SERUM IgG ANTIBODY RESPONSES TO PERIODONTAL PATHOGENS AMONG PEDIATRIC SUBJECTS

Ronan Allen

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements of the Master of Science in the Department of Periodontology, School of Dentistry.

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Abstract

RONAN ALLEN: Serum IgG Antibody Responses to Periodontal Pathogens among Pediatric Subjects
(Under the direction of Ceib Philips, Ph.D., Jessica Lee, D.D.S., Ph.D., David Paquette, D.M.D., D.M.Sc., M.P.H).

The first part of this thesis details the results from an observational study of 303 children 9-11 years of age. An immuno-checkerboard assay was used to analyze IgG levels for 18 different bacteria. Nearly half of the subjects tested positive for at least one orange complex bacteria and to \textit{A. actinomycetemcomitans}. A higher proportion of females had positive IgG responses to 14 of the 18 bacteria. A significantly higher proportion of African Americans exhibited IgG responses for \textit{P. gingivalis, T. denticola, P. intermedia, P. nigrescens, A. actinomycetemcomitans and C. rectus} (P<0.05). This cross sectional study indicates that a sizable proportion of young patients may be exposed and elicit an immune response to known periodontal pathogens. Recent evidence suggests that obesity and nutrition may be associated with periodontal disease. These two modifiable risk factors and their effects on periodontal disease are discussed in detail in the second part of this thesis.
I would like to thank and acknowledge the efforts of:

Catherine Champagne  
Ceib Philips  
Jessica Lee  
See He Kim  
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for all their encouragement, guidance and support.

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Abstract

The aim of this study is to assess prevalence of detectable serum immunoglobulin G (IgG) levels specific for periodontal bacteria in children and to compare the prevalence between gender and ethnic subcohorts. A subset of children 9-11 year olds were selected from the Cardiovascular Health in Children and Youth III (CHIC III) study, 75 with glucose levels $\geq 100\text{mg/dL}$ and 228 with glucose levels $< 100\text{mg/dL}$. Collected blood samples were analyzed for IgG levels for 18 different bacteria (oral and non-oral) using an immuno-checkerboard assay. Within this young cohort, 51.6% had positive IgG responses to at least one orange complex pathogen. Nearly half of the subjects tested positive for IgG to $A$.actinomycetemcomitans (46.6%). A higher proportion of females had positive IgG responses to 14 of the 18 bacteria ($P<0.05$). A higher proportion of African Americans exhibited IgG responses to 14 of the bacteria. Differences were statistically significant for $P$.gingivalis, $T$.denticola, $P$.intermedia, $P$.nigrescens, $A$.actinomycetemcomitans and $C$.rectus ($P<0.05$). This cross sectional study indicates that a sizable proportion of young patients may be exposed to and elicit an immune response to known periodontal pathogens. Within this study, a higher proportion of female and African American subjects had immune responses compared to males and Caucasians respectively.
INTRODUCTION

The prevalence of periodontal disease in medically healthy children in North America varies from 0.4 - 0.8% for aggressive and 2.0 - 5.0% for chronic periodontitis (Albandar et al. 2002). Prevalence rates for periodontitis prior to puberty vary from 0.84% to 26.9%. This variability is due to the limited number of case reports available for this age group (Oh et al. 2002). Gingivitis is affects a much greater proportion of children than periodontitis with estimates of greater than 70% for most populations studied (Oh et al. 2002). Prevalence rates for childhood periodontitis are higher among developing countries (MacGregor et al. 1986) and variations exist for different race and ethnic groups. Prevalence estimates for early onset or aggressive periodontitis range from 0.1 - 0.2% in Caucasians to as high as 3.0% in Africans and African Americans (Albandar et al. 2002). The effect of gender as a risk factor for periodontitis and the exact mode of inheritance of the disease remain uncertain. The available evidence suggests that young females in the peripubertal age may be more affected than males (Hørmand & Frandsen 1979); however, this effect is reduced or reversed with increasing age (Loe & Brown, 1991).

The oral bacterial biofilm is the primary etiological agent responsible for both gingivitis and periodontitis in children and adults (Löe et al. 1965, Moore et al. 1987). Although the oral biofilm can harbor a wide spectrum of bacterial types, it seems that only certain bacteria initiate and stimulate progression of periodontal disease (Socransky
et al. 1998). Putative periodontal pathogens in children include *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum* and *Treponema denticola* (Van Winkelhoff et al. 2005). Within periodontitis subjects, these bacteria are recovered in higher numbers (Slots and Rams 1992), are associated with disease progression (Albandar et al. 1997) and possess potent virulence factors (Fives-Taylor et al. 1999). Among the pathogenic bacteria, *A. actinomycetemcomitans* and *P. gingivalis* have been well documented as major players in the pathogenesis of aggressive forms of periodontal disease in children (Van Winkelhoff et al. 1989).

The oral cavity becomes exposed to bacteria very early, and many commensal organisms can be detected prior to tooth eruption (Bimstein & Matsson, 1999). The acquisition of oral bacteria includes horizontal and vertical transmissions as well as cross-infection (Van Winkelhoff et al. 2005). Colonization of periodontal pathogens in the biofilm of young children is thought to be infrequent (Könönen et al. 2000). When it does occur, transmission and infection is thought to occur via maternal source (Watson et al. 1996). In a cohort of 2 to 12-year-old children the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* in plaque samples was 4.8% in healthy and 20.0% for periodontitis subjects respectively (Okada et al. 2000). However, higher levels of colonization have been recently documented with 71% of 18 to 48-month-old children harboring at least one periodontal pathogen (Yang et al. 2002). The presence of periodontal pathogens in the biofilm may not be indicative of disease initiation and progression (Frisken et al. 1990, Okada et al. 2000). While, as their presence is necessary
for disease, their identification is still an inherent risk indicator for future periodontal diseases in a susceptible host (Socransky et al 1998).

Immunoglobulin G antibodies (IgG) provide the majority of antibody-based immunity against invading pathogens and contribute to the inhibition of bacterial adherence and colonization, enhancing bacterial phagocytosis, via opsonization and agglutination (Kinane et al. 1999). Elevated serum IgG antibodies to *A. actinomycetemcomitans* have been reported in juvenile periodontitis (Zambon et al. 1988), and to *Porphyromonas gingivalis, Campylobacter rectus, Prevotella intermedia*, and *Tanerella forsythia* in adult periodontitis subjects compared to periodontally healthy subjects (Haffajee et al. 1995). Increases in antibody to colonizing pathogenic bacteria can be associated with subsequent progressing disease (Mouton et al. 1987, Ebersole & Taubman 1994).

IgG antibodies produced in response to periodontopathogens are increased in patients with clinical manifestations of periodontitis, and this reflects the subgingival colonization by the specific bacteria (Gunsolley et al. 1990, Nakagawa et al 1994a). Papapanou et al. 2001 reported 84% sensitivity and 57.5% specificity when using checkerboard assessments of serum IgG antibodies to oral bacteria as a surrogate for periodontal disease status. In particular serum IgG levels to *P. gingivalis, A. actinomycetemcomitans* and *T. forsythensis* have been shown to be elevated in adult patients with periodontitis compared to subjects without disease (Kinane et al. 1999, Persson et al. 2000). Interestingly, reductions in serum IgG levels to *P. gingivalis* have been observed following successful therapy in patients with severe chronic periodontitis (Mooney et al. 1994).
It has been suggested that localized aggressive periodontitis patients produce elevated levels of IgG compared to generalized aggressive patients suggesting a protective role of the host in localizing attachment loss to a few teeth (Califano et al. 1992). Other reports indicate elevated levels of serum antibodies with disease progression possible indicating production of an ineffective class of antibody (Albandar et al. 2001).

*A. actinomycetemcomitans* and *P. gingivalis* have strong associations with chronic and aggressive forms of periodontitis and elevated serum IgG antibodies to these bacteria have been reported in children (Celenligil et al. 1998). Pathogenic strains of bacteria can be found in significant levels in the plaque of young children, eliciting a serum antibody response that increases with age, and may be related to incipient signs of periodontal disease (Bimstein et al. 2004). Differences in serum antibody response to periodontopathogens have been reported for different ethnic populations. Serum antibodies to *A. actinomycetemcomitans* were reported to be higher in African-Americans than in Caucasians (Gunsolley et al. 1991).

The majority of studies examining IgG responses have focused on only a select few bacteria and no studies to date have reported the prevalence of serum IgG responses to a broad range of periodontopathogens in young children. More information on the acquisition of specific periodontal bacteria with concomitant immune responses in children could help to elucidate the development and pathogenesis of periodontitis. The aim of this cross sectional study is to measure the prevalence of serum IgG antibody responses against a broad range of oral and one non-oral bacteria for a population of 9-11 year-old-children.
MATERIALS AND METHODS

Sample

Subjects for this cross sectional, observational study were selected from the Cardiovascular Health in Children and Youth III (CHIC III) study. A detailed description of the study design and objectives of the CHIC III study has previously been published (Ondrak et al. 2007). Briefly, 1,420 non-Hispanic African American or Caucasian participants were recruited from 34 schools that included a high proportion of minority students located in rural areas three regions (Eastern Coastal, Central Piedmont, and Western Mountain) in North Carolina. Demographic data were collected between January 2000 and February 2003 at participants’ respective schools by teams of trained and calibrated research assistants. Consent was given for use of data and stored specimens in future research.

A subset of 320 children ranging in age from 9 to 11 years were selected for the analysis of IgG responses to periodontal bacteria after provision of assent and parental consent reviewed and approved by the Institutional Review Board of UNC-CH. The inclusion criteria consisted of ability to read and write English; and having at least one natural relative available to report family history. The exclusion criteria were
being physically handicapped as reported by parents, teachers, school nurses, or self; suffering from any serious illness such as type 1 diabetes requiring insulin, renal disease, or moderate to severe asthma as reported by parents, teachers, school nurses, or self; having any major developmental disability as reported by parents. Seventy-five children in the cohort had elevated fasting glucose levels (≥100mg/dL). For each of these “prediabetic” children, three children matched for normal fasting glucose levels (≤100mg/dl), age, sex, and race were randomly selected.

Analysis of serum IgG Responses

Serum samples were obtained by sterile venipuncture from each participant early in the morning after an overnight fast. Fasting compliance was confirmed by on-site research assistants. The blood samples (10ml) were allowed to clot for 2 hours at room temperature before centrifuging (20 minutes at approximately 2000 x g). Plasma and serum were separated and stored at -80°C until analysis. Specific IgG responses for periodontal pathogens were measured using an immuno-checkerboard (Sakellari et al. 1997). Accordingly, pure colonies of known bacteria were grown on agar plates. Known bacteria from these colonies were added to 1 ml of phosphate buffered saline (PBS) in a 1.5 ml Eppendorf tube. Bacterial cells were disrupted for 10 sec with an ultrasonic device. Nitrocellulose membranes were prepared, and the Miniblotter apparatus was pre-chilled for 4 hours at 4°C. Membranes were equilibrated by soaking first in distilled water and then PBS for 10 minutes. Each membrane was loaded with bacterial suspensions (in duplicate) at 125 µl and protein A at 10µg/ml as a positive control (Boehringer, Germany). Miniblotter devices were incubated overnight at 4°C. Thereafter,
channels were emptied and washed with 500 ml TBS-T (NaCl, Trizma base at pH 7.6 and Tween 20). Membranes were dried at 37°C, blocked, washed, incubated for one hour with 100 ml of a solution containing distilled water (105 ml), 100% methanol (30 ml) and 30% hydrogen peroxide (15 ml), and then washed and blocked again. Miniblotters were mounted and rotated 90°, and channels were loaded with 130 µl of the unknown serum samples (diluted at 1/50, 1/1000 and 1/2000) and standards (IgG at various concentrations). Devices were incubated for one hour at room temperature, and then incubated with secondary antibody (Fab goat-anti-human IgG conjugated to HRP, Boehringer, Germany) for another hour at room temperature on a rotating table. Membranes were removed and washed with TBS-T. Equal volumes (25 ml) of ECL-Western-blotting detection reagents (Amersham, Buckinghamshire, United Kingdom) were mixed and added to the membranes. Membranes were quickly wrapped in plastic, photographed and developed. Images were scanned, and the IgG response to each of 18 bacteria, 17 periodontal pathogens (P. gingivalis, Prevotella intermedia, Prevotella nigrescens, Tanerella forsythensis, Treponema denticola, A. actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Peptostreptococcus micros, Capnopytophagia ochracea, Vionella parvula, Streptococcus intermedius, Streptococcus oralis, Streptococcus sanguis, Actinomyces viscosis, Streptococcus noxia) and one non-oral pathogen associated with gastritis and gastric ulcer (Helicobacter pylori) was scored dichotomously as detectable or not detectable (detectable threshold level >269.2 ng/mL was used). In addition to the individual bacteria responses, combinations of detectable bacteria were noted as present or absent for each subject. The combinations considered were: two or more red complex
bacteria; two or more of the orange complex bacteria; at least one orange complex bacteria plus *A. actinomycetemcomitans*; at least one red complex bacteria plus *A. actinomycetemcomitans*; at least one orange complex bacteria plus at least one red complex bacteria.

**Statistical Analysis**

Univariate statistics were used to summarize responses for the demographic and bacteria data. The Chi-square test was used to compare the detectable proportion of each bacteria between males and females and between African-American and Caucasian children. Fisher’s exact test was used to compare the presence of the combinations of bacteria between gender and ethnic subcohorts.
RESULTS

Description of Sample

Seventeen of the initial 320 subjects could not be included due to incomplete sample collection, such as inadequate serum volume to perform IgG analysis. From the remaining 303 children studied, 72 (23.1%) were nine, 184 (59%) ten, and 56 (17.9%) eleven years old respectively. Forty-five percent (n=135) were female and 58% (n=176) were African-American (Table 1). Four percent of the children reported having smoked. Mean fasting plasma glucose was calculated at 93.3 mg/dL ± SD 11.4 for the sample and 24.8% (n=75) of the children were labeled as “pre-diabetic” with a fasting blood glucose of ≥100 mg/dL.

Prevalence of serum IgG response to individual bacteria

The proportion of children with detectable bacterial threshold levels for all species examined ranged from 2.3% for *F. nucleatum* to 51.6% for *P. intermedia* (Table 2). The percentage of IgG responses for red complex bacteria ranged from 5.9% for *P. gingivalis* to 14.5% for *T. denticola* with orange complex responses highest for *P. intermedia* (51.6%) and lowest for *F. nucleatum and S. noxia* (both 2.3%). The percentage of children with IgG response to *A. actinomycetemcomtans* was 4.66%. A higher proportion of girls had a positive IgG response to 14 of the individual pathogens (Table 2). Compared to the boys, girls had elevated proportions of detectable thresholds for all 3
red complex bacteria although this did not reach statistical significance. Within the other bacterial complexes statistically significant higher proportions of girls had IgG responses to two of the orange (P. micros and A. actinomycetemcomitans), one of the green (C. oralis), two of the yellow complex bacteria (S. sobrinos, S. intermedia), and the gut bacterium H. pylori (p<0.05).

Table 1. Demographics of study sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55.4%</td>
</tr>
<tr>
<td>Female</td>
<td>44.6%</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>58.1 %</td>
</tr>
<tr>
<td>Caucasian</td>
<td>41.9%</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>22.8%</td>
</tr>
<tr>
<td>10</td>
<td>58.7%</td>
</tr>
<tr>
<td>11</td>
<td>18.5%</td>
</tr>
<tr>
<td>Hyperglycemic (glucose ≥ 100mg/dL)</td>
<td>24.8%</td>
</tr>
<tr>
<td>Smokers (current and former)</td>
<td>3.9 %</td>
</tr>
</tbody>
</table>

Notably, a higher proportion of African Americans had a positive response to 14 of the individual pathogens (Table 2). For the red complex bacteria, significantly higher proportions of IgG responses to T. denticola were found in African American children.
(19.9%) compared to Caucasians (7.1%) (P<0.005). IgG responses for *P. gingivalis* were also significantly elevated in African Americans when compared to Caucasians (P<0.05). Statistically significant elevations in the proportions of detectable thresholds were also observed for five of the orange complex bacteria (*C. rectus*, *P. intermedia* *P. negriscens*, and *S. noxia*) (P<0.05). IgG responses to *P. nigrescens* and *P. intermedia* were found to occur in more than twice the number of African Americans than Caucasians while IgG response to *S. noxia* was not detected in Caucasians but found in seven (4%) of the African American children. In addition, *C. rectus* was also detected in a low proportion of Caucasians (1.6%) while African Americans had significantly higher proportions (15.3%) (P<0.005). IgG specific for *A. actinomycetemcomitans* was observed in 99 (56.3%) of African Americans versus only 42 (33.1%) of Caucasians (P<0.005) The difference in the proportion of detectable thresholds was not statistically significant for either the purple and yellow complex bacteria.
**Table 2.** Frequencies (n) of subjects with detectable levels of IgG

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>All (n=303)</th>
<th>Males (n=168)</th>
<th>Females (n=135)</th>
<th>p-value</th>
<th>Caucasian (n=127)</th>
<th>African American (n=176)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pg</em></td>
<td>5.9%(18)</td>
<td>4.8%(8)</td>
<td>7.4%(10)</td>
<td>0.33</td>
<td>1.6%(2)</td>
<td>9.1%(16)</td>
<td>0.01*</td>
</tr>
<tr>
<td><em>Tf</em></td>
<td>9.6%(29)</td>
<td>7.1%(12)</td>
<td>12.6%(17)</td>
<td>0.11</td>
<td>6.3%(8)</td>
<td>11.9%(21)</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Td</em></td>
<td>14.5%(44)</td>
<td>14.3%(24)</td>
<td>14.8%(20)</td>
<td>0.90</td>
<td>7.1%(9)</td>
<td>19.9%(35)</td>
<td>0.002*</td>
</tr>
<tr>
<td><strong>Orange complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cr</em></td>
<td>9.6%(29)</td>
<td>7.7%(13)</td>
<td>11.9%(16)</td>
<td>0.23</td>
<td>1.6%(2)</td>
<td>15.3%(27)</td>
<td>0.0001*</td>
</tr>
<tr>
<td><em>Pi</em></td>
<td>51.6%(157)</td>
<td>49.4%(83)</td>
<td>54.8%(74)</td>
<td>0.35</td>
<td>33.1%(42)</td>
<td>65.3%(115)</td>
<td>0.0001*</td>
</tr>
<tr>
<td><em>Pn</em></td>
<td>15.5%(57)</td>
<td>15.5%(26)</td>
<td>15.6%(21)</td>
<td>0.98</td>
<td>7.9%(10)</td>
<td>21.0%(37)</td>
<td>0.002*</td>
</tr>
<tr>
<td><em>Pm</em></td>
<td>12.5%(38)</td>
<td>8.3%(14)</td>
<td>17.8%(24)</td>
<td>0.01*</td>
<td>9.5%(12)</td>
<td>14.8%(26)</td>
<td>0.17</td>
</tr>
<tr>
<td><em>Fn</em></td>
<td>2.3%(7)</td>
<td>2.4%(4)</td>
<td>2.2%(3)</td>
<td>0.93</td>
<td>0.8%(1)</td>
<td>3.4%(6)</td>
<td>0.13</td>
</tr>
<tr>
<td><em>Aa</em></td>
<td>46.6%(141)</td>
<td>41.1%(69)</td>
<td>53.3%(72)</td>
<td>0.03*</td>
<td>33.1%(42)</td>
<td>56.3%(99)</td>
<td>0.0001*</td>
</tr>
<tr>
<td><em>Sn</em></td>
<td>2.3%(7)</td>
<td>2.4%(4)</td>
<td>2.2%(3)</td>
<td>0.93</td>
<td>0%(0)</td>
<td>4.0%(7)</td>
<td>0.02*</td>
</tr>
<tr>
<td><strong>Purple complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Vp</em></td>
<td>2.3%(7)</td>
<td>1.8%(3)</td>
<td>3.0%(4)</td>
<td>0.50</td>
<td>1.6%(2)</td>
<td>2.8%(5)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Green complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ec</em></td>
<td>6.3%(19)</td>
<td>4.8%(8)</td>
<td>8.2%(11)</td>
<td>0.23</td>
<td>3.2%(4)</td>
<td>8.5%(15)</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Co</em></td>
<td>22.7%(69)</td>
<td>17.9%(30)</td>
<td>28.9%(39)</td>
<td>0.02*</td>
<td>17.3%(22)</td>
<td>26.7%(47)</td>
<td>0.04*</td>
</tr>
<tr>
<td><strong>Yellow complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Si</em></td>
<td>39.8%(121)</td>
<td>32.7%(55)</td>
<td>48.9%(66)</td>
<td>0.004*</td>
<td>42.5%(54)</td>
<td>38.1%(67)</td>
<td>0.44</td>
</tr>
<tr>
<td><em>So</em></td>
<td>18.8%(57)</td>
<td>16.1%(27)</td>
<td>22.2%(30)</td>
<td>0.17</td>
<td>14.2%(18)</td>
<td>22.2%(39)</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Ss</em></td>
<td>5.6%(14)</td>
<td>1.8%(3)</td>
<td>8.2%(11)</td>
<td>0.009*</td>
<td>4.7%(6)</td>
<td>4.6%(8)</td>
<td>0.94</td>
</tr>
<tr>
<td><em>Av</em></td>
<td>4.6%(14)</td>
<td>5.4%(9)</td>
<td>3.7%(5)</td>
<td>0.50</td>
<td>4.7%(6)</td>
<td>4.6%(8)</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>Non-oral</strong></td>
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</tr>
<tr>
<td><em>Hp</em></td>
<td>6.3%(19)</td>
<td>2.4%(4)</td>
<td>11.1%(15)</td>
<td>0.002*</td>
<td>6.3%(8)</td>
<td>6.3%(11)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Denotes statistically significance (Chi-squared test)
Prevalence of serum IgG response to combinations of bacteria

Twenty-one subjects (6.9%) had a positive IgG responses to at least two red complex bacteria, similarly, 69 (22.8%) subjects had IgG responses to at least two of the orange complex. A higher proportion of females were IgG positive for 6 of the 7 combinations of bacteria, but girls and boys differed statistically only for all three of the red complex bacteria (p<0.05). Over one-third of the subjects (33.7%) had IgG responses to at least one orange complex bacteria in combination with *A. actinomycetemcomitans*. In contrast, 43 of the subjects (14.8%) had detectable IgG specific for at least one red complex pathogen in combination with *A. actinomycetemcomitans* (Table 3).

**Table 3.** Frequencies (n) of subjects with detectable levels of IgG to different combinations of bacteria

<table>
<thead>
<tr>
<th>Groups of Bacteria</th>
<th>Subject groups</th>
<th>All (n=303)</th>
<th>Males (n=168)</th>
<th>Females (n=135)</th>
<th>p-value</th>
<th>Caucasian (n=127)</th>
<th>African American (n=176)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Complex</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least 2</td>
<td></td>
<td>6.9% (21)</td>
<td>4.8% (8)</td>
<td>9.6% (13)</td>
<td>0.10</td>
<td>3.2% (4)</td>
<td>9.7% (17)</td>
<td>0.03*</td>
</tr>
<tr>
<td>All 3</td>
<td></td>
<td>1.0% (3)</td>
<td>0% (0)</td>
<td>2.2% (3)</td>
<td>0.04*</td>
<td>0.8% (1)</td>
<td>1.1% (2)</td>
<td>0.76</td>
</tr>
<tr>
<td>Orange complex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least 2</td>
<td></td>
<td>22.8% (69)</td>
<td>20.2% (34)</td>
<td>25.9% (35)</td>
<td>0.24</td>
<td>11.8% (15)</td>
<td>30.7% (54)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>3 or more</td>
<td></td>
<td>8.9% (27)</td>
<td>8.9% (15)</td>
<td>8.9% (12)</td>
<td>0.99</td>
<td>0.8% (1)</td>
<td>14.8% (26)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Other combinations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least 1 Red + Aa</td>
<td></td>
<td>14.2% (43)</td>
<td>13.7% (23)</td>
<td>14.8% (20)</td>
<td>0.78</td>
<td>5.5% (7)</td>
<td>20.5% (36)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>At least 1 Orange +Aa</td>
<td></td>
<td>33.7% (102)</td>
<td>29.2% (49)</td>
<td>39.3% (53)</td>
<td>0.06</td>
<td>18.9% (24)</td>
<td>44.3% (78)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>At least 1 Orange +at least 1 red</td>
<td>18.8% (57)</td>
<td>17.9% (30)</td>
<td>20.0% (27)</td>
<td>0.64</td>
<td>7.9% (10)</td>
<td>26.7% (47)</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

*Denotes statistically significance (Fisher’s exact test)
Detectable IgG responses to one orange and one red complex bacteria was evident in 18.8% of the study sample. Again, a higher proportion of the African American children were positive for all seven of the combinations of bacteria with a statistically significant difference (P<0.05) observed for one of the seven combinations and highly significant (P<0.001) for five of the combinations of bacteria analyzed (Table 3).
DISCUSSION

The overall hypothesis of our study is that exposure to periodontal pathogens and overweight/obesity is associated with increased systemic inflammation and hence increased diabetes risk in children. This first paper documents the prevalence of positive IgG responses to a number of periodontal pathogens. The rationale is that IgG antibody response represents an estimate of the overall systemic burden induced by periodontal infection. When combined with clinical periodontal measurements, these immune and other inflammatory responses could provide better estimates of the overall systemic burden imposed by an oral infectious challenge in chronic diseases such as diabetes.

The major finding of the present exploratory study is that the children in this cohort were exposed to and exhibited detectable immune responses to a broad range of known periodontal pathogens at an early age. This immune response against specific microorganisms can be used as evidence for exposure and impact of the microbial etiology on the pathogenesis of periodontitis (Kinane et al. 1999). Significantly more girls 9-11 years of age were likely to have IgG responses to one or more orange complex bacteria and *A. actinomycetemcomitans* as compared to boys. In addition, significantly more African Americans had IgG responses to both red and orange complex bacteria as well as *A. actinomycetemcomitans* versus white children.

Early colonization of periodontal pathogens in the oral biofilm of children has been previously chronicled (Morinushi et al. 2000). Controversy still remains as to the percentages of children who harbor pathogenic bacteria, elicit an immune response and if
they are likely to develop periodontal disease. In this study, the prevalence of positive IgG responses to *P. gingivalis*, *T. forsythensis* and *T. denticola* (red complex bacteria) was 5.9% (n=18), 9.6% (n=29) and 14.5% (n=44) respectively. Significant numbers of children exhibited IgG responses to at least one of the orange complex bacteria (51.6%). When combinations of bacteria were analyzed, it was apparent that over one-third of the children exhibited immune IgG responses to at least one orange complex bacteria in combination with *A. actinomycetemcomitans*. The last bacterium is most closely associated with localized aggressive periodontitis (Van Winkelhoff et al. 1989) and IgG responses were present in nearly half of the study population (46.6%). Children with *A. actinomycetemcomitans* associated aggressive periodontitis have an elevated IgG response in a high proportion of children (> 90%) (Ebersole et al. 1987).

The prevalence of pathogenic bacteria colonizing the oral biofilm of young subjects shows wide variation. Okada et al. 2004 reported low levels of *P. gingivalis* and *A. actinomycetemcomitans* as well and absence of *P. intermedia* in healthy children between the ages of 2 and 12 years; however, these percentages increased to over 20% in the biofilm of subjects with clinical periodontitis as defined by attachment loss of greater than 3 mm on at least one site of 4 teeth. Other studies have found high levels of periodontopathgens in periodontally healthy subjects with no differences in levels among periodontal healthy and diseased subjects. (Gafan et al. 2004, Lamell et al. 2000). Since periodontal examinations were not performed in this study, a positive IgG response is not indicative of a clinical diagnosis of periodontitis.

The majority of studies have shown an association between IgG responses and presence of pathogens in the biofilm of children (Savitt et al. 1991, Kojima et al. 1997,
Periodontitis status in children has also been positively associated with IgG response especially with *P. gingivalis* and *A. actinomycetemcomitans* for whom higher levels were noted with increasing disease severity (Kojima et al. 1997, Bimstein et al. 2004, Donley et al. 2004). However, other studies have found no relationship between serum IgG responses and presence of bacteria in the plaque biofilm (Morinushi et al. 2000, Bimstein et al. 2004) or periodontal status (Doty et al. 1982).

Longitudinal analysis in young children recognize that exposure to periodontal pathogens occurs early but may be episodic (Ebersole et al. 1991). After exposure to periodontal pathogens serum IgG levels become elevated and usually remain stable (Donley et al. 2004) even when bacteria are not found in the biofilm. This may be due to protection by an appropriate immune response or failure of bacteria to survive in non-anaerobic conditions in children (shallow pocket). Another factor that is not addressed in this present or the Donley et al. (2004) study is the presence or absence of disease. High levels of antibody production with presence of disease may indicate production of ineffective IgG antibodies and this may even contribute to disease severity (Kojima et al. 1997). Magnusson et al. 1991 found elevated serum IgG levels to *P. gingivalis, A. actinomycetemcomitans* and *E. Corrodens* in refractory periodontitis patients.

Smoking is a known risk factor for periodontitis accounting for increased disease prevalence and severity in adults (Tomar et al. 2000). Self reported smoking represented only a small proportion of this young cohort of children (3.9%). Duration and dose of smoking increase disease risk; however, due to the younger age of the subjects neither of these factors was reported in this study. Few studies exam the association between smoking and periodontitis risk in children. A recent study in 12–21 year old Chilean
students estimated the risk for chronic periodontitis in smokers and nonsmokers to be the same (Lopez et al. 2001). The study sample however smoked on average 5.4 cigarettes per day for 3 years, which is a relatively low but early exposure to cigarette smoking. It is known that adult periodontitis patients who smoke exhibit a reduction in total serum IgG levels and associated increase in periodontal destruction (Graswinkel et al. 2004). This suggests a reduction of the protective effect of serum IgG against periodontal pathogens.

*H. pylori* infection is considered to be etiologic for gastritis and peptic ulcers and is a risk factor for gastric cancer (Graham, 1991). Approximately 10% of the population are affected by gastric ulceration throughout their lifetime, and more than 50% of people are carriers of this bacterium (Souto et al. 2008). Previous studies suggest that childhood is a high risk time for acquiring *H. pylori* infection. Mode of transmission of *H. pylori* is uncertain, many of the current theories suggest that the most probable pathways of horizontal transmission of *H. pylori* are through oral-oral, fecal-oral, or gastric-oral contact. Factors such as low socioeconomic level, poor hygienic conditions and overcrowding have been implicated (Wong et al. 2005). *H. pylori* has been detected in the oral biofilm of 41% of adult periodontitis subjects; hence, periodontal pocketing and inflammation may favor the colonization by this species in the oral cavity (Dye et al. 2008). CVD risk has also been shown to be increased in subjects exhibiting positive IgG responses to *H. pylori* (Whincup et al. 2000). Immune responses to this bacterium may further compound the overall chronic systemic inflammatory burden. In this group of young people 6.9% exhibited an IgG response to *H. pylori* with girls having statistically significant higher proportion of detectable thresholds than males (P<0.01).
For this study, the assumption was that an antibody response indicates systemic exposure to an oral organism independent of the periodontal status. Due to majority of studies supporting the association between IgG response and periodontal status, comparisons of IgG responses as a surrogate marker of exposure are appropriate. For children, the majority of studies have focused on a few of the more commonly associated periodontal pathogens, including *P. gingivalis* and *A. actinomycetemcomitans*. The present study found IgG response to *P. gingivalis* in 5.8% of subjects which is lower than other studies who had positive IgG responses in 52% of subjects aged 2-12 years (Donley et al. 2004). These differences may be associated with smaller sample size and the demographic and health and periodontal status of the subjects studied. Among the bacteria studied, *P. intermedia* were responsible for an IgG response in over 50% of the children. Nakagawa et al. (1994b) found a statistically significant increase in the proportions of *P. intermedia* and serum antibody levels against *P. intermedia* among pre-pubertal gingivitis subjects. Serum levels of testosterone in boys and estradiol and progesterone in girls was positively correlated with levels of *P. intermedia* and *P. nigrescens* and increasing gingival inflammation.

Studies analyzing epidemiological data on periodontitis report slight increased prevalence of localized aggressive forms among young Caucasian females over males (Albandar et al. 2002). More females in this study had significantly higher IgG responses to *P. micros* and *A. actinomycetemcomitans*, which may reflect a difference in bacterial composition or host response from that in males due to the earlier onset of puberty. It is known that hormonal variations which occur near puberty increase the prevalence of gingivitis in some individuals (Nakagawa et al. 1994b; Mariotti et al. 1999). These
changes may encourage the development of pathogenic strains, and increased numbers of bacteria which could lead to an increased immune response as evidenced by the increased IgG response to bacteria in females (see Table 3). Hørmand & Frandsen, (1979) found that females were five times more likely to develop localized aggressive periodontitis between 12 and 18 years old. They attributed this to the earlier eruption pattern of 1st molars and incisors in girls.

In this current study, African Americans were more likely to produce IgG antibody to pathogens than Caucasians. Increased levels of IgG to red and orange complex bacteria as well as *A. actinomycetemcomitans* may confer higher risk for initiation and progression of periodontitis (Craig et al. 2002). This is in accordance to the increased prevalence of periodontitis reported in this racial group (Albandar et al. 2002). According to this data from the NHANES III survey, aggressive and chronic forms of periodontitis were reported to be more prevalent in African-American children than in Caucasians. There also appear to be differences in the bacterial subgingival colonization in African Americans and similarly, an increased prevalence and severity of periodontitis (Craig et al. 2002). Differences in host response to bacterial colonization, including serum antibody levels, have also been demonstrated among the various ethnic/racial groups (Gunsolley et al. 1990) with African Americans having elevated serum antibody to *A. actinomycetemcomitans*. The differences in bacterial colonization and host response point to mechanisms that may account for the increased prevalence and severity of periodontitis observed in African Americans.

The early colonization by periodontal pathogens and the associated elevated immune response may either offer protection to the host or the immune response may be
insufficient or possibly contributory to the disease process manifesting in attachment loss and bone loss (Albandar et al. 2001). Despite focused research in this area, the relationship between specific IgG antibody and protection against periodontal pathogens remains obscure. Some studies found high antibody levels against periodontal pathogens to be associated with disease stability and other studies detect no apparent antibody effect on disease activity (Kinane et al. 1999). From this group of children, in which a number were overweight/pre-diabetic or healthy, it can be concluded that gender and race may represent increased risk for elevated immune responses to the periodontal pathogens. Obesity and diabetes are known risk factors for periodontal disease (Genco et al. 2005) and the increase in IgG response in this group may be because of the high number of cases presenting with overweight +/- prediabetes. This will be reported in subsequent papers (Champagne et al. in preparation).

A potential for improving periodontal risk assessment especially in the mixed dentition when traditional clinical examination may be difficult due to eruption patterns, would be to also assess the IgG responses to known periodontopathogens. Knowledge of the patient’s pathogenic exposure may alter the maintenance/intervention strategies for these at risk groups. Prevention and reduction of the inflammatory burden caused by periodontitis may have a profound effect on not only prognosis of periodontal disease but other systemic inflammatory diseases. A number of studies have recently correlated elevated serum IgG responses of *A. actinomycetemcomitans*, *P. gingivalis* and *C. rectus* and increased risk of cardiovascular disease (Beck et al. 2005, Pussinen et al. 2004, Colhoun et al. 2008).
To help understand the mechanisms involved in initiation and progression of periodontitis, it makes sense to monitor children from an early age, to elucidate both the exact role of individual bacteria and the host immune response. As this paper reports, the immune/inflammatory burden starts at a very early age and the long term affects on overall periodontal and systemic health remains to be seen. The objective of the present study was to examine the nature of the immune response to periodontal pathogens and help to identify other risk factors in a cohort of children. It was observed that even in this young cohort there are variations in immune responses to periodontal pathogens with respect to race and gender. The information gained from this exploratory study may help to identify persons with disease/disease risk earlier and enable us to implement preventive measures for these patients. Longitudinal studies combining clinical and immune responses are necessary to unravel the complexities of periodontal disease acquisition, initiation and progression. Further research is needed to fully comprehend the immune response in periodontitis and its contribution to systemic inflammation, especially in patients with other inflammatory disease processes.

This observational study revealed a surprisingly high number of 9-11-year-old children exhibiting IgG responses to known periodontopathogens. Significantly higher proportions of IgG immune responses were noted for girls and African Americans. This analysis shows that the immune-inflammatory burden may start very early with potential long term local and/or systemic consequences. Future studies are necessary to ascertain the impact of low grade chronic immune responses to periodontal pathogens and how these responses influence periodontal, as well as systemic health in children.
REFERENCES


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PART 2:

PERIODONTITIS: THE ROLE OF OBESITY, NUTRITION AND SYSTEMIC INFLAMMATION

Abstract

Recent studies have suggested that obesity and nutrition may be associated with periodontal disease. There has been an alarming increase in the prevalence of obesity in the United States and across the world. Obesity is a risk factor for many serious systemic diseases including CVD and diabetes. The chronic systemic inflammatory and immune effects induced by obesity have been linked with many of these morbidities. Malnutrition and reduction in antioxidant intake have also been observed to affect inflammatory and immune pathways. These two conditions may also increase the host’s susceptibility to periodontal disease through its impact on inflammatory and immune parameters. The aim of this review is to provide a detailed update on both obesity and nutrition, examining the evidence and biological plausibility regarding the impact of nutrition and obesity on periodontal disease.
INTRODUCTION

The initiation and progression of periodontitis is influenced by several determinants including patient, social and behavioral factors, systemic aspects, genetics, tooth related factors, microbial composition and other “emerging” factors (Nunn, 2003). Among the emerging factors that affect periodontal disease, obesity and nutritional intake have recently been identified as modifiable factors which could alter the course of periodontal disease progression (Genco et al. 2005, Schifferle, 2005).

Presently the pathogenesis of periodontitis is based on the concept that there is an interaction of the plaque bacteria and the host at the gingival crevice leading to the destruction of the periodontal supporting tissues (Offenbacher, 1996). The innate defense is the first line directed against these pathogenic bacteria and comprises neutrophils that phagocytose the bacteria and limit the inflammation to the marginal gingiva (gingivitis). In certain susceptible individuals, this bacterial biofilm can evade the host defense mechanisms and gain access to the underlying periodontium. This leads to lipopolysaccharide (LPS) presentation to the monocyte lymphocyte axis which induces an inflammatory response as evidenced by the production of inflammatory mediators such as prostaglandin E$_2$ (PGE$_2$), interleukin 1- beta (IL-1B) and tumor necrosis factor – alpha (TNF-α) (Offenbacher, 1996). These inflammatory cytokines are released from
monocytes and macrophages, which can lead to further inflammation and tissue destruction. This tissue destruction is evidenced clinically as periodontitis where gingival inflammation is accompanied by loss of collagen fiber attachment to the root cementum and migration of the junctional epithelium apically. If the pathological process is allowed to continue or there is robust host response, then resorption of supporting alveolar bone may occur (Armitage, 1995). The host response is integral to the clinical presentation of periodontitis and other diseases have been postulated to upregulate this inflammatory response. This “priming” of the defense cells has been observed when individuals have contributing exposures such as diabetes, IL-1 gene polymorphisms and increased stress (Kornman, 2008).

It has been observed that obesity and malnutrition have significant effects on immune, inflammatory and metabolic processes (Fantuzzi et al. 2005, Chapple et al. 2009). Periodontitis may be exacerbated by the increase in systemic inflammatory burden caused by either of these two maladies. Obesity and nutritional habits are usually formed in childhood, and concomitant inflammatory burden early on may have detrimental effects on periodontal progression. The aim of this review is to discuss the association between obesity and nutrition with periodontal disease and to highlight some avenues for future research.
Definition and Classification

Obesity is defined as a condition in which excess body fat has accumulated to such an extent that overall health may be negatively affected (Kopelman, 2000). Body Mass Index (BMI) is the most commonly used method for assigning weight classification. It is routinely used in doctors’ offices as well as in epidemiological and clinical studies to assess obesity status. BMI is a measure of body mass or weight in relation to height and calculated by dividing the weight in kilograms by height in meters squared (kg / m²). A BMI of greater than 25.0 is considered overweight and a BMI of greater than 30 is considered obese (Kopelman, 2000). (See table 1 for definitive classification). Childhood obesity is defined as a body mass index for age and gender that is greater than the 95th percentile (Fig.1) (Barlow et al. 1998).

Table 1. Classifications for Body Mass Index (BMI). Modified from Kopelman et al. 2000

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Weight classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5</td>
<td>Underweight</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>Normal weight</td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>Overweight</td>
</tr>
<tr>
<td>30.0–34.9</td>
<td>Obese class I</td>
</tr>
<tr>
<td>35.0–35.9</td>
<td>Obese class II</td>
</tr>
<tr>
<td>40.0+</td>
<td>Obese class III</td>
</tr>
</tbody>
</table>
BMI scores have been criticized due to overestimation of obesity levels especially among more muscular men who usually have an increased BMI without accompanying body fat increase (Kopelman, 2000). Abdominal obesity (waist circumference of > 40 inches) or waist-to-hip ratio (WHR), which looks at the proportion by which fat is distributed around the torso, may be more accurate measures of obesity-related morbidities and mortalities (Janssen, 2009).

![Fig. 1 Growth chart used to calculate BMI for a 10 year old boy (CDC, 2006)](image)

Other methods of assessing obesity involve using calipers to assess skinfold thickness at various sites, is an accurate measure of subcutaneous fat (Jackson et al 1978).

More recently, bioelectrical impedance analysis (BIA) has been used for estimating body composition. BIA can determine the electrical impedance, or opposition to the flow of an electric current through body tissues which can then be used to calculate
an estimate of total body water (TBW). TBW can be used to estimate fat-free body mass and, by difference with body weight, body fat percentage (Kopelman, 2000).

Unfortunately, methods other than BMI and WHR have not proven to be superior, and current evidence suggests they are of limited use. Body fat percentage measurement techniques used mainly for research include computed tomography (CT), magnetic resonance imaging (MRI), and dual energy X-ray absorptiometry (DEXA). These techniques provide very accurate measurements, but it can be difficult to scan the severely obese due to weight limits of the equipment and insufficient diameter of the CT or MRI scanner. It is generally agreed that men with more than 25% body fat and women with more than 33% body fat are obese (Haslam, 2000)

Prevalence and Causes

The prevalence of obesity and diabetes is increasing in the United States and around the world not only in adults but also in children and adolescents as well. For the United States, prevalence of obesity among adults has doubled, and the prevalence of overweight among children and adolescents has tripled over the last 20 years. Approximately 31% (59 million) of adults in America are now classified as obese and 15.8% of children aged 6–11 years, and 16.1% of adolescents aged 12–19 years, are overweight. In 1990, four states had obesity prevalence rates of 15–19% and no states had rates at or above 20%. In 2005, only four states had obesity prevalence rates less than 20%, while 17 states had prevalence rates of 25% or greater (fig.3)
Obesity is mainly caused by high calorie diet and physical inactivity. In recent years, the trend for increasing obesity has been linked to larger portion sizes, processed foods, diets high in fat and increased consumption of sweetened drinks (Harnack et al. 2000). In children the reduced participation in physical exercise due to health and safety issues at school and the increased watching of television and video games have also added to the problem (Dollman et al. 2005). The risk of obesity is 5.3 times higher in children who watch 5 hours of television compared to those that watch 2 hours or less (Gortmaker et al. 1996). Genetic variability as well as metabolic rate although minor have been implicated in the current obesity epidemic (Haslam et al. 2005).
Obesity has been associated with increased risk for type 2 diabetes, high blood pressure, cardiovascular disease, osteoarthritis, respiratory disorders, gall bladder disease and nonalcoholic fatty liver (Gregg et al. 2005). Increases in body fat alter the body's response to insulin, potentially leading to insulin resistance. Mortality risks appear to be directly related to body mass index, but only in younger and middle-aged, but not older, males and females (Freedman et al. 2006). This observation may reflect weight loss associated with illness in later stages of life. Therefore, death rates are higher in thinner people, a phenomenon known as reverse causation. (Kopelman, 2000). Obesity on average reduces life expectancy by 6–7 years (Fontaine et al. 2003). Severe obesity (BMI >40) reduces life expectancy by 20 years for men and 5 years for women (Haslam et al. 2005). According to the Framingham Study, a 2% increase in the death rate occurred with each extra pound of weight gained between ages of 50 to 62 years (Hubert et al. 1986).
Associations between obesity and type 2 diabetes are consistent in the literature. The risk of developing type 2 diabetes is increased 10-fold for obese women and 11.2-fold for obese men (Field et al. 2001). In the Nurses Health Study, BMI was an independent risk factor for diabetes (Colditz et al. 1995). Compared with women with stable weight the relative risk for diabetes mellitus among women who had a weight gain of 5.0 to 7.9 kg was 1.9, 95% CI, 1.5 to 2.3. The corresponding relative risk for women who gained 8.0 to 10.9 kg was 2.7, 95% CI, 2.1 to 3.3. Abdominal obesity appears to represent an increased diabetic risk with waist circumference over 40 inches producing a 3.5-fold increase risk of diabetes (Seidell et al. 1997). Obesity is also associated with “metabolic syndrome”, a combination of maladies including hypertension, insulin resistance, dyslipemia and atherosclerosis which are all risk factors for cardiovascular disease. Accordingly, obesity may cause up to 12% of heart failure cases in North America (Kenchaiah et al. 2004)

**Metabolic Syndrome**

Metabolic syndrome is recognized as a cluster of risk factors associated with type 2 diabetes and cardiovascular disease (Alberti et al. 2005). It affects a large number of people, and prevalence increases with age. Some studies estimate the prevalence in the US to be up to 25% of the population (Alberti et al. 2005). Central obesity is a major constituent of metabolic syndrome. The National Cholesterol Education Program (NCEP) definition of metabolic syndrome requires the presence of abdominal obesity (waist circumference > 88 cm or BMI >30 kg/m²) along with 2 or more of the following factors, 1) triglycerides > 150 mg/dL; 2) decreased serum high-density lipoprotein (HDL)
cholesterol (< 50 mg/dL); 3) systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg; 4) fasting plasma glucose > 110 mg/dL (NCEP, 2001).
OBESITY, DIABETES AND SYSTEMIC INFLAMMATION

The adipose tissue (fat) in the body is either stored subcutaneously or around organs. It is only in recent years that adipose tissue has been recognized as a complex secretory organ participating in physiologic and pathologic processes, including immunity and inflammation. Of the cells in adipose tissue, adipocytes are the most abundant and secrete a variety of bioactive molecules, known collectively as “adipokines”. These protein molecules either act locally or are released systemically where they function as signaling molecules to other tissues and organs (Trayhurn & Wood, 2004). The most studied adipokines, leptin and adiponectin have a number of functions within the body. Leptin, which is predominately secreted from adipocytes assists in the maintenance of energy expenditure by decreasing appetite or increasing metabolism (Friedman, 2000). Leptin acts through the hypothalamus of the central nervous system where its receptors are highly expressed. Leptin levels are vital in regulating body mass, imitating some of the actions of insulin (by altering glucose uptake in muscle and fat cells) and lowering glucose production in the liver (Matsuzawa, 2005). It appears that obese persons have elevated leptin levels that do not suppress appetite. This leptin resistance may contribute to pathological processes’ associated with obesity (Blüher et al. 2009). Leptin resistance has been associated clinically with hypertension, atherosclerosis and cardiovascular disease (Reilly et al. 2004). Other than appetite, immunological functions of leptin include stimulation of cytokines and increases
macrophage phagocytosis (Torpy et al. 1998). Leptin has also been shown to protect T lymphocytes from apoptosis and regulate T-cell proliferation and activation (Farooqi et al. 2002).

Another adipokine, adiponectin is secreted from adipose tissue into the systemic circulation, but interestingly, levels are decreased in obese subjects. The actions of adiponectin within the body include glucose maintenance, insulin sensitivity and fatty acid breakdown. Adiponectin possesses anti-inflammatory properties. It has been shown to inhibit interleukin-6 (IL-6) production, and induce release of interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) (Ouchi et al. 2003). Adiponectin also reduces induction of intracellular adhesion molecule -1 (ICAM-) and vascular intracellular adhesion molecule (VCAM) which helps protect against hypertension and atherosclerosis (Farooqi et al. 2002).

Type II diabetics also exhibit low serum levels of adiponectin. It is hypothesized that TNF-α production, which is increased in obese subjects, leads to reduction in circulating adiponectin levels (Fantuzzi, 2005). Low levels of adiponectin are also associated with an increased risk of CVD and other features of the metabolic syndrome (Kumada et al. 2003).

Adipose tissue of obese individuals contains an increased number of macrophages and these macrophages appear to be hyperactive in their amount of cytokine secretion. Macrophages in adipose tissue of obese individuals produce higher levels of TNF-α, IL-6, and chemokines compared with those in non-obese persons. IL-6 and TNF-α are consistently elevated in the serum and adipose tissue of obese subjects (Fantuzzi, 2005). Increased levels of IL-6 are a potential cause for the increase in acute-phase proteins,
such as C-reactive protein (CRP) observed in obese subjects. Elevated CRP levels (>3 mg/ml) in obese patients are associated with risk of progression to type 2 diabetes and increased risk of cardiovascular disease (Pradhan, 2007). The pro-coagulant properties of IL-6 may lead to elevated plasma concentrations of fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein which may explain the increased risk of cardiovascular events in obesity (Ridker et al. 2000). Increased levels of PAI-1 are associated with accumulation of adipose tissue especially in central obesity (Mavri et al. 2001). PAI-1 is produced by adipocytes and stromal cells and prevents the dissolution of the fibrin clot by inhibiting fibrinolysis. The development of type 2 diabetes and atherogenesis have been directly linked to the increased production of PAI-1 (De Taeye et al. 2005). TNF-α can directly lead to insulin resistance by inducing serine phosphorylation of the insulin receptor, which inhibits insulin signaling (Simons et al. 2005).

Abdominal obesity is associated with reduced uptake of insulin by the liver, increased hepatic glucose production (gluconeogenesis), release of free fatty acids (FFA), resulting in elevation of triglycerides, and insulin resistance (Fantuzzi et al. 2005). Increased blood glucose levels from ingestion of excess carbohydrates leads to formation of free fatty acids (via increased liver synthesis of free fatty acids) and the formation of triglycerides within adipocytes. Insulin secretion in response to increased carbohydrate levels also reduces lipolysis further increasing adiposity (Musso et al. 2009). Increased plasma FFAs lead to further increase in hepatic gluconeogenesis and increased peripheral insulin resistance with down regulation of insulin receptors. Elevated FFA’s have also
been shown to induce apoptosis of B-cells of the pancreas via de novo ceramide formation and increased nitric oxide production (Genco et al. 2005).

These elevations in blood glucose levels also induce oxidative stress, thus further compounding insulin resistance. Chronic hyperglycemia leads to the formation of advanced glycation end products (AGEs) by nonenzymatic glycation of proteins, which on binding to their surface receptor for AGEs (RAGEs), induce more ROS and proinflammatory events (Lindsey et al. 2009). Although research has not fully elucidated the exact mechanisms underlying obesity and systemic inflammation and diabetes it is likely that adipokines and cytokines produced by adipose tissue play a central role. The increased production of circulating inflammatory cytokines in obesity is hypothesized to alter the inflammatory response, a potential mechanism linking periodontitis and obesity.
OBESITY AND PERIODONTAL DISEASE

One of the earliest studies evaluating how obesity modified periodontal response in obese was conducted in obese-hypertensive rats (Perlstein et al. 1977). This study demonstrated that the obese rats were more likely to have periodontal disease than healthy rats. They concluded that the hypertrophy and hyperplasia of the walls of the blood vessels in the periodontium, could possibly alter the vascular, inflammatory and immune pathways. Since this initial animal study, several human cross sectional studies have elucidated a significant increase in periodontitis risk in obese individuals. One Japanese study showed that BMI and waist to hip ratio were positively associated with increased risk for periodontitis. Among the 241 healthy Japanese adults, a BMI $\geq 30$ correlated with an adjusted relative risk (RR) 8.6, 95% CI 1.4-51.4 (Saito et al. 1998). It also appears that upper body obesity, which increases risk of diabetes and CVD (Haslam et al. 2005) is also associated with periodontitis risk (Saito et al. 2001). For this study, 643 Japanese subjects were examined for obesity using BMI levels, body fat (DEXA), and waist to hip ratio. Analysis demonstrated significant risk for periodontitis only in those subjects with high BMI levels or increased body fat as well as high waist to hip ratios. These results suggest that the upper body fat accumulation is more closely related to risk for periodontitis. In the US population, analysis of the NHANES III data (Wood et al. 2003) showed correlations between body mass index, waist to hip ratio and periodontal indices such as clinical attachment loss (CAL), probing depth (PD), gingival...
bleeding even after adjusting for age, gender, diabetes, smoking and socioeconomic status. WHR has the highest F ratio (F=253.32), followed by BMI (F=19.651) however, subcutaneous fat was not significant, suggesting distribution of fat may be a important factor in the association of obesity with periodontal disease.

More recently, Dalla Vecchia et al. 2005 investigated the association between overweight/obesity and periodontal status in a representative population in southern Brazil. The 706 subjects were 30 to 65 year old and were defined as having periodontitis if ≥30% of teeth present had CAL ≥5mm. Obesity (BMI>30 kg/m²) significantly correlated with periodontitis for females only of which 80% were more likely to develop periodontitis. Obese non-smoking females were 3.4 times more likely to be diagnosed with periodontitis than normal weight non-smoking females. These results were postulated to occur because of the proportion and distribution of fat and muscle among males and females. This study potentially highlights the need for additional methods for diagnosing obesity other than BMI.

Recently, Khader et al. 2009 looked at the effect of obesity on prevalence of periodontitis in a group of Jordanian patients. The results of this study revealed prevalence of periodontitis in 14% of normal weight subjects, whereas 29.6% of overweight and 51.9% and obese subjects had periodontitis. In this analysis increased waist circumference and high waist-to-hip ratio were more strongly associated with periodontitis risk than BMI. Torrungruang et al. (2005) indicated that BMI was not associated with CAL among a Thai population 50 to 73 years old, however they concluded that older age diluted the association of obesity and periodontitis due to potential confounders, such as increased systemic disease and the high number of
edentulous patients in this group. Alabdulkarim et al. 2005 showed that, in a case control study, alveolar bone loss is related to obesity (BMI ≥ 30) before the age of 40, but not when subjects are 40 years and older.

**Table. 2** Summary of a number of studies demonstrating positive association of obesity and periodontal disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Type</th>
<th>Subjects</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saito et al. 1998</td>
<td>Cross-sectional</td>
<td>241 healthy Japanese adults</td>
<td>BMI ≥ 30 = OR 8.6</td>
</tr>
<tr>
<td>Nishimura et al. 2000</td>
<td>Case-series</td>
<td>N = 79 Subjects with NIDDM</td>
<td>BMI was associated with periodontitis in NIDDM</td>
</tr>
<tr>
<td>Saito et al. 2001</td>
<td>Cross-sectional Convenience</td>
<td>N = 643 (512 females, 131 males) 19–79 years</td>
<td>WHR, BMI, and body fat were associated with periodontitis</td>
</tr>
<tr>
<td>Wood et al. 2003</td>
<td>Cross-sectional</td>
<td>NHANES III N = 8842</td>
<td>WHR and BMI associated with CAL</td>
</tr>
<tr>
<td>Al-Zahrani et al. 2005</td>
<td>Cross-sectional</td>
<td>NHANES III N = 13665 18–90 years</td>
<td>BMI &gt; 30 and high waist associated with periodontitis especially in younger adults (18–34 years)</td>
</tr>
<tr>
<td>Buhlin et al. 2003</td>
<td>Case-control N ¼ 96</td>
<td>50 periodontitis and 46 periodontally healthy subjects, 36–70 years</td>
<td>BMI &gt; 26 in men and &gt;25 in women was associated with periodontitis</td>
</tr>
<tr>
<td>Nishida et al. 2005</td>
<td>Cross-sectional</td>
<td>N = 372, 20–59 years</td>
<td>BMI &gt; 26 was associated with periodontitis</td>
</tr>
</tbody>
</table>

*Adopted from Saito et al. 2007

Al-Zahrani et al. 2003 examined the relationship between BMI, waist circumference and periodontal disease in a representative United States sample (NHANES III). The results were significant only for younger subjects with obesity (BMI≥30) being associated with greater risk for periodontitis (OR = 1.76, 95% CI, 1.187-2.612). They also found that high WC and being of young age had an OR of 2.27 (95% CI, 1.48- 3.48). These results again emphasize the influence of fat distribution in the
analysis and also, older age may potentially confound the relationship between obesity and periodontitis. The increase in other medical conditions and poly-pharmacy associated with aging may detract from the relationship (Al-Zahrani et al. 2005).

Linden et al. (2007) investigated the association between obesity and periodontal status among Northern Irish men 60 to 70 years old. This study evaluated whether high body mass index (BMI) in early life predicted poor periodontal status in later life. Of the 2748 subjects obesity was associated with a 77% increase in prevalence of periodontitis defined as least two teeth with >6mm CAL loss and PD ≥ 5mm. This cohort study showed that weight gain (≥30%) during adulthood increased risk for periodontitis, but BMI at 21 years old had no effect on periodontitis risk. In contrast, Reeves et al. 2006 recently analyzed the NHANES III data set using a sample of non-smokers ranging in age from 13-21 years old. Of the 2,452 in the cohort, adolescents aged 17-21 years old had increased risk of periodontitis per 1 kg increase in body weight (adjusted OR 1.06, 95% CI 1.01-1.09).

Obesity is associated with various health related choices that impact periodontal status (e.g., diabetes, oral hygiene and frequency of maintenance visits) (Al Zahrani et al. 2005). The association between obesity and periodontal disease has also been shown to be independent of type 2 diabetic status (Saito et al. 2005). Although obesity in itself has been independently associated with periodontitis risk, a model linking obesity, diabetes and periodontal disease has been recognized. Genco et al. 2005 using again a large sample from NHANES III (12,367 subjects) were able to demonstrate that obese individuals (BMI>30 kg/m²) had statistically significant increased severity of attachment loss (P<0.001). The authors concluded that obesity was linked with insulin resistance and
associated with high plasma TNF-α level which produced a hyperinflammatory state leading to increased risk of periodontitis. However, Saito et al. 2005 analyzed 584 Japanese women for obesity, glucose tolerance and periodontitis. This study established associations for obesity and deep probing depths (OR = 4.3, 95% 2.1-8.9), however, neither impaired glucose tolerance nor diabetes were significantly associated with deep probing depths. This association has also been observed in children. A case control study by Lalla et al. (2006) using 182 children with type 1 diabetes and 180 healthy controls found that within this group of six to 18 year old diabetics, BMI was significantly correlated with destruction of connective tissue and bone, but duration of diabetes and mean hemoglobin A1c (HbA1c) were not.

A recent study (Haffajee et al. 2009) with analysis of 574 subjects using logistic regression analysis demonstrated OR of 2.3 (95% CI 1.2-4.5), for an obese individual to exhibit periodontitis after adjustment for age, gender and smoking status. Only *Tannerella forsythia* was found in elevated levels in the biofilm of obese individuals with healthy periodontal tissues or gingivitis. This may reflect an overgrowth of this organism that might increase initiation and progression of periodontitis. Offenbacher et al. 2009 when analyzing their data related to the Periodontitis and Vascular Events (PAVE) pilot study discovered that reduction in serum high sensitivity C- reactive protein hs-CRP levels were reduced after preventative or periodontal therapy as compared to the no treatment group. However, within this group of 303 subjects who had periodontitis and recent history of cardiovascular event, when obesity was considered in the adjusted odds ratio, treatment had no effect on reduction of hs-CRP levels. This study demonstrates the
impact of obesity on systemic inflammation and need for not only periodontal intervention but also obesity counseling to reduce cardiovascular risk.
BIOLOGICAL PLAUSABILITY

Obesity is a risk factor for numerous chronic inflammatory diseases such as type 2 diabetes, arteriosclerosis and cholelithiasis (Haslam et al. 2005). The potential observational evidence for obesity to increase the risk for periodontitis, another chronic inflammatory disease has been discussed above. The proposed mechanisms underlying this association are not fully elucidated at present but some lines of evidence exist relating the pathogenesis of these diseases. From early animal experiments it was shown that obese rats were more likely to develop periodontitis than normal rats (Perlstein et al. 1977). This group histologically demonstrated the increased thickening of capillaries of the periodontium, causing reduced blood flow and reduction of influx of PMNs for defense against periodontal pathogens. Obesity also leads to elevated levels of the pro-coagulant PAI-1 which may further decrease blood flow in the periodontium. (Mavri et al. 2001).

Obesity is commonly associated high circulating free fatty acids levels which have been shown to directly cause proliferation of the junctional epithelium and bone loss in rat periodontitis lesions (Tomofuji et al. 2005). As mentioned earlier, adipose tissue, especially visceral adipose tissue, is an important organ that produces several systemically active substances known as adipocytokines. The secretion of these cytokines may affect periodontal tissues. Tumor necrosis factor-α (TNF-α) is secreted directly from adipose tissue and may perpetuate periodontal disease progression. One study analyzed
gingival crevicular fluid levels of tumor necrosis factor-α in young subjects (Lundin et al. 2004). This study pointed to a correlation between TNF-α production and BMI ≥ 40. Although the subjects did not have periodontal destruction, it was hypothesized that TNF-α secreted from adipose tissue in young adults may influence the periodontal status in later life. Some studies have hypothesized that the association of obesity with periodontitis is inextricably linked with insulin resistance and diabetes. (Genco et al. 2005). This group postulates that obesity leads to increased TNF-α production from adipocytes which inhibit insulin signaling leading to insulin resistance. This altered insulin resistance eventually culminates in diabetic associated hyperinflammatory state which is further antagonized by chronically increased circulating FFAs in obesity which modulate production of TNF-α and IL-10 (Bradley et al. 2008). It is hypothesized that this priming of the inflammatory response that causes the immune system to exhibit an exaggerated immune reaction to periodontal pathogens. As already mentioned there is evidence for the independent association of obesity with periodontitis (Saito et al. 2005) and this may be due to the fact that obesity itself produces enough proinflammatory cytokines to tip the balance in favor of hyperreponsiveness to periodontal bacteria. Proinflammatory cytokines play essential roles in the inflammatory reaction in periodontal disease. This influence on surrounding cells and stimulation of lymphocytes culminates in a cascade of events leading to upregulation of osteoclasts and bone resorption via the receptor activator of nuclear factor-kappa B (RANK)–RANK ligand (RANKL)–osteoprotegerin (OPG) axis (Cochran, 2008).

The adipokine leptin stimulates the immune system by increasing cytokine production and phagocytosis by macrophages and increasing oxidative stress (Fantuzzi et
al. 2005). In obesity leptin upregulates adhesion molecules on endothelial cells, leading to transmigration of monocytes and therefore increased numbers of macrophages. The macrophages associated with adipose tissue produce increased levels of TNF-α and IL-6 and receptors which could lead to a lead to a hyperinflammatory state increasing the risk of periodontitis (Nishimura et al. 2001). The adipokines have also been shown to induce reactive oxygen species (ROS) production leading to upregulation of redox-sensitive gene transcription factors, such as nuclear factor–kappa beta (NF-κB) and activating protein-1 (AP-1) further enhancing inflammatory cascades and oxidative stress (Chapple & Matthews, 2007a).

Although reports consistently point to an association between obesity and periodontitis, more longitudinal randomized controlled studies are necessary to further elucidate the complex role of obesity in periodontitis. However, due to increased risks for diseases associated with obesity and the potential impact on periodontal tissues, counseling on obesity is recommended as an important component of patient management in the periodontal office. Management and education of obese patients may necessitate provision of nutritional information which may also impact periodontal health (Chapple, 2009).
Components of a balanced healthy lifestyle usually include maintaining an average weight, participating in an appropriate exercise routine, and consuming a balanced healthy diet (Al-Zahrani et al. 2005). These lifestyle choices have been shown to help reduce the risk for developing several chronic diseases such as coronary heart disease and stroke (DHHS, 2000). As already discussed research has shown that obesity can be significantly associated with increased risk of systemic (Kopelman et al. 2000) and periodontal disease (Saito et al. 2001). However, until recently interest on the effects of nutrition in the periodontal literature has been minimal. Revival of interest in the association between periodontitis and malnutrition has been largely mitigated through the early hypothesis that deficiencies in certain micronutrients may lead to increased oxidative stress and a decrease in the body’s ability to combat infection. A significant association between poor overall diet quality and higher periodontitis prevalence has been recently reported (Al-Zahrani et al. 2005).

Due to the multifactorial etiology of the disease, it can be difficult to find direct associations between risk factors and periodontitis. Problems are further increased with a potential risk factor like nutrition due to conflicting opinions on what constitutes an appropriate balanced diet, the use of self-reported diet analysis in epidemiological studies and complications in assessing micronutrient deficiencies among individuals. These issues were present in earlier studies, which reported conflicting results regarding
associations between several individual micronutrients and prevalence of periodontitis (Slade et al. 1976, Jacob et al. 1987). Dietary questionnaires have been found to have weak associations with serum biochemistry levels of micronutrients and with a lack of intervention studies, our ability to draw any solid conclusions on causality have been limited (Knutsen et al. 2001).

What Constitutes a Balanced Diet?

Kennedy et al. (1995) developed the healthy eating index which is a useful tool in assessing calorie intake with correct proportions of carbohydrates, proteins and fats as well as micronutrient and vitamin consumption. Although the nutritional value of these recommendations are adequate for most individuals, small variations in micronutrients such as vitamins and minerals may have an effect on periodontal tissues (Chapple, 2009). Much of the basic research thus far has tested nutrient mechanisms in chronic disease processes other than in periodontal disease; hence, there is a need to consider the influence of nutrient mechanisms and inflammation associated with periodontal disease.

Dietary choices are usually based on price, ease of preparation and taste with frank disregard to supplying nutrients to the body (Howarth et al. 2007). Although malnutrition will most likely result in macro- and micro-nutrient deficiency, it is also important to note that obese individuals are more likely to consume a diet high in fatty acids and refined carbohydrates and lacking essential vitamins and antioxidants (Mann, 2002). Using data from the National Health Interview Survey it was estimated that on any given day, nearly 50% of the population did not eat any fruit, 80% consumed no vegetables, and most diets were low in vitamin A and C rich foods as well as dietary fiber.
(Patterson et al. 1995). Based on these findings it is likely that periodontal patients are likely to be making poor dietary choices.
The mechanism underlying the association between diet and periodontitis could be related to the local and/or systemic effects of nutrition on periodontal health. The type of food consumed has been linked to the development and survival of plaque biofilm, by providing a direct nutrient source or by altering its surrounding environment (Bowden et al. 1997). Also a diet composed of course, firm foods (i.e., nuts and vegetables) has been suggested to reduce plaque accumulation, while a softer diet may promote plaque accumulation and therefore contribute to the periodontal disease progression (Newman et al. 1974). Nutrition is acknowledged to have a significant impact on optimal functioning of the immune response (Boyd et al. 2003).

Malnutrition is characterized by depletion of antioxidants, immune dysfunction, reduction of T lymphocytes and hormonal disturbances with increased cortisol levels in blood and saliva (Hughes et al. 2008). Periodontal infections may be adversely affected by malnutrition with alterations in the oral microbial flora as well as reductions in saliva production and its antibacterial components (Enwonwu, 1995). Studies linking nutritional status and periodontitis have largely focused on a number of micronutrients including the Ascorbic acid (vitamin C), vitamin B-complex, and calcium levels.

Of the vitamins, ascorbic acid has received the majority of attention largely due to its historical association with scurvy, which has pronounced effects on the oral cavity (Fain et al. 1998). Other forms of periodontal destruction have been noted with the
reduction of vitamin C intake. Melnick et al. (1988) examined 60 necrotizing ulcerative gingivitis (NUG) patients comparing them to 60 matched controls. Those patients with a history of NUG had a statistically significant reduction in ingestion and plasma levels of ascorbic acid. Initial studies on vitamin C deficiency in Guinea pigs were carried out by Glickman, (1948). Histology revealed that animals on a vitamin C deficient diet for 30 days developed deeper probing depths, edema and hemorrhage in the periodontal tissues. Lack of Vitamin C was proposed to cause a reduction in connective tissue, inflammatory cells, and inhibit fibroblast proliferation. A study conducted by Waerhaug, (1958) on vitamin C-deficient monkeys demonstrated increased osteoclastic activity leading to increased alveolar bone resorption.

**Table 3.** Studies showing positive associations with nutritional deficiencies and periodontal disease

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Type</th>
<th>Nutrient Deficiency</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glickman et al. 1948</td>
<td>Animal; Guinea Pig</td>
<td>Vitamin C (none-30 days)</td>
<td>Increased periodontal inflammation and destruction</td>
</tr>
<tr>
<td>Vogel et al. 1979</td>
<td>Cross sectional, 35 periodontitis subjects matched with 1222 controls</td>
<td>Vitamin C (4 day diet analysis)</td>
<td>Periodontitis patients had significantly lower vit. C consumption</td>
</tr>
<tr>
<td>Nishida et al. 2000</td>
<td>Cross-sectional using 12,976 from NHANES III</td>
<td>Vitamin C (24hr diet analysis) and periodontal exam</td>
<td>Low vit. C levels showed increased periodontitis OR 1.19, 95% CI 1.05-1.33</td>
</tr>
<tr>
<td>Dreizen et al. 1977</td>
<td>Animal; Monkeys</td>
<td>Vitamin B3 (diet deficient)</td>
<td>Animals developed NUG and NUP</td>
</tr>
<tr>
<td>Oliver et al. 1969</td>
<td>Animals; Rats</td>
<td>Calcium deficient</td>
<td>Osteoporotic alveolar bone changes and reduction in periodontal ligament fibers</td>
</tr>
<tr>
<td>Nishida et al. 2000</td>
<td>Cross Sectional</td>
<td>Calcium (24hr dietary recall)</td>
<td>Statistically significant, relationship to periodontitis (OR: 1.84–1.99)</td>
</tr>
</tbody>
</table>
One early study (Vogel et al. 1979) analyzed United States data from NHANES I to discern if nutritional status had an impact on periodontitis risk. A four day diet analysis was conducted on a small sample of 35 patients (19 females 16 males) between 22 to 59 years old and presenting with generalized moderate to severe periodontitis. They calculated the amount of calories carbohydrates, fat, protein, sodium, calcium, phosphorus, thiamine, niacin, folic acid, fluoride, cholesterol, vitamins A, C, E, and B12 consumed by periodontitis subjects and compared these with 1,222 individuals of the general population without periodontitis. Results demonstrated significantly lower levels of riboflavin and vitamin C consumption by the periodontitis subjects compared to the general population but levels were above the recommended daily allowance. Interestingly, a large percentage of periodontitis subjects showed deficiency in calcium, magnesium, iron, vitamin E and folic acid. Ismail et al. (1983) also found a weak association between periodontal disease and vitamin C deficiency in the analysis of nutritional and periodontal health data collected from a representative sample of the United States. This study also found that intake of ascorbic acid in amounts larger than those recommended by the dietary standards does not seem to be associated with better periodontal health.

More recently, Nishida et al. (2000a) utilized NHANES III data to assess the association between vitamin C consumption and periodontitis. Analysis of over 12,000 subjects using a 24-hour dietary recall but without information on supplement use was conducted to determine the effect of low vitamin C on periodontitis risk. The results showed an inverse relationship with vitamin C intake and periodontitis risk (OR 1.19, 95% CI 1.05 to 1.33). Current and former tobacco users who were taking less dietary
vitamin C showed an increased risk of periodontal disease with an OR of 1.28, 95% CI 1.04 - 1.59 for former smokers and an OR of 1.21, 95% CI 1.02 - 1.43 for current tobacco users.

Studies evaluating the role of vitamin C on periodontal status appear to indicate that a weak, but statistically significant effect may be present. In contrast, studies evaluating the effects of vitamin C supplementation on the response to periodontal therapy have largely failed to show any preventable benefit or strong association between such supplements and clinically relevant improvements in therapeutic results. Woolfe et al. (1984) found that the use of supplemental doses greater than the RDA of vitamin C in normal human subjects does not have a predictable or strong effect on the gingival response to initial therapy. Similarly, ascorbic acid supplementation did not influence PMN chemotaxis or responses to experimental gingivitis (Vogel et al. 1986). The role of Vitamin C in protection from periodontitis may be related to antioxidant properties which can neutralize free radicals associated with increased oxidative stress in periodontitis subjects. Vitamin C has also been shown to suppress macrophage production of free radicals and is a primary cofactor in collagen synthesis as seen with scurvy, an ulcerative condition of the gingival tissues under conditions of severe vitamin C deficiency (Fain et al. 1998). Studies of vitamin C and periodontal disease have produced mixed results. Although studies have not shown a clear relation between plasma ascorbate levels and inflammatory periodontitis, this epidemiologic evidence of vitamin C intake and periodontal disease, especially among smokers, may be of significance and warrant further prospective randomized controlled trials.
Vitamin B₃ (niacin) deficiency in humans is associated with a disease known as pellagra, which is endemic in regions such as Africa, China and Mexico. It manifests with dementia, diarrhea, dermatitis and eventually death. Although oral manifestations are usually confined to the tongue (glossitis), Dreizen et al. (1977) observed the periodontal effects of Vitamin B₃ deficiency in monkeys. The results showed that the animals developed a syndrome similar to pellagra as well as stomatitis. The stomatitis also produced a necrotizing gingivitis and periodontitis and an ulcerative and atrophic glossitis. Pack et al. (1984) using a double-blind study to determine the effects of a folate mouthwash on gingivitis in adults showed that the mouthwash improved gingival health topically rather than systemically. The same group however (Pack et al. 1986) showed no statistically significant reduction of experimental gingivitis with a 0.1% vitamin B-complex mouthwash. Intuitively, more longitudinal studies are necessary to demonstrate prolonged affects of B-complex vitamins on gingival and periodontal health.

Calcium and vitamin D deficiencies have been evaluated with respect to effects on the periodontal disease. Initial animal experiments involving rats found a reduction in the amount of periodontal ligament fibers along with reduction in alveolar bone density when animals where fed a diet deficient of calcium and vitamin D (Oliver et al. 1969). When dietary calcium intake was analyzed Using the NHANES III data, it was estimated that low calcium intake was associated with increased periodontitis with an odds ratio of OR 1.84, 95% CI 1.36 to 2.48 for young males, OR 1.99, 95% CI 1.34 to OR 2.97 for young females, and 1.90, 95% CI 1.41 to 2.55 for the older males (Nishida et al. 2000b). A longitudinal study demonstrated decreased tooth loss in subjects receiving supplemental calcium and vitamin D over 5 years (Krall et al. 2001). However
supplemental calcium did not have any effect on periodontal indices in patients with untreated periodontal disease (Uhrbom et al. 1984).

The results of these studies seem to suggest that low dietary intake of calcium may result in increased risk for periodontal disease but that the effects of taking dietary supplemental calcium on arresting periodontal disease or as adjunctive aid in its treatment have not been thoroughly evaluated.
NUTRITION, ANTIOXIDANTS AND INFLAMMATION

Although initiated by plaque biofilm the majority of the destruction seen in periodontitis is mediated through an inflammatory immune response (Offenbacher, 1996). The causes of this hyperinflammatory state are multifactorial and at present not fully understood. It is possible that dietary constituents or deficiencies may alter the hyperinflammatory phenotype causing a shift in the balance towards a proinflammatory or anti-inflammatory response.

The initial host defense against periodontal pathogens comes from the polymorphonuclear leukocytes (PMNs) (Kornman, 2008). After migration through the junctional epithelium, PMNs, prompted by bacterial antigens produce reactive oxygen species (ROS) during phagocytosis such as superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) (Miyasaki, 1991). Not only are these free radicals released into the phagosome, but are also emitted into the extracellular matrix. These ROS contribute to tissue destruction by causing lipid peroxidation and upregulation of inflammatory cytokines by monocytes and macrophages through activation of the transcription factor nuclear factor κB (Chapple et al. 2007a). It has been demonstrated that adult periodontitis patients generate higher levels of superoxide in their gingival fluid than healthy controls (Guarnieri et al. 1991).

Antioxidants are molecules designed to limit oxidation reactions which transfer electrons to an oxidizing agent. Antioxidants interact with each other and with other metabolites either independently or synergistically (Knight et al. 1998). It is therefore
difficult to ascertain the exact role of individual antioxidants as each may depend on the function of other members of the group. This leads to controversy when trying to determine the effects of depletion of individual antioxidants on periodontal inflammation. Most research has therefore focused on the relationship of periodontal disease and total plasma antioxidant concentrations. Chapple et al. (2007b) demonstrated that reduced levels of total plasma antioxidants and vitamin C resulted in increasing prevalence of periodontitis.

Several studies have demonstrated a correlation between ROS and periodontal disease activity (Marton et al. 1993, Schmidt et al. 1996). Antioxidants reduce damage caused by ROS by removing free radicals, seizing transition metal ions, and inducing oxidation of other molecules (Linke et al. 2005). Well known antioxidants include vitamin C, vitamin E (tocopherol), carotenoids, and reduced glutathione. Vitamin C is a powerful scavenger of free radicals and protects against oxidants in cigarette smoke (Chapple et al. 2007a). Vitamin E stops the free radical reactions and stabilizes membranes but due to limited mobility, it may have reduced antioxidant ability. Studies have found that vitamin E may reduce periodontal disease and associated breakdown of collagen fibers (Ritchie & Kinane, 2003, Battino et al. 1999).

People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, have been shown to have lower mortality rates and suffer less chronic disease (Diplock, 1998). Carotenoids function as radical-trapping antioxidants. Other than Papillon-Lefevre syndrome (PLS), very little research exists looking at the role of carotenoids in periodontitis. Recent evidence suggests that defects in polymorphonuclear leukocyte enzymes involved in oxidative burst are to blame for the syndrome (Noack et
al. 2008). The enzyme, cathepsin C, which is defective in the syndrome, plays a major role in the formation of ROS (Noack et al. 2008). Affected individuals present with lower antioxidant levels and very high superoxide concentrations, therefore it has been suggested that a specific antioxidant therapy could be a promising approach in treating some PLS subjects (Battino et al. 2001).

Omega-3 polyunsaturated fatty acids (ω-3PUFAs) found in fish oil also possesses anti-inflammatory properties. They have been shown to increase antioxidants and reduce bone resorption activity (Schubert et al. 2007). ω-3PUFAs have been shown to reduce bone loss associated with experimental periodontitis with *Porphyromonas gingivalis* (Kesavalu et al. 2006). ω-3PUFAs supplements have been shown to reduce TNFα production by monocytes in patients who expressed high baseline production (Grimble et al. 2002). Serhan et al. (2008) have shown reduction of bone loss from experimental periodontitis in the rabbit model with the novel Use of lipid mediators termed resolvins and protectins derived from the ω-3PUFAs, Eicosapentanoic acid and Docosahexanoic acid. These resolvins and protectins stimulate resolution of inflammation by preventing neutrophil penetration, phagocytosing dead neutrophils, enhancing clearance of inflammation and promoting cellular regeneration (Van Dyke et al. 2008). Another study examined the effect of a botanical formulation, which included rose hips, a blueberry and blackberry mixture, and a grapevine extract (Kornman et al. 2007). Healthy adults with elevated C-reactive protein (CRP) who were either positive (IL-1+) or negative (IL-1-) for the at-risk IL-1 gene polymorphism randomly received the formulation or a placebo. After 12 weeks, IL-1β gene expression was significantly lower than at baseline and than
in placebo for IL-1+ and IL-1- subjects. IL-1+ subjects had achieved a statistically significant reduction in CRP with the botanical mixture than with placebo.
OBESITY, NUTRITION AND PERIODONTAL DISEASE

It is interesting to acknowledge that a diet high in saturated fats or refined carbohydrates commonly associated with obesity may also contribute to systemic inflammation due to formation of free fatty acids. As noted earlier FFA formation contributes to insulin resistance and diabetes, which may both impact on the hyperinflammatory response and potentially increase periodontal destruction (Genco et al. 2005). Diets composed of mainly processed foods high in refined carbohydrates and fats lead to increased blood glucose and fatty acids levels, which create ROS faster than antioxidants can be produced (Yamato et al. 2007). The resultant oxidative stress over time may result in chronic systemic inflammation. When glucose and fatty acids are chronically elevated the Krebs cycle and electron transport chain become overloaded. The excess electrons formed cause reduction of oxygen and superoxide formation with resultant elevations in ROS (O’Keefe et al. 2008). This dietary oxidative stress has been associated with increases in CRP and proinflammatory cytokines (Monnier et al. 2006, Bradley et al. 2008). Oxidative stress caused by dietary fats leads to lipid peroxidation with resultant formation of low density lipoproteins which become oxidized and bind to toll-like receptors on inflammatory cell membranes, triggering NF-κB activation (Chapple et al. 2007a). The combination of obesity mediated systemic inflammation and a decrease in antioxidant rich diet could further exacerbate the systemic inflammatory state. The oxidative stress and hyperinflammatory condition associated with these two risk factors may further predispose patients to increased risk of periodontitis by causing an exaggerated response to periodontal pathogens.
Figure 4. Model linking nutrition, obesity, systemic inflammation and periodontitis

Figure 4 depicts the potential deleterious effects of periodontal disease on systemic inflammation and oxidative stress. The immune responses elicited from exposure to periodontal pathogens have been well established (Gunsolley et al. 1990). Periodontal disease has been linked to numerous inflammatory conditions, such as diabetes (Genco et al. 2005), cardiovascular disease (Beck et al. 2005) and rheumatoid arthritis (de Pablo et al. 2008). The inflammatory burden caused by chronic periodontal disease is postulated to be one of the reasons leading to associations with systemic diseases. IgG immune responses to periodontal pathogens have had been associated with increased risk for CVD in a number of adult studies (Beck et al. 2005; Pussinen et al. 2004). The high prevalence of immune responses in children reported in the initial portion of document is cause for concern. The long-term exposure to these pathogens and
concomitant immune responses may have profound influence on development of inflammatory diseases such as CVD and diabetes.
SUMMARY AND CONCLUSIONS

Obesity and malnutrition have significant effects on inflammatory, immune and metabolic processes. Periodontitis may be exacerbated by the increase in systemic inflammatory burden caused by either of these two maladies. The dentist is in a prime location to counsel on risk factors for periodontitis which include educating patients on maintaining appropriate weight and encouraging a balanced nutritious diet. Reduction in both of these modifiable risk factors may have a beneficial effect not only on the patient’s periodontal health but on systemic health as well. The increased prevalence of obesity in children is a serious health concern. Poor nutritional habits, which are usually established in childhood, have the potential to further exacerbate systemic inflammation and oxidative stress. The long term exposure of this inflammatory burden, coupled with increased immune responses to periodontal pathogens in young children, as reported in the observational study at the beginning of this document, may further compound the issue.

Additional longitudinal research is needed to ascertain the role that obesity and nutrition play in the initiation and progression of periodontitis and the molecular mechanisms involved in its pathogenesis. In summary, nutrition factors in terms of nutrient intake and adiposity may play an important role in periodontal disease. Nutritional intervention studies in patients with inflammatory periodontal diseases are needed, and such interventions offer great potential as novel therapeutic strategies.
However, until more data becomes available from micronutritional intervention studies on periodontitis, advice should be limited to reducing obesity and encouragement of healthy balanced diets containing fruits, vegetables and fish oils.
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