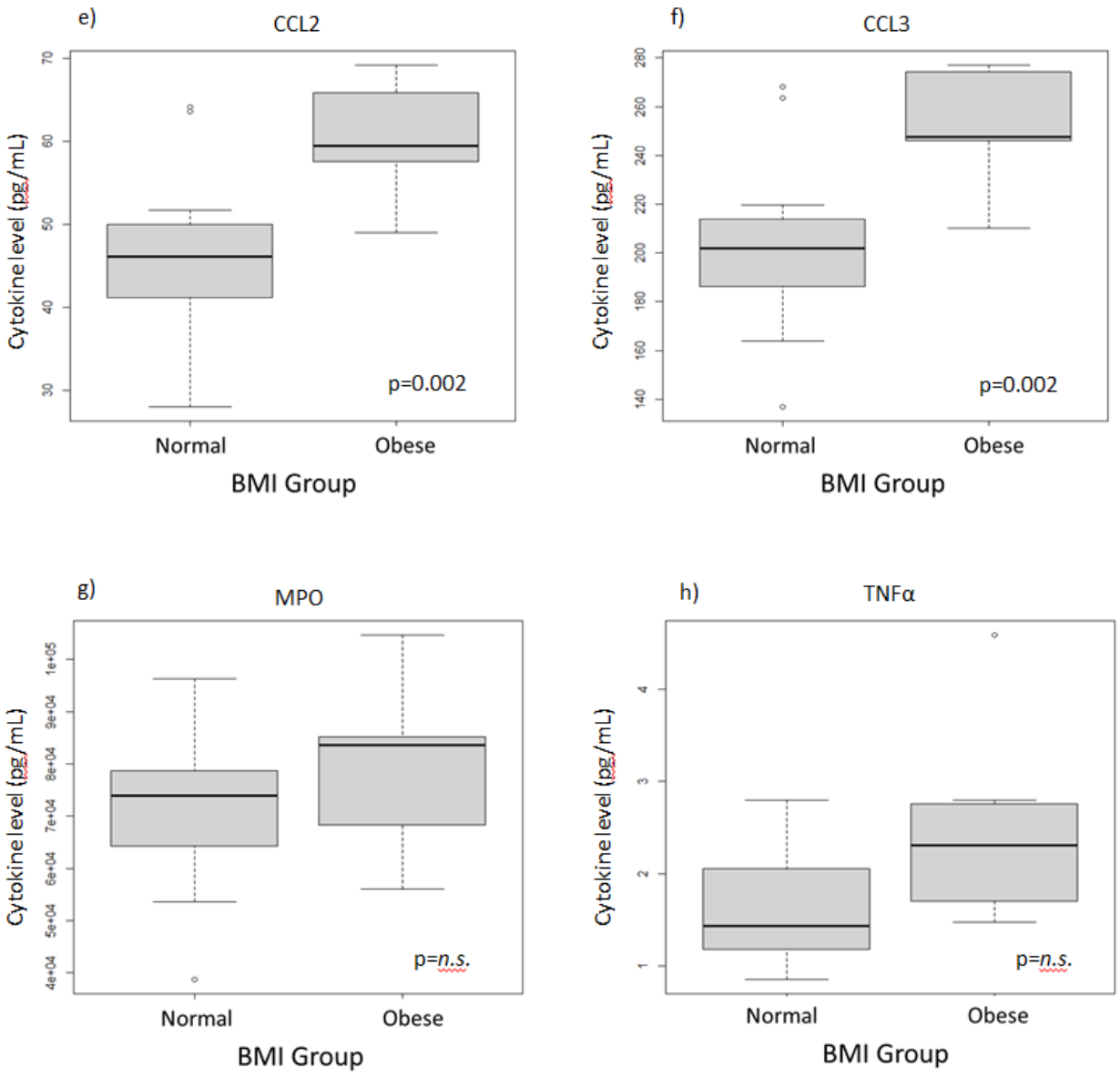


Figure 3. Pro(a,b,c,e,f,g,h)- and anti(d) -inflammatory cytokines in obese and normal weight subjects (n=20.) Only CCL2 and CCL3 remain statistically significant after Bonferroni correction indicating that these two pro-inflammatory cytokines are elevated in children with obesity. All other cytokines are suggestive of higher levels of cytokines in obese children, but were not statistically significant (p-values listed; n.s. = p>0.05).

IV. DISCUSSION

IV.A Childhood obesity and dental providers

With such currently high rates of childhood obesity, early dental intervention may be more necessary amongst providers. Dental cleanings and examinations are indicated for children every 6 months and are imperative for evaluation of developing dentition.[39] Dentists have a unique ability to approach diet from an oral health standpoint and aid parents to a better understanding of nutrition and overall health.[39] Early intervention by dentists may be as simple as patient/parental education, or as complex as early orthodontic treatment with fixed oral appliances. In order to learn how to guide families down a path of proper oral and overall health, the etiology of childhood obesity must be evaluated and understood. There are numerous factors that can contribute to childhood obesity such as diet, socioeconomic status, parental history of obesity, and exercise habits.[2,3,5] Access to healthy foods and quality of lunches during school hours also can contribute to children's overall health. [40] Further, childhood obesity has been shown to increase the likelihood of obesity in adulthood.[4] Given the multifactorial nature of childhood obesity, dentists should evaluate pediatric patients from a wholesome perspective to properly aid in overall health.

IV.B Childhood obesity, oral hygiene, and oral health

Periodontitis is one of the long-term problems associated with poor oral health. Periodontitis is a estimated to be present in half of the United States population and can lead to infection, pain, or early tooth loss.[41] The primary etiology of periodontal disease is bacterial presence within dental plaque.[41] While this current study focused on gingival plaque in obese

children, it is important to recognize the long term effects that poor oral hygiene and dietary habits may have. The American Dental Association has developed nutritional tools, such as Mouth Healthy™, to guide children down a path of healthy diet choices.[42] One of the purposes of this study was to evaluate whether obese children have poorer oral health by using gingival bleeding and plaque score as a proxy for oral health.

In recent years, various studies have evaluated oral inflammation in obese children. In a similar study of 100 school children aged 7-12, Sfasciotti et al. (2017) found an average full-mouth plaque score of 21.86% in normal weight subjects compared to a score of 50.08% in overweight/obese subjects.[27] Gomes Ferraz et al. (2014) used a combination of oral hygiene index and gingival bleeding index to compare hygiene in children aged 6-14 years and found that obese children had statistically significant decreased level of oral hygiene compared to normal weight children.[28] This current study was completed in an urban Medicaid population in a pediatric dental clinic. In the overall sample and cytokine subsample, there was a statistically significant higher plaque score in the obese group compared to the non-obese group ($p<0.001$). It is worth noting that statistical metrics between the overall sample and cytokine subsample were similar. Within the overall sample obese group, the minimum plaque score noted was 50%. Descriptively, this suggests that all 23 of the obese subjects had gingival plaque on over half of their dental surfaces.

The presence of gingival bleeding during plaque removal is a known indicator of tissue inflammation[43] and was therefore used as a proxy in this study. While in adult populations bleeding upon periodontal probing is often used as an indicator of gingival inflammation[43] , this study employed bleeding during prophylactic treatment, which is defined as the removed of plaque from the gingival margin using dental instruments. Within the overall sample and cytokine data set, there was significantly higher bleeding upon treatment in obese subjects compared to non-

obese subjects ($p<0.001$, $p=.0042$). Prior studies evaluating gingival bleeding and obesity in children have shared similar statistically significant results.[27,44] The presence of gingival plaque is multifactorial. Optimal oral hygiene requires quality of brushing as well as quantity. Within the demographic questionnaire (Appendix A) given to each subjects' parent, information was collected related to oral hygiene frequency per day. When we controlled for parent-reported frequency of tooth brushing, a strong positive correlation between BMI percentile and plaque score remained ($p<0.001$).

IV.C Oral inflammation in obese children

The presence of oral inflammation was statistically evaluated by measuring levels of pro- and anti-inflammatory cytokines within the obese and non-obese groups in a subsample (n=20). This study compared gingival crevicular fluid levels of 8 cytokines between groups (IL-10, CCL2, IL-1b, CCL3, mpo, TNFa, IL-6, IL-8). IL-10, IL-1b, mpo, TNFa, IL-6, and IL-8 levels were not statistically significant when compared across obese and non-obese groups following a Bonferroni correction.

Suresh et al. (2016) found an increased levels of the pro-inflammatory cytokine resistin in obese subjects with periodontal disease compared to non-obese subjects with healthy periodontium.[45] In a similar prospective clinical cohort study, Saloom et al. (2017) noted increased levels of adipokines leptin and resistin in obese their obese subjects compared to normal weight subjects.[37] MPO and RANKL were also at significantly higher levels in gingival crevicular fluid within the obese group.[37] This prior study suggested that known inflammatory factors associated with bone breakdown were higher in obese children.[37] In our current study, there were significantly

higher levels of CCL2 and CCL3 in the obese group compared to the non-obese group. Chemokine ligand 2 (CCL2, also known as MCP1) can be released by endothelial cells, fibroblasts, and epithelial cells and is known to attract myeloid and lymphoid cells via chemotaxis.[46] CCL2 is active in immune response as well as in pathological conditions including obesity and diabetes.[46] Given what is known about CCL2, it seems unsurprising that levels may be significantly higher in an obese population with higher levels of oral inflammation based on multiple proxies (bleeding, plaque score). Chemokine ligand 3 (CCL3, also known as MIP-1α) is involved in the acute inflammatory process, and stimulates the activation of polymorphonuclear leukocytes.[47] The presence of CCL3 has also been shown to activate osteoclastic and osteoblastic activity, suggesting it is a marker for bone remodeling.[47] In this study, increased levels of CCL2 and CCL3 among obese subjects may suggest increased oral inflammation with the possibility of increased bone turnover.

IV.D Limitations of this study

- A limited sample size of only 54 subjects was included in the overall sample, and 20 total subjects were included in the cytokine data
- Dietary data collected from each subject was not included in this analysis, and therefore may be a confounding variable
- Oral hygiene behavior (tooth brushing) was parent-reported and did not include information on flossing.
- Plaque scores were not calculated using the most standard methods (gingival index, plaque index)[43] and thus may not be easily comparable to other published work

IV.E Future studies

Future studies may benefit from including data in a different population to evaluate whether relationships between obesity and oral health transcend between communities. In the current sample, the predominant racial/ethnic identity is Hispanic Caucasian. However, this label applies to a broad range of cultural/ethnic groups and may obscure more culture-specific differences in diet and oral health behaviors. While we collected socio-economic data and food insecurity data, this was not fully analyzed for the current study, and future analysis of this data may provide further insights. As stated, plaque scores can be multifactorial in nature and it may be beneficial to control for specific dietary habits prior to evaluating hygiene. There are also ways to track tooth brushing behavior in real time, such as through the use of specialized "smart" electric toothbrushes. Further, increasing the sample size of this data set will likely increase the accuracy of the cytokine data. The current cytokine analyses are preliminary and clearly demonstrate the need for further work employing a larger sample.

V. Conclusion

- Obese children displayed significantly higher levels of plaque compared to non-obese children
- Obese children displayed significantly increased levels of bleeding upon treatment compared to non-obese children
- Obese children displayed higher levels of pro-inflammatory cytokines CCL2 and CCL3 compared to non-obese children

CITED LITERATURE

1. Mobley C, Marshall TA, Milgrom P, Coldwell SE. The Contribution of Dietary Factors to Dental Caries and Disparities in Caries. *Acad Pediatr*. 2009;9(6):410-414. doi:10.1016/j.acap.2009.09.008

2. Hales CM. Prevalence of Obesity Among Adults and Youth: United States, 2015–2016. 2017;(288):8.

3. Kumar S, Kelly AS. Review of Childhood Obesity. *Mayo Clin Proc*. 2017;92(2):251-265. doi:10.1016/j.mayocp.2016.09.017

4. Whitlock EP. Screening and Interventions for Childhood Overweight: A Summary of Evidence for the US Preventive Services Task Force. *PEDIATRICS*. 2005;116(1):e125-e144. doi:10.1542/peds.2005-0242

5. Martínez-Villanueva J, González-Leal R, Argente J, Martos-Moreno GÁ. La obesidad parental se asocia con la gravedad de la obesidad infantil y de sus comorbilidades. *An Pediatría*. 2019;90(4):224-231. doi:10.1016/j.anpedi.2018.06.013

6. Ogden CL, Carroll MD, Lawman HG, et al. Trends in Obesity Prevalence Among Children and Adolescents in the United States, 1988-1994 Through 2013-2014. *JAMA*. 2016;315(21):2292-2299. doi:10.1001/jama.2016.6361

7. Prevalence | CLOCC. Accessed June 25, 2020. http://www.clocc.net/about-childhood-obesity/prevalence/

8. Margellos-Anast H, Shah AM, Whitman S. Prevalence of Obesity Among Children in Six Chicago Communities: Findings from a Health Survey. *Public Health Rep*. 2008;123(2):117-125.

9. Thanakun S, Pornprasertsuk - Damrongsri S, Izumi Y. Increased oral inflammation, leukocytes, and leptin, and lower adiponectin in overweight or obesity. *Oral Dis*. 2017;23(7):956-965. doi:10.1111/odi.12679

10. Ekuni D, Mizutani S, Kojima A, et al. Relationship between increases in BMI and changes in periodontal status: a prospective cohort study. *J Clin Periodontol*. 2014;41(8):772-778. doi:10.1111/jcpe.12273

11. Highfield J. Diagnosis and classification of periodontal disease. *Aust Dent J*. 2009;54:S11-S26. doi:10.1111/j.1834-7819.2009.01140.x

12. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *The Lancet*. 2005;366(9499):1809-1820. doi:10.1016/S0140-6736(05)67728-8

13. Lal S, Cheng B, Kaplan S, et al. Accelerated Tooth Eruption in Children With Diabetes Mellitus. *PEDIATRICS*. 2008;121(5):e1139-e1143. doi:10.1542/peds.2007-1486

14. Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol*. 1967;38(6):610-616. doi:https://doi.org/10.1902/jop.1967.38.6.610

15. Modesto A, Jacas CA, Kim S (Michelle), et al. Childhood Obesity, Genetic Variation, and Dental Age. *Pediatr Dent*. 2019;41(2):132-135.

16. Hilgers KK, Akridge M, Scheetz JP, Kinane DF. Childhood Ohesity and Dental Development. *Pediatr Dent*. Published online 2006:6.

17. Must A, Phillips SM, Tybor DJ, Lividini K, Hayes C. The Association Between Childhood Obesity and Tooth Eruption. *Obesity*. 2012;20(10):2070-2074. doi:https://doi.org/10.1038/oby.2012.23

18. Mack KB, Phillips C, Jain N, Koroluk LD. Relationship between body mass index percentile and skeletal maturation and dental development in orthodontic patients. *Am J Orthod Dentofacial Orthop*. 2013;143(2):228-234. doi:10.1016/j.ajodo.2012.09.015

19. Lehtinen A, Oksa T, Helenius H, Rönning O. Advanced dental maturity in children with juvenile rheumatoid arthritis. *Eur J Oral Sci*. 2000;108(3):184. doi:10.1034/j.1600-0722.2000.108003184.x

20. Nicholas CL, Kadavy K, Holton NE, Marshall T, Richter A, Southard T. Childhood body mass index is associated with early dental development and eruption in a longitudinal sample from the Iowa Facial Growth Study. *Am J Orthod Dentofacial Orthop*. 2018;154(1):72-81. doi:10.1016/j.ajodo.2017.10.033

21. Murakami S, Mealey BL, Mariotti A, Chapple ILC. Dental plaque–induced gingival conditions. *J Periodontol*. 2018;89:S17-S27. doi:10.1002/JPER.17-0095

22. Goodson JM. Disease reciprocity between gingivitis and obesity. *J Periodontol*. 2020;91(Suppl 1):S26-S34. doi:10.1002/JPER.20-0046

23. Casavalle PL, Lifshitz F, Romano LS, et al. Gingivitis and Insulin Resistance in Obese Children. *Diabetes Care*. 2016;39(12):e216-e217. doi:10.2337/dc16-0708

24. Halder S, Kaul R, Angrish P, Saha S, Bhattacharya B, Mitra M. Association between Obesity and Oral Health Status in Schoolchildren: A Survey in Five Districts of West Bengal, India. *Int J Clin Pediatr Dent*. 2018;11(3):233-237. doi:10.5005/jp-journals-10005-1517

25. Kesim S, Çiçek B, Aral CA, Öztürk A, Mazıcıoğlu MM, Kurtoğlu S. Oral Health, Obesity Status and Nutritional Habits in Turkish Children and Adolescents: An Epidemiological Study. *Balk Med J*. 2016;33(2):164-172. doi:10.5152/balkanmedj.2016.16699

26. Martinez-Herrera M, Silvestre-Rangil J, Silvestre F. Association between obesity and periodontal disease. A systematic review of epidemiological studies and controlled clinical trials. *Med Oral Patol Oral Cirugia Bucal*. Published online 2017:0-0. doi:10.4317/medoral.21786

27. Sfasciotti GL, Marini R, Pacifici A, Ierardo G, Pacifici L, Polimeni A. Childhood overweight-obesity and periodontal diseases: is there a real correlation? *Ann Stomatol (Roma)*. 2017;7(3):65-72. doi:10.11138/ads/2016.7.3.065

28. Gomes Ferraz E, Rodrigues Silva L, Almeida Sarmento V, et al. Association between childhood obesity and oral hygiene status. *Asoc ENTRE Obes Infant EL ESTADO Hig ORAL*. 2014;30(2):253-259. doi:10.3305/nh.2014.30.2.7476

29. Anand N, Suresh M, Chandrasekaran SC. Effect of Obesity and Lifestyle on the Oral Health of Pre Adolescent Children. *J Clin Diagn Res JCDR*. 2014;8(2):196-198. doi:10.7860/JCDR/2014/6694.4058

30. Virtanen JI, Muikku T, Similä T, Cinar AB, Pohjola V. Physical activity, BMI and oral health behaviour among adolescents: Finnish School Health Promotion Study. *Eur J Public Health*. 2019;29(2):296-302. doi:10.1093/eurpub/cky193

31. Increased BMI in children—an indicator for less compliance during orthodontic treatment with removable appliances | European Journal of Orthodontics | Oxford Academic. Accessed February 9, 2021. https://academic.oup.com/ejo/article/40/4/350/4876039

32. Manohar N, Hayen A, Fahey P, Arora A. Obesity and dental caries in early childhood: A systematic review and meta-analyses. *Obes Rev*. 2020;21(3):e12960. doi:https://doi.org/10.1111/obr.12960

33. Ravaghi V, Rezaee A, Pallan M, Morris AJ. Childhood obesity and dental caries: an ecological investigation of the shape and moderators of the association. *BMC Oral Health*. 2020;20(1):338. doi:10.1186/s12903-020-01329-7

34. Farsi DJ, Elkhodary HM, Merdad LA, et al. Prevalence of obesity in elementary school children and its association with dental caries. *Saudi Med J*. 2016;37(12):1387-1394. doi:10.15537/smj.2016.12.15904

35. Hooley M, Skouteris H, Millar L. The relationship between childhood weight, dental caries and eating practices in children aged 4–8 years in Australia, 2004–2008. *Pediatr Obes*. 2012;7(6):461-470. doi:https://doi.org/10.1111/j.2047-6310.2012.00072.x

36. Trikaliotis A, Boka V, Kotsanos N, Karagiannis V, Hassapidou M. dmfs and BMI in pre-school Greek children. An epidemiological study. *Eur Arch Paediatr Dent*. 2011;12(3):176-178. doi:10.1007/BF03262802

37. Saloom HF, Papageorgiou SN, Carpenter GH, Cobourne MT. Impact of Obesity on Orthodontic Tooth Movement in Adolescents: A Prospective Clinical Cohort Study. *J Dent Res*. 2017;96(5):547-554. doi:10.1177/0022034516688448

38. Carneiro LG, Nouh H, Salih E. Quantitative gingival crevicular fluid proteome in health and periodontal disease using stable isotope chemistries and mass spectrometry. *J Clin Periodontol*. 2014;41(8):733-747. doi:10.1111/jcpe.12262

39. American Academy of Pediatric Dentistry. Guideline on periodicity of examination, preventive dental services, anticipatory guidance/counseling, and oral treatment for infants, children, and adolescents. *Pediatr Dent*. 2013;35(5):E148-156.

40. Mallonee LF, Boyd LD, Stegeman C. A scoping review of skills and tools oral health professionals need to engage children and parents in dietary changes to prevent childhood obesity and consumption of sugar-sweetened beverages. *J Public Health Dent*. 2017;77(S1):S128-S135. doi:https://doi.org/10.1111/jphd.12237

41. Harvey JD. Periodontal Microbiology. *Dent Clin North Am*. 2017;61(2):253-269. doi:10.1016/j.cden.2016.11.005

42. MouthHealthy - Oral Health - American Dental Association. Accessed March 15, 2021. https://www.mouthhealthy.org/en

43. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? *J Clin Periodontol*. 1986;13(6):590-596. doi:10.1111/j.1600-051X.1986.tb00852.x

44. Martens L, De Smet S, Yusof MYPM, Rajasekharan S. Association between overweight/obesity and periodontal disease in children and adolescents: a systematic review and meta-analysis. *Eur Arch Paediatr Dent*. 2017;18(2):69-82. doi:10.1007/s40368-017-0272-1

45. Suresh S, Mahendra J, Singh G, Pradeep A, Sundaravikram, Sekar H. Comparative Analysis of GCF Resistin Levels in Obese Subjects with and without Periodontal Disease. *J Clin Diagn Res JCDR*. 2016;10(5):ZC71-ZC74. doi:10.7860/JCDR/2016/19066.7802

46. Gschwandtner M, Derler R, Midwood KS. More Than Just Attractive: How CCL2 Influences Myeloid Cell Behavior Beyond Chemotaxis. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.02759

47. The effect of CCL3 and CCR1 in bone remodeling induced by mechanical loading during orthodontic tooth movement in mice | Elsevier Enhanced Reader. doi:10.1016/j.bone.2012.09.036

VI. APPENDICES

A. Parental Questionnaire – English

Demographic/Health History Questionnaire

Please help us by telling us current information about your household's socioeconomic status and your child's dental health. The information will be extremely helpful in comparing the results of our study with other studies in both the U.S. and abroad. As always, when we use the information we will remove any personal identifiers and only report group summary information (your name and other identifying information will not be linked to the answers to these questions).

1. What is your child's race? (you may circle more than one)
    a. Black/African-American
    b. White/Caucasian
    c. Asian
    d. Native American
    e. Other _____

2. Is your child Hispanic/Latino?
    a. Yes
    b. No

3. What is the highest level of education achieved by the parents/guardians of your child?
    a. Parent/guardian 1:
        i. Some high school
        ii. High school diploma or GED
        iii. Some college
        iv. 2 year degree, technical/beauty school technical/beauty school
        v. Bachelor's degree
        vi. Post-graduate or professional degree degree
    b. Parent/guardian 2 (if applicable)
        i. Some high school
        ii. High school diploma or GED
        iii. Some college
        iv. 2 year degree,
        v. Bachelor's degree
        vi. Post-graduate or professional

4. Which of the following best describes your total household income for the last year before taxes (include salaries, wages, tips, interest, etc.)?
    a. Less than $20,000 per year
    b. $20,000 – $39,999 per year
    c. $40,000 – $59,999 per year
    d. $60,000 – $79,999 per year
    e. $80,000 – $110,000 per year
    f. More than $110,000 per year

5. How often does your child usually brush his or her teeth?
    a. Never
    b. Rarely
    c. A few times a week
    d. 1 time a day
    e. 2 times a day (morning and night)
    f. 3 times a day

[MORE QUESTIONS ON NEXT PAGE]

6. Has your child ever been treated by an orthodontist (a dentist who does braces/Invisalign)?
    a. Yes
    b. No

7. Is your child a diabetic?
    a. No
    b. Yes – Type 1 diabetic
    c. Yes – Type 2 diabetic

8. If your child is female, has she had her first menses/first menstrual period?
    a. Yes
    b. No

    8a. If yes, please indicate approximately what age she was when her first menstrual period occurred _____

Please indicate whether the following statements are often true, sometimes true, or never true for your household in the last 12 months.

9. "Within the past 12 months, I/we worried whether our food would run out before we got money to buy more."

[ ] Often true [ ] Sometimes true [ ] Never true [ ] Don't Know

10. “Within the past 12 months, the food we bought just didn’t last and we didn’t have money to get more.”

[ ] Often true [ ] Sometimes true [ ] Never true [ ] Don’t Know

[MORE QUESTIONS ON NEXT PAGE]

11. Do you or members of your household buy healthy food (like fruits, vegetables, whole grains and lean meats) as often as you would like to?

[ ] Yes [ ] No [ ] I(We) could buy healthier food, but prefer not to change our diet.

[ ] Don’t Know

12. If your answer to #11 was NO, what prevents you or your household from buying healthy food? Check all that apply.

[ ] Transportation (no car available or long CTA rides)

[ ] Healthy food is not available where we live

[ ] The fruit/vegetables in stores where we live are low quality

[ ] Don’t have enough money

[ ] Don’t always feel safe in my/our neighborhood

[ ] Don’t have childcare

[ ] Work schedule

[ ] Restrictions of Food Assistance Program (like SNAP)

[ ] Other:_____

13. What are some of the healthy foods that you, or members in your household, eat?
14. Are there any foods which you would like to eat more often to make your diet healthier?

15. In your opinion, do you think access to healthy food could be improved in your community? If yes, how do you think access to healthy food could be improved?

B: Parental Questionnaire – Spanish

Cuestionario de historial de salud /demográfico

Por favor ayúdenos con la información socioeconómica de la gente que vive en su hogar y la salud dental de su hijo/a. La información adquirida será de gran ayuda para comparar los resultados de nuestro estudio con otros estudios que se han realizado en los E.E.U.U y en el extranjero. Como siempre, la información que usted nos provea no tendrá ningún vínculo con su identidad y solo se presentará la información como un resumen de todos los cuestionarios que se han repartido (Su nombre y su información proveída no será vinculada con sus respuestas dadas en el cuestionario).

1. ¿De que raza es su hijo/a? (Puede circular más de una)
   a. Negro/ Afro-americano
   b. Blanco/ Caucásico
   c. Asiático
   d. Indígena nativo americano
   e. Otro _____

2. ¿Su hijo/a es hispano/latino?
   a. Sí
   b. No

3. ¿Cuál es el nivel de educación más alto que han recibido los padres/guardianes de su hijo/a?

a. Padre/ guardián 1:	b. Madre/ guardián 2:
i. No completó la secundaria / highschool	i. no completó la secundaria
ii. Completo la secundaria o recibió un GED	ii. complete la secundaria/ highschool o recibió un GED
iii. no completó la universidad	iii. no completó la universidad
iv. certificado del colegio técnico/escuela de belleza (Carrera)	iv. certificado del colegio técnico/escuela de belleza (Carrera)
v. completó la Universidad	v. completó la Universidad
vi. completó un posgrado o certificado de profesión	vi. completó un posgrado o certificado de profesión

4. ¿Cuál de las siguientes cifras describe el total de su salario anual del año pasado antes de la deducción de impuestos (incluye salario, propinas, beneficios, etc.)?

a. Menos de $20,000 anual
b. $20,000 – $39,999 anual
c. $40,000 – $59,999 anual
d. $60,000 – $79,999 anual
e. $80,000 – $110,000 anual
f. Más de $110,000 anual

[Más preguntas a continuación de la página]

5. ¿Con que frecuencia su hijo/a se cepilla los dientes?
    a. Nunca
    b. Con poca frecuencia
    c. Algunas veces por semana
    d. 1 vez al día
    e. 2 veces al día (mañana y noche)
    f. 3 veces al día

6. ¿Su hijo/a ha recibido tratamiento de un especialista en ortodoncia (un odontólogo que aplica /frenos?/aparatos dentales?/ Invisalign)
    a. Sí
    b. No

7. ¿Su hijo/a es diabético/a?

    a. No
    b. Sí - diabetes tipo 1
    c. Sí - diabetes tipo 2

8. ¿Si su hija/o es hembra, ha comenzado a menstruar/ ha tenido su primera menstruación?
    a. Sí
    b. No

    8a. ¿Si su respuesta fue sí, aproximadamente a que edad comenzó a menstruar/ recibió su primera menstruación su hija?

    _____

Por favor indique si las siguientes frases son ciertas, a veces cierta, o nunca cierta para su hogar a través de los últimos 12 meses.

9. “En los últimos 12 meses, Yo/ nosotros nos preocupamos por alimentos o de no tener suficientes alimentos hasta tener efectivo para comparar más”

[ ] Cierto [ ] A veces cierto [ ] Nunca cierto [] No se

10. “En los últimos 12 meses, la comida que hemos comprado no nos ha alcanzado o no hemos tenido efectivo para comparar más.”

[ ] Cierto [ ] A veces cierto [ ] Nunca cierto [] No se

[Más preguntas a continuación de la página]

11. Usted o las personas en su hogar compran alimentos saludables (como frutas, verduras, trigo y carne magra [carne del animal que contiene poca grasa])

[ ] Sí [ ] No
[ ] Yo (nosotros) pudiéramos comprar comida saludable, pero preferimos no cambiar nuestra dieta alimenticia
[ ] No sé

12. ¿Si su respuesta a la pregunta #11 fue NO, que le prohíbe a usted o a la gente de su hogar comprar comida saludable? Señale todos los factores que implica.

[ ] Transportación (no tiene un vehículo disponible o el viaje en la CTA es muy largo)
[ ] No hay comida saludable cerca de donde vivimos.
[ ] Las verduras (frutas/ vegetales) son de mala calidad cerca de donde vivimos.
[ ] No nos alcansa el dinero
[ ] No siempre me siento seguro donde vivo.
[ ] Falta de servicios de cuidado infantil
[ ] El horario de trabajo
[ ] Restricciones del programa de asistencia alimentaria (SNAP)
[ ] Otras inconveniencias:
_____

13. ¿Cuáles son algunas de las comidas saludables que usted, o personas en su hogar, consume?

14. ¿Hay algunas comidas que le gustaría comer más a menudo para para que haga su dieta más saludable?

15. ¿En su opinión, cree que se pudiera mejorar el acceso a comidas saludables en su vecindario? ¿Si sí, cómo cree que el acceso a comida saludable debe ser mejorado?

C: Plaque Scoring Method Description

Plaque Scoring Method:

1. Four teeth were assessed for the presence or absence of plaque at the gingival margin (two molars and two incisors).

2. Plaque was observed to be present or absent on the four following surfaces: mesial, distal, facial, and lingual.

3. The number of surfaces with a presence of plaque were summed together and divided by total number of surfaces.

4. For example: 8 total plaque surfaces out of 16 total surfaces = 8/16 = 0.5 = 50% plaque score for this patient.

D. Raw Data, All Subjects

ID	BMI	Age	Age_Years	Sex	Race	Hispanic	Height_m	Weight_lb	Weight_kg	BMI2	BMI_Perc	Plaque	Bleeding
NDT001	Normal	10	10.08333333	M	Caucasian	Yes	1.397002794	66.6	30.20408163	15.476441	22	75	Y
NDT002	Normal	12	11.91666667	M	Caucasian	No	1.524003048	85	38.54875283	16.597346	29	30	N
NDT003	Normal	10	10.08333333	M	Caucasian	No	1.524003048	60	27.21088435	11.715774	1	75	Y
NDT004	Normal	12	11.16666667	F	Caucasian	Yes	1.498602997	102	46.2585034	20.597684	83	70	N
NDT005	Normal	12	11.66666667	F	Caucasian	Yes	1.57480315	103	46.71201814	18.835454	63	40	N
NDT006	Normal	12	11.66666667	F	Caucasian	Yes	1.549403099	101	45.80498866	19.080245	66	30	N
NDT007	Normal	11	10.83333333	F	African American	Yes	1.397002794	80	36.28117914	18.590319	67	50	Y
NDT008	Normal	12	11.5	F	African American	No	1.524003048	88	39.90929705	17.183135	40	40	Y
NDT009	Normal	12	11.83333333	F	Native American	Yes	1.498602997	93	42.17687075	18.780241	61	30	N
NDT010	Normal	11	11	M	Caucasian	Yes	1.320802642	56	25.3968254	14.558062	5	20	N
NDT011	Normal	13	12.75	F	NA	NA	1.524003048	96	43.53741497	18.745238	52	15	N
NDT012	Normal	12	11.58333333	M	NA	Yes	1.524003048	80	36.28117914	15.621032	15	20	Y
NDT013	Normal	12	12.25	F	Caucasian	Yes	1.498602997	74	33.5600907	14.943418	5	20	N
NDT014	Normal	13	12.91666667	M	Caucasian	Yes	1.57480315	96	43.53741497	17.555374	35	30	N
NDT015	Normal	12	12.08333333	M	African American	No	1.6002032	86	39.00226757	15.231392	7	20	N
NDT016	Normal	11	10.66666667	M	Caucasian	Yes	1.498602997	98	44.44444444	19.789931	84	50	Y
NDT017	Normal	10	10.25	M	African American	No	1.447802896	87	39.45578231	18.823127	79	50	N
NDT018	Normal	12	12.16666667	M	Caucasian	Yes	1.549403099	107	48.5260771	20.213725	79	40	N
NDT019	Normal	10	10.41666667	M	Caucasian	Yes	1.346202692	60	27.21088435	15.014876	13	40	N
NDT020	Normal	12	12.5	M	Caucasian	Yes	1.422402845	69	31.29251701	15.466615	7	30	N
NDT021	Normal	11	11	M	Caucasian	No	1.447802896	72	32.65306122	15.57776	19	25	N
NDT022	Normal	10	10	F	Caucasian	Yes	1.473202946	73	33.10657596	15.254188	21	45	N
ODT001	Obese	12	12.75	F	Caucasian	Yes	1.524003048	168	76.19047619	32.804167	-	90	Y
ODT002	Obese	12	12.08333333	F	African American	no	1.549403099	147	66.66666667	27.770257	97	80	Y
ODT003	Obese	13	12.33333333	F	African American	NA	1.6002032	212	96.14512472	37.547152	99	70	N

ODT004	Obese	10	10.25	M	Caucasian	Yes	1.473202946	129	58.50340136	26.956032	99	80	Y
ODT005	Obese	12	12	F	African American	No	1.6002032	151	68.48072562	26.74349	97	60	N
ODT006	Obese	11	10.91666667	F	African American	No	1.57480315	135	61.2244898	24.687245	96	60	Y
ODT007	Obese	12	11.58333333	M	Caucasian	No	1.625603251	160	72.56235828	27.458845	98	60	Y
ODT008	Obese	12	11.91666667	F	Caucasian	Yes	1.6002032	139	63.03854875	24.61818	94	70	Y
ODT009	Obese	11	11.33333333	M	Caucasian	Yes	1.57480315	138	62.58503401	25.23585	97	70	N
ODT010	Obese	11	11.66666667	F	Caucasian	Yes	1.549403099	188	85.26077098	35.515703	99	70	Y
ODT011	Obese	10	10.5	M	Asian	No	1.447802896	109	49.43310658	23.582998	96	75	Y
ODT012	Obese	10	10	M	Caucasian	Yes	1.447802896	112	50.79365079	24.232072	97	70	Y
ODT013	Obese	12	12.4167	M	Caucasian	Yes	1.524003048	123	55.78231293	24.017337	95	65	Y
NDT023	Normal	11	11.66	M	Asian	No	1.498602997	95	43.08390023	19.184117	72	70	N
ODT014	Obese	11	11.25	M	Caucasian	yes	1.498602997	131	59.41043084	26.453888	98	80	Y
ODT015	Obese	12	12.83	F	Caucasian	Yes	1.57480315	191	86.62131519	34.92788	93	80	Y
NDT024	Normal	12	12.916	F	Black	No	1.6002032	92	41.72335601	16.294047	22	60	N
NDT025	Normal	11	11.33	F		Yes	1.549403099	98	44.44444444	18.513505	65	75	Y
NDT026	Normal	12	12.5	M	Caucasian	No	1.651003302	145	65.75963719	24.124789	94	75	N
NDT027	Normal	11	11.916	M	Caucasian	Yes	1.524003048	113	51.24716553	22.064708	93	50	N
NDT028	Normal	12	12.583	M	Native America	Yes	1.473202946	107	48.5260771	22.358879	91	93	Y
NDT029	Normal	10	10.25	M		Yes	1.397002794	92	41.72335601	21.378867	93	50	N
ODT016	Obese	10	10.66	M	Black	No	1.422402845	106	48.07256236	23.760307	97	100	Y
ODT017	Obese	11	11.166	M	Caucasian	No	1.422402845	115	52.15419501	25.777692	98	75	Y
ODT018	Obese	10	10.916	M	Caucasian	Yes	1.422402845	122	55.32879819	27.346768	99	93	Y
ODT019	Obese	12	12.33	F	Black	No	1.57480315	185	83.90022676	33.830669	>99	100	Y
NDT030	Normal	12	12.583	M		Yes	1.473202946	93	42.17687075	19.433418	85	50	N
ODT020	Obese	10	10.33	F	Caucasian	Yes	1.371602743	92	41.72335601	22.178008	93	75	Y
ODT021	Obese	10	10.83	M	Caucasian	Yes	1.549403099	166	75.28344671	31.35961	99	93	N
NDT031	Normal	10	10.166	F	Black/Taino	Yes	1.320802642	81	36.73469388	21.057197	90	75	Y
ODT022	Obese	11	11.75	M	Caucasian	Yes	1.524003048	137	62.13151927	26.751017	98	100	Y
ODT023	Obese	10	10.916	M	Caucasian	Yes	1.498602997	140	63.49206349	28.271331	99	50	N

E. Raw Data, Cytokine Subset

ID	BMI	Age	DOB	DOS	Age_Years	Sex	Race	Hispanic	Height_In	Height_m	Weight_lb	Weight_kg	BMI2	BMI_Perc	Plaque	Bleeding
NDT007	Normal	11	39597	43538	10.83333333	F	African American	Yes	55	1.397002794	80	36.28117914	18.59032	67	50	Y
NDT008	Normal	12	39336	43538	11.5	F	African American	No	60	1.524003048	88	39.90929705	17.18314	40	40	Y
NDT011	Normal	13	38898	43564	12.75	F	NA	NA	60	1.524003048	96	43.53741497	18.74524	52	15	N
NDT012	Normal	12	39348	43565	11.58333333	M	NA	Yes	60	1.524003048	80	36.28117914	15.62103	15	20	Y
NDT013	Normal	12	39122	43586	12.25	F	Caucasian	Yes	59	1.498602997	74	33.5600907	14.94342	5	20	N
NDT014	Normal	13	38907	43608	12.91666667	M	Caucasian	Yes	62	1.57480315	96	43.53741497	17.55537	35	30	N
NDT015	Normal	NA	39174	43608	12.08333333	M	African American	No	63	1.6002032	86	39.00226757	15.23139	7	20	N
NDT016	Normal	11	39742	43627	10.66666667	M	Caucasian	Yes	59	1.498602997	98	44.44444444	19.78993	84	50	Y
NDT017	Normal	10	39916	43664	10.25	M	African American	No	57	1.447802896	87	39.45578231	18.82313	79	50	N
NDT018	Normal	12	39227	43664	12.16666667	M	Caucasian	Yes	61	1.549403099	107	48.5260771	20.21372	79	40	N
ODT001	Obese	NA	38772	43431	12.75	F	Caucasian	Yes	60	1.524003048	168	76.19047619	32.80417	-	90	Y
ODT002	Obese	12	39070	43489	12.08333333	F	African American	no	61	1.549403099	147	66.66666667	27.77026	97	80	Y
ODT003	Obese	13	39018	43529	12.33333333	F	African American	NA	63	1.6002032	212	96.14512472	37.54715	99	70	N
ODT004	Obese	10	39802	43531	10.25	M	Caucasian	Yes	58	1.473202946	129	58.50340136	26.95603	99	80	Y
ODT007	Obese	12	39380	43615	11.58333333	M	Caucasian	No	64	1.625603251	160	72.56235828	27.45885	98	60	Y
ODT008	Obese	12	39287	43627	11.91666667	F	Caucasian	Yes	63	1.6002032	139	63.03854875	24.61818	94	70	Y
ODT010	Obese	11	39449	43678	11.66666667	F	Caucasian	Yes	61	1.549403099	188	85.26077098	35.5157	99	70	Y
ODT011	Obese	10	39886		10.5	M	Asian	No	57	1.447802896	109	49.43310658	23.583	96	75	Y
ODT012	Obese	10	40091	43754	10	M	Caucasian	Yes	57	1.447802896	112	50.79365079	24.23207	97	70	Y
ODT013	Obese	12	39233		12.4167	M	Caucasian	Yes	60	1.524003048	123	55.78231293	24.01734	95	65	Y

F. Raw Data, Cytokine Levels

Sample ID	il10	ccl2	il1b	ccl3	mpo	TNFa	IL6	IL8
Healthy 1	4.21	64.18	175.39	268.23	83116.96	2.797837	1.567428	100.7771
Healthy 2	2.77	46.12	131.86	202.06	74001.96	2.564473	0.554635	177.7215
Healthy 3	2.02	41.55	64.76	187.49	64619.59	0.854117	0.149078	30.56897
Healthy 4	2.25	43.86	164.80	191.71	71835.33	-	-	25.50824
Healthy 5	2.97	48.27	281.56	207.20	78650.88	1.320355	0.62768	18.22504
Healthy 6	2.97	51.74	170.73	219.68	77932.52	1.048384	-	38.32958
Healthy 7	0.80	28.00	122.59	136.79	38731.93	1.475722	0.337063	85.78102
Healthy 8	4.45	63.60	115.01	263.64	96248.25	1.39818	-	5.149813
Healthy 9	1.69	33.89	29.13	163.98	53620.54	1.553457	0.578145	90.70632
Healthy 10	2.33	40.74	62.46	184.81	63859.31	7.477396	6.334038	282.447
Obese 1	2.492119	47.56877	59.34206	208.1982	78674.12907			
Obese 2	4.29	69.23	137.35	274.25	83551.75	1.942164	0.385022	195.971
Obese 3	3.75	56.93	146.21	243.48	104744.61	4.589226	4.325993	265.2503
Obese 4	3.83	60.64	126.80	257.33	81778.57	2.331031	0.337063	115.0151
Obese 5	2.41	48.99	106.60	210.16	68336.54	1.475745	-	99.38845
Obese 6	4.37	65.89	204.03	275.71	99772.62	1.475722	0.97047	57.6411
Obese 7	3.51	58.20	257.44	246.02	65092.15	2.292247	0.678292	189.9693
Obese 8	3.36	59.43	120.90	247.62	85139.10	2.720022	3.294453	146.1499
Obese 9	3.20	57.58	117.12	246.02	85127.73	2.797837	0.626719	302.7877
Obese 10	4.21	67.58	261.49	277.21	56111.89			

VITA

NAME: Chad Mitchell Silver

EDUCATION: B.S., Psychology, Indiana University, Bloomington, IN, 2015

D.M.D., University of Illinois Chicago School Of Dentistry, Chicago, IL, 2019

Certificate in Pediatric Dentistry, University of Illinois Chicago, College of Dentistry, Chicago, IL, 2021

M.S., Oral Sciences, University of Illinois at Chicago, College of Dentistry, Chicago, IL, 2021

PROFESSIONAL MEMBERSHIP: American Academy of Pediatric Dentistry
American Dental Association
Illinois Society of Pediatric Dentistry
Chicago Dental Society

ABSTRACTS: Silver, C., Truskoski D., Thalji G., Naqvi A., Alrayyes S., Nicholas, C., The Influence of Oral Inflammation on Timing of Dental Eruption: A Review of the Literature. University of Illinois Chicago, College of Dentistry, Clinic and Research Day 2020.

Silver, C., Truskoski D., Wu, N., Naqvi A., Alrayyes S., Nicholas, C., The Influence of Oral Inflammation on Timing of Dental Eruption: A Review of the Literature. University of Illinois Chicago, College of Dentistry, Clinic and Research Day 2021.

Silver, C., Naqvi A., Wu C., Alrayyes S., Nicholas C., The Influence of Oral Inflammation on Timing of Dental Eruption. University of Illinois Chicago, College of Dentistry, Clinic and Research Day 2020. American Academy of Pediatric Dentistry 2020 Annual Conference.

PRESENTATIONS: Poster Presentation, University of Illinois Chicago, College of Dentistry, Clinic and Research Day, March 12, 2020
Title: The Influence of Oral Inflammation on Timing of Dental Eruption: A Review of Literature.
Co-Authors: Christina Nicholas, Afsar Naqvi, Sahar Alrayyes, Ghandeer Thalji

Poster Presentation, University of Illinois Chicago, College of Dentistry, Clinic and Research Day, 2021
Title: The Influence of Oral Inflammation on Timing of Dental Eruption
Co-Authors: Christina Nicholas, Afsar Naqvi, Sahar Alrayyes, Christine Wu

Poster Presentation, American Academy of Pediatric Dentistry (AAPD) 74th Annual Session, Virtual, May 26, 2021
Title: Title: The Influence of Oral Inflammation on Timing of Dental Eruption
Co-Authors: Christina Nicholas, Afsar Naqvi, Sahar Alrayyes, Christine Wu