Body Fat Indices and Biomarkers of Inflammation: A Cross-Sectional Study with Implications for Obesity and Peri-implant Oral Health

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**Purpose:** To examine the relationships between three measures of body fat—body mass index (BMI), waist circumference (WC), and total body fat percent—and markers of inflammation around dental implants in stable periodontal maintenance patients. **Materials and Methods:** Seventy-three subjects were enrolled in this cross-sectional assessment. The study visit consisted of a physical examination that included anthropologic measurements of body composition (BMI, WC, body fat %); introral assessments were performed (full-mouth plaque index, periodontal and peri-implant comprehensive examinations) and peri-implant sulcular fluid (PISF) was collected on the study implants. Levels of interleukin (IL)-1\(\alpha\), IL-1\(\beta\), IL-6, IL-8, IL-10, IL-12, IL-17, tumor necrosis factor-\(\alpha\), C-reactive protein, osteoprotegerin, leptin, and adiponectin in the PISF were measured using multiplex proteomic immunoassays. Correlation analysis with body fat measures was then performed using appropriate statistical methods. **Results:** After adjustments for covariates, regression analyses revealed statistically significant correlation between IL-1\(\beta\) in PISF and WC (\(R = 0.33\); \(P = .0047\)). **Conclusion:** In this study in stable periodontal maintenance patients, a modest but statistically significant positive correlation was observed between the levels of IL-1\(\beta\), a major proinflammatory cytokine in PISF, and WC, a reliable measure of central obesity. Int J Oral Maxillofac Implants 2014;29:1429–1434 doi: 10.11607/jomi.3758

**Key words:** body composition, cytokines, dental implants, interleukins, obesity, peri-implantitis

Obesity is a growing social and medical problem throughout the world.\(^1\) In 2007, more than 1 billion people worldwide were considered overweight and close to 321 million were considered obese.\(^2\) Prospective studies have clearly shown that obese subjects have significantly higher risks of developing chronic systemic illnesses such as cardiovascular disease or diabetes.\(^3\) Chronic periodontitis, a host-mediated chronic inflammatory disease of specific bacterial origin that affects the attachment apparatus of teeth, is a highly prevalent condition estimated to affect close to 47% of the U.S. population.\(^4\) If left untreated, it progressively leads to a sustained systemic inflammatory burden that may affect the homeostasis of multiple systems.\(^5\) In addition, chronic systemic diseases, such as diabetes, may in turn have a detrimental effect on the oral tissues, including the periodontium.\(^6\)

Several animal and human cross-sectional studies have shed light on the association between obesity and chronic periodontitis. This association was later confirmed by longitudinal studies and further validated by recently published systematic reviews on this topic.\(^7\)–\(^9\) It is widely accepted that smoking and uncontrolled diabetes can influence treatment outcomes, and obesity has also been proposed as an
independent predictor of poor outcomes following nonsurgical periodontal therapy.\(^9\)

Obesity has been linked with an exacerbated systemic inflammatory status, induced either directly or via the release of adipokines from the adipose tissue, an altered microbial profile in obese individuals, or the increased free fatty acids that in turn increase insulin resistance.\(^{10\text{-}12}\) Although the scientific evidence linking obesity and periodontitis is mounting, to the current authors’ knowledge, there have been no studies exploring the impact of obesity on peri-implant health. The authors therefore hypothesized that obesity will have a negative influence on peri-implant health by inducing a proinflammatory response in the tissues surrounding and supporting functionally loaded implants. The specific aim of this cross-sectional study was to assess the relationship between body fat indices and the expression of molecular markers of inflammation around dental implants in a stable periodontal maintenance (recall) population.

**MATERIALS AND METHODS**

**Subject Identification and Recruitment**

Institutional review board approval was obtained from the University of Iowa's Human Subjects Office (#201109878). Subjects who were enrolled in a maintenance therapy program at the College of Dentistry and who had at least one moderately rough-surfaced implant in function for a minimum of 6 months were identified using electronic health records and contacted. To be eligible, subjects had to be 18 years or older, current nonsmokers, and enrolled in the collegiate periodontal maintenance program. Pregnant or nursing women, edentulous subjects, and subjects with blade-type or smooth-surface implants or who had taken medications known to alter oral inflammatory status or hormonal levels for 3 months prior to the study visit were excluded. Subjects with a history of aggressive periodontitis were also excluded from the study.

**Body Composition and Systemic Evaluation**

On the day of the study visit, height (in meters), weight (in kilograms), and waist circumference (WC, in centimeters) were measured and recorded. Body mass index (BMI) was calculated using the Quetelet Index (weight [kg]/height [meter]\(^2\)). Based on the BMI, subjects were categorized as underweight (BMI < 18.5), normal (BMI 18.5 to 24.99), overweight (BMI \(\geq\) 25.00 to 29.99), or obese (BMI \(\geq\) 30) for descriptive purposes. The WC of the subject was recorded using a measuring tape (RJL System) at the level of the umbilicus after exhalation, with the tape kept parallel to the floor (Fig 1a). Body fat content was measured noninvasively using a bioimpedance-based body fat analyzer (RJL System) (Figs 1b to 1d). The bioimpedance analyzer measures resistance, reactance, and phase angle for each subject. Along with age, height, weight, body frame, and daily activity parameters, total body fat percentage was calculated using Body Composition Analysis Software (RJL System). The bioimpedance analyzer measures body water content, which is used to estimate fat-free mass, which, when subtracted from the subject’s total weight, yields an estimate of fat mass.\(^{13}\) During the same visit, fasting blood glucose level was measured using a point-of-care glucometer (One Touch Ultra 2 Blood Glucose Meter), and blood pressure was measured in duplicate on each arm while the subjects were seated; systolic and diastolic blood pressures were averaged separately.

**Peri-implant Sulcular Fluid Collection and Analysis**

After the physical examination, peri-implant sulcular fluid (PISF) was collected noninvasively from the sulcus around the target dental implant using Periopaper strips (Oraflow) (Figs 1e and 1f). In patients with multiple implants, a maxillary implant was preferred over a mandibular implant and a posterior implant was preferred over an anterior implant, as implants placed in the posterior maxilla seem to have more biologic complications than implants at other sites.\(^{14}\) Briefly, after the subject rinsed the mouth and cotton rolls were placed in the buccal and lingual positions at the study site, the area was dried for 5 seconds with compressed air. The paper strip was gently placed into the mucosal crevices around the dental implant at four sites (mesiobuccal, distobuccal, mesiolingual, distolingual) for 30 seconds per site. This allowed the collection of a sufficient volume of PISF for analysis. After 30 seconds, the strips were removed and the volume of fluid collected in each strip was measured (Periotron, Oraflow). After it was confirmed that an adequate volume had been obtained, the four paper strips from each implant were pooled and transferred into labeled microfuge tubes and stored at \(-80°C\) for later use. At the time of the analysis, paper strips (per implant) were suspended in 0.01 mol/L sodium phosphate buffer (pH 7.2) prepared using pyrogen-free water containing protease inhibitors, and the concentrations (pg/30 seconds) of interleukin-1\(\alpha\) (IL-1\(\alpha\)), IL-1\(\beta\), IL-6, IL-8, IL-10, IL-12, IL-17, tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), C-reactive protein (CRP), osteoprotegerin (OPG), leptin, and adiponectin were measured using multiplexed fluorescent bead-based immunoassays (Millipore) in the Luminex 100 IS Instrument (Luminex). These biomarkers were selected on the basis of their relationship to inflammation, obesity, and osseous remodeling. All measurements were taken in
using Pearson correlations. Plots were generated, utilizing both raw and natural log-transformed values, to evaluate linearity. After identification of candidate cytokines, a linear model was fit separately for each cytokine at each site (control and implant) to evaluate the potential association with each obesity measure while controlling for age, whole-mouth plaque index, days since last cleaning, and brushing and flossing habits. Model selection was performed by backward elimination, retaining only those terms that were significant at the 5% level. The final models determined whether a significant relationship remained between cytokine level and obesity measure. Model assumptions were assessed using standard residual analyses, including evaluation of normality via the Shapiro-Wilk procedure.

RESULTS

Of the 341 invitations sent to potential subjects, 210 responded; of these, 88 subjects fulfilled the inclusion criteria and were scheduled for a study visit. Of the 88 subjects scheduled, body fat measurements, clinical examination, and sample collection were completed in 73 subjects. The descriptive data, including mean age, plaque index, and obesity measures, are displayed in Table 1. Of the 73 subjects who completed the study, 26 were obese and 47 were either overweight or normal. The mean values (and standard deviations) for triplicate and the median of the replicates was used in analyses.

Periodontal and Peri-implant Health Evaluation

Following collection of PISF, plaque levels were recorded using the modified Quigley-Hein Plaque Index, followed by a comprehensive periodontal and peri-implant examination. To reduce the chance of errors in data collection, one calibrated examiner (GAO) did the intraoral examinations for all study participants. For teeth, probing pocket depth, recession, presence or absence of bleeding on probing and suppuration at six points, as well as mobility, pain on percussion or palpation, and width of facial keratinized mucosa (KM) (if any), were assessed and recorded. The same parameters were recorded for dental implants, with the exception of recession, given the absence of a reliable landmark that could be utilized across different implant systems and restorative modalities. Subjects were questioned regarding their brushing and flossing frequency, time since last prophylaxis, past history of periodontal therapy, familial history of periodontal disease, any complications during or after implant placement, and whether bone grafting was performed prior to or simultaneously with implant placement.

Statistical Analyses

Bivariate correlational analyses were used to identify promising ($P < .10$) relationships between measures of obesity and natural log-transformed cytokine levels using Pearson correlations. Plots were generated, utilizing both raw and natural log-transformed values, to evaluate linearity. After identification of candidate cytokines, a linear model was fit separately for each cytokine at each site (control and implant) to evaluate the potential association with each obesity measure while controlling for age, whole-mouth plaque index, days since last cleaning, and brushing and flossing habits. Model selection was performed by backward elimination, retaining only those terms that were significant at the 5% level. The final models determined whether a significant relationship remained between cytokine level and obesity measure. Model assumptions were assessed using standard residual analyses, including evaluation of normality via the Shapiro-Wilk procedure.
BMI, WC, and total body fat % in the study population were 28.2 ± 4.8, 91.4 ± 15.5 mm, and 31.4% ± 7.8%, respectively. Analyses performed to evaluate the correlations among the different measures of obesity employed (BMI, WC, and fat %) indicated a strong correlation between each of the measures that reached statistical significance (P < .0001) (data not shown).

The concentrations of the biomarkers in PISF are shown in Table 2. After adjustment for covariates, the results of the statistical modeling indicated a modest but statistically significant positive correlation between WC and the levels of IL-1β in PISF (P < .05) (Fig 2). Additionally, of the covariates assessed, a statistically significant association was found between plaque index and the levels of IL-1α in PISF (P < .05). Interestingly, a statistically significant correlation was observed between age and IL-12 levels in PISF (P < .05) (Fig 3). Except for this age relationship with IL-12, in none of these models did any of the covariates other than body composition have a significant association with cytokine levels.

DISCUSSION

Obesity has reached epidemic proportions worldwide. In an adult population with stable periodontal maintenance therapy, this study has demonstrated for the first time a modest but statistically significant correlation between WC, a well-established measure of obesity, and IL-1β expression in the PISF. Apart from WC, no correlations were observed between the other measures of obesity, namely, BMI and body fat %, and the levels of any of the cytokines measured in the PISF.
With regard to the prognostic value of the levels of key mediators in the PISF in assessing peri-implant health, a recent study concluded that IL-1β, TNF-α, macrophage inflammatory protein-1α, and IL-8 in PISF could be reliable prognostic markers of early stages of peri-implant mucositis, a condition similar to gingivitis around natural teeth. This was later confirmed in another study that showed that the differential expression of biomarkers related to local inflammation and bone remodeling in PISF may help clinicians to differentiate peri-implant mucositis from healthy peri-implant tissue. Other studies demonstrated that inflammatory cytokines, such as IL-1α and IL-1β, are present in higher concentrations in sites with existing or progressing peri-implantitis, a condition that resembles periodontitis around implants, as compared to healthy sites. IL-1β is an important cytokine with physiologic and pathologic functions that acts as a gatekeeper of inflammation. Although the current findings indicate a significant correlation between WC and levels of IL-1β, the cross-sectional nature of this study does not allow further assessment of the potential relationship between the elevated levels of IL-1β and the initiation of peri-implant pathologies.

Of the several anthropometric measures of obesity, WC appears to be the most reliable measure of visceral fat content and a good predictor of obesity-related comorbidities, including mortality. In contrast, BMI has been shown to be inaccurate in athletes, older persons, and people of Asian descent. In addition, it is well known from the medical literature that visceral fat is more detrimental and contributes independently to mortality and that BMI does not capture the visceral fat content and distribution as accurately as WC. Oral hygiene habits, smoking, diabetes, and educational levels are possible confounders in the association between periodontitis and obesity. This study attempted to address as many of these as reasonably feasible. The subjects in this study were all nonsmokers at the time of the visit and were already participating in a maintenance recall program, which had a positive impact on oral hygiene, as illustrated by the average plaque index of this population (0.8 ± 0.5). The findings indicate that a large proportion of patients followed a strict oral hygiene regimen, since close to 74% of subjects reported brushing two or more times per day and 78% reported flossing at least once a day at the time of the visit. With regard to physical activity, the majority of patients participated in light to moderate daily activity (79%). The educational attainment of the subjects was not recorded. A large majority of the subjects had a normal fasting blood glucose level of less than 100 mg/dL (82%), while the rest exhibited prediabetic levels (100 to 125 mg/dL); none were diabetic (>126 mg/dL).

Other factors that could affect the expression of cytokines in PISF were the relative position of the implant shoulder in relation to the bone crest and the possible influence of the variation in the thickness of KM. A recently published study reported that the position of the implant-abutment microgap with regard to marginal bone level has a strong influence on the levels of cytokines, with more proinflammatory mediators present at sites where the microgap is closer to the bone. A limitation of this study is that the effect of microgap position in the final concentrations of biomarkers was not assessed. The decision not to include it in the analysis was made on the basis of the variety of implant systems and restorative modalities presented in the selected population, which would have added a high degree of heterogeneity to the analyses. Moreover, to minimize radiation, new radiographs were obtained only in subjects who had not had radiographs of the implant taken within the past 2 years. Some of the existing radiographs were not standardized and therefore could not be included in such analysis. Another anatomical factor that can influence cytokine levels is the width of KM (KMW). Interestingly, it has been reported that lower levels of TNF-α were detected around implants with at least 2 mm of KMW, compared to no KM. The current study did not assess the direct effect of KMW on the explored association, given the fact that approximately 92% of the included implant sites presented ≥ 2 mm of KM.

In this study, additionally, a statistically significant correlation was observed between IL-12 (p40) in PISF and the age of the subject. This is the first study in dentistry to report such a finding. IL-12 is a potent proinflammatory molecule that bridges innate immunity with adaptive immunity. Previous animal studies have indicated that IL-12 expression increases with age in both diabetic and insulin-treated nondiabetic rats. Another study found that IL-12 expression is increased in aged mice compared to younger mice, and this was shown to be a major player in the age-related dysregulation of several cytokines. A human study observed that chronic periodontitis patients presented higher concentrations of IL-12 in the gingival crevicular fluid in diseased sites. Based on these findings, the correlation observed here between age and the expression of IL-12 warrants further investigation.

Finally, it is worth noting that only one representative implant per subject was assessed; there is a possibility of overestimating or underestimating the overall peri-implant inflammatory status in individuals with multiple implants. The cross-sectional nature of this study precluded the assessment of causality in the explored association, which is another limitation. Hence, the authors acknowledge the need for prospective studies with larger study populations and selected
outcomes of interest. Future studies should take into account factors such as educational attainment, gingival index, KMW, and microgap position relative to the peri-implant marginal bone level. In addition, although invasive, it would be interesting to look at the association of histologic parameters/indices of obesity such as large adipocytes (%) with markers of peri-implant inflammation.

**CONCLUSION**

This study provides evidence that a modest but statistically significant correlation exists between waist circumference, a reliable marker of central obesity measuring visceral fat content, and the levels of interleukin-1β in peri-implant sulcular fluid obtained from patients in a periodontal maintenance program, after controlling for a majority of confounding factors.

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