Comparison of Alveolar Bone Loss, Alveolar Bone Density and Second Metacarpal Bone Density, Salivary and Gingival Crevicular Fluid Interleukin-6 Concentrations in Healthy Premenopausal and Postmenopausal Women on Estrogen Therapy

Charles F. Streckfus, Roger B. Johnson, Todd Nick, Audrey Tsao, and Michelle Tucci

Departments of Diagnostic Sciences, Health Sciences, and Orthopedic Surgery, University of Mississippi Medical Center, School of Dentistry, Jackson.

Background. Osteoporosis is an age-related metabolic bone disease characterized by decreased mass and increased susceptibility to fracture. The literature suggests a relationship between oral bone loss and skeletal osteoporosis; however, most studies have produced conflicting results. The purpose of this study was to determine if a relationship exists among alveolar bone loss, alveolar bone density, second metacarpal density, salivary and gingival crevicular fluid interleukin 6 (IL-6), and IL-8 concentrations in premenopausal and postmenopausal healthy women receiving estrogen therapy.

Methods. Twenty-eight healthy women (aged 23–78) were evaluated for this study. A vertical bitewing and hand radiographs were taken, and the subjects were evaluated for the presence of active periodontitis. The bitewing and hand radiographs were digitized, and measurements were made from the cemento-enamel junction to the alveolar crest from both arches. Bone density was evaluated in the maxillary and mandibular alveolar process and at the mid-shaft of the second metacarpal. Percent cortical area and the moment of inertia measurements were also determined. Stimulated whole saliva was collected for a 5-min period using a cube of paraffin as a stimulant and was analyzed for total protein by a colorimetric reaction and IL-6 and IL-8 by ELISA.

Results. The results of the study showed that postmenopausal women on estrogen therapy had more alveolar bone loss, more missing teeth, and reduced alveolar and second metacarpal bone density than premenopausal women. In addition, postmenopausal women on estrogen therapy had higher salivary IL-6 concentrations than premenopausal women. Alveolar bone densities were also strongly correlated to second metacarpal densities.

Conclusions. The results of the study suggest that changes in alveolar bone density and levels of bone resorptive cytokines in saliva may be secondary to changes in menopausal status. These changes may predispose loss of alveolar bone with resultant loss of teeth.
to find that independent investigators report conflicting results (4–10).

One might assume that loss of bone mass or decrease in bone mineral density would be evident throughout the body in patients with osteoporosis. However, studies relating oral bone to skeletal bone status have proved inconclusive and often yielded conflicting results. Elders et al. (6), in their study of 286 healthy postmenopausal women, found no relationship between missing teeth and density of the vertebral and metacarpal thickness. In contrast, Krall et al. (7), in a study of 329 healthy postmenopausal women, found a correlation between the number of missing teeth and lumbar vertebral and radial bone densities. In studies using women with a confirmed diagnosis of osteoporosis, Kribbs et al. (8) found correlations between densities of the vertebra and radius with the mandible, using microdensitometry technique to assess bone densities of the alveolar process. In addition, in another study by Kribbs et al. (9), women older than 50 years also demonstrated a correlation between mandibular and vertebral/radius bone mineral densities. Von Wowern et al. (10), in contrast, found no relationship between mandibular bone density and that of the radius and vertebra in 18 individuals diagnosed with nephritis.

The evaluation of the significance of oral bone mass to the diagnosis of osteoporosis measures is complicated by this variation in skeletal parameters. As a consequence, it may be necessary to facilitate specific biochemical markers associated with age and menopause in conjunction with radiographic and densiometric determinations. One such group of biochemical markers associated with age and menopause in bone is interleukin 6 (IL-6) (11–14).

Bone mass is regulated by systemic growth factors and hormones and by molecules produced within the adjacent tissue. Cytokines are important components of the tissue microenvironment, and several are reported to regulate development and activity of osteoblasts and osteoclasts (11–14). Precise coordination of bone deposition and resorption is required to maintain adequate bone mass. Lack of coordination of these processes results in metabolic bone disease. When the rate of bone resorption exceeds deposition, osteopenia may develop. Bone resorption occurs due to osteoclast activity. There are recent evidence from rodent studies to suggest a relationship between serum levels of estradiol and tissue levels of IL-6 on osteoclast development and activity and resultant bone resorption (12,13). IL-6 plays an important role in regulation of bone resorption due to its ability to stimulate osteoclast formation in bone marrow (14,15). Decreased 17-β estradiol levels result in upregulation of IL-6 production by bone marrow cells. This is due to the inhibition of IL-6 gene transcription through an estrogen receptor-mediated effect on the transcriptional activity of the proximal 225-bp sequence of the promoter. Thus, precise measurement of IL-6 levels could signal potential episodes of skeletal bone resorption (15).

The purpose of this study was to determine if radiographic determinations of alveolar bone mass and density, radiographic measurements of the second metacarpal, and salivary and gingival crevicular fluid concentrations of IL-6 are affected by menopausal status (premenopausal vs postmenopausal plus estrogen). The investigators also wanted to determine the possible association between these bone and biochemical parameters.

METHODS AND MATERIALS

Population

Twenty-eight healthy women of varying ages were evaluated for this study. These women were not taking medications, were of the same socioeconomic strata, and utilized comprehensive dental care on a regular basis at the University of Mississippi Medical Center School of Dentistry. The women were interviewed regarding their past dental history and were asked if they ever had periodontal disease. In addition, the subjects with missing teeth were asked how they lost their teeth. To the best of their knowledge and with the support of their dental records, the participants reported comprehensive care throughout their lives and had never experienced an episode of periodontal disease. To the best of their recollection, all teeth were lost due to either trauma or tooth decay and not periodontal disease. All subjects were nonsmokers and had at least one premolar and molar per quadrant.

Blood specimens for assay of 17-β estradiol levels were not available for this study. As a consequence, data concerning the participants' menopausal status were collected by interview. Menopausal status was defined after review of the subjects' answers to the medical history questionnaires and a review of their past medical records. The gynecological interview also included their past pharmacological, surgical, and medical histories. Premenopausal women were defined as having a regular menstrual cycle, with normal flow, and without any periods of amenorrhea. Postmenopausal status was defined by the absence of menses for 12 consecutive months or the cessation of menses by surgical intervention of hysterectomy and/or bilateral oophorectomy.

Height and weight determinations were made for all the participants. The mean body index (BMI) was calculated for each individual (BMI = weight/[height]²).

Oral Radiography

Intra-oral variables. — Alveolar bone loss (ABL) and alveolar bone densities (ABD) were measured from a vertical bitewing radiograph. The projection geometry was fixed using a cephalostat as described by Jeffcoat et al. (16). The film positioner also included an aluminum wedge (see Figures 1 and 3) for bone density determinations (17). The aforementioned techniques controlled for elongation and foreshortening, providing investigators with consistent radiographs suitable for computerized histomorphometry and densiometry (16).

ABL measurements were made from the cemento-enamel junction (CEJ) to the alveolar crest (Figure 1) by one calibrated examiner (C.F.S.). These measurements were taken from the mesial and distal aspects of each tooth. Teeth with restorations and/or decay that obliterated the CEJ were not measured. In addition, the teeth in each arch were measured and an average ABL was determined for the maxillary
(Mxabl) and mandibular (Mdabl) arch. A total ABL (TABL) for the entire oral cavity was also computed. The images were analyzed on a computer using PerioPro software (University of Alabama, Birmingham) and were expressed as millimeters. Alveolar bone measurements performed on standardized radiographs are accurate to ±0.2 mm (16).

ABD measurements were made using the SigmaProScan software (Jandel, San Rafael, CA). The interproximal bone (see Figure 1) was measured from alveolar crest to the borders of the periodontal ligaments to the base of the film. The area and density were recorded along with the area and density of the wedge. An average ABD was determined by combining all the measurements together for both the maxillary (MXDEN) and mandibular (MdDEN) arches. A total ABD (TABD) for the entire oral cavity was also computed. Densities were expressed as millimeters per aluminum.

**Periodontal clinical measurements.** — The presence of periodontal disease can be a confounding factor in any study regarding oral bone loss. Clinically, the periodontal health of an individual is determined by evidence of gingival bleeding, presence of supra- and sub-gingival calculus, and the loss of periodontal attachment and (most importantly) alveolar bone. Clinical measurements for the presence of periodontal disease included gingival and calculus assessments and probing pocket depths. The examination procedures used for these assessments were those as defined in the Oral Health Surveys of the National Institute of Dental Research Diagnostic Criteria and Procedures Manual (18) and were performed by one calibrated examiner (C.F.S.). Six sites (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual) on all the teeth were evaluated. To control for occult periodontal disease and confirm the visual findings of the periodontal exam, IL-8, a pro-inflammatory cytokine associated with periodontal disease, was also assayed in both the stimulated whole saliva and the gingival crevicular fluid (GCF).

**Radiographic measurements of the second metacarpal.** — All measurements were made on the second metacarpal bones of the nondominant hand (19) and were to be taken postero-anteriorly at an average exposure of 1.0 sec at 100 Ma and 60 KVP without intensifying screens as described by Garn (20). An aluminum wedge was simultaneously radiographed. The wedge permitted densiometric determinations of the second metacarpal (17).

Percent cortical area (PCA) and the cross-sectional moment of inertia (CMI) were determined as described by Garn (20), Fox et al. (21), Ward and Manson (22), and Roy et al. (19). All PCA and CMI determinations were made at the mid-shaft of the second metacarpal (19-22). The formula used for the calculation of PCA was as follows: PCA = [(total width$^2$ - medullary width$^2$)/total width$^2$] × 100. The formula used for the calculation of CMI was as follows: CMI = ($\pi/64$) (total width$^4$ - medullary width$^4$).

Second metacarpal density determinations were made at the midshaft of the second metacarpal (Figure 2). Density determinations were made on the ulna cortical bone (UCBDEN), the radial cortical bone (RCBDEN), and the medullary space (MBDEN). Measurements were 5 mm proximal and 5 mm distal from the mid-shaft (10 mm total length). Data were expressed as millimeters per aluminum.

**Specimen Collections**

**Gingival crevicular fluid sample collection.** — GCF specimens were taken from the mesiobuccal sites of the maxillary and mandibular premolars. These sites were selected because the premolars are the least likely teeth to
have periodontal disease and therefore would provide sites with bone loss possibly due to aging and/or menopausal status rather than periodontal disease (23). All teeth were assessed for plaque and gingival bleeding (18). Clinically detectable supragingival plaque was carefully removed without touching the gingiva that might stimulate fluid flow (24). This procedure minimizes contamination of the strip from plaque. The crevicular fluid was collected by placing 2 × 5 mm filter paper strips of Periopaper (Pro Flow, Amityville, NY) at the entrance to the gingival sulcus (24). The strip was held in place for 30 sec (24). On removal, the volume was determined by measurement in a capacitance flow meter, the Periotron 8000 (Pro Flow, Amityville, NY), and comparison to a calibration curve. The samples were frozen in liquid nitrogen prior to analysis. Results were expressed as microliters per 30 sec.

Saliva sample collection. — Stimulated whole saliva (SWS) collections were made as described by Navasesh and Christensen (25). A standard piece of paraffin (1.5 g) was placed in the subject’s mouth. The patient was asked to swallow any accumulated saliva and was then instructed to chew the wax at a regular rate and expectorate into a pre-weighed plastic cup. The samples were then weighed and the volume and physical characteristics recorded (25). Volumes were expressed as milliliters per minute.

Laboratory Techniques and Measurements

The two maxillary GCF samples and the two mandibular GCF samples were thawed, and the two strips per arch were eluted in 400 μL of phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA). During the elution process, the strips were centrifuged to enhance the process. The eluted samples were analyzed to determine levels of IL-6 and IL-8 and for total protein content. The analyses were performed by one previously calibrated technician.

The frozen SWS samples were thawed and centrifuged at 500–1500 × g for 20 min to precipitate cells and large macromolecules. The supernatants were analyzed for IL-6 and IL-8 and for total protein content.

Protein assay. — Samples (100 μL) of GCF and saliva were assayed for protein using the bicinchoninic acid method (Pierce Chemical Co., Rockford, IL), which is a highly sensitive and selective detection reagent for the cuprous ion. This method measures protein concentrations from 0.5 to 20 μg. In this assay, bicinchoninic acid serves as a chelating agent for Cu²⁺, forming a color complex in the presence of protein. Aliquots of GCF and saliva were placed in microtiter plates, and the Pierce BCS protein assay reagent was added. Samples were incubated for 30 min at 37 °C and the optical density read at 562 nm in a microplate spectrophotometer. The final concentration of each substance was derived from a standard curve, and data were expressed as micrograms per milliliter.

IL-6 and IL-8 enzyme-linked immunosorbant assay (ELISA). — Wells were coated with diluted saliva and reacted with rabbit-anti-human IL-6 (Sigma Chemical Co., St. Louis, MO) or goat-anti-human IL-8 (Genzyme Co., South San Francisco, CA) overnight. They were then washed three times with PBS + .05% Tween (vol/vol), and peroxidase-conjugated anti-rabbit IgG (IL-6) (Sigma Chemical) was added to each well and incubated at room temperature for 30 min. Wells were then washed with PBS + .05% Tween, developed (10 mg phenylenediamine dissolved in 1.0 mL absolute methanol added to 99 mL distilled water and 10 μL hydrogen peroxide (30%), and stopped with 25 μL of 8 M sulfuric acid. The absorbance of each well was determined using a microplate spectrophotometer at 490 nm versus substrate blank. The cytokine concentration was determined by reference to a standard curve constructed with each assay. Appropriate positive and negative controls were included with each test. The cytokine content of the samples was expressed as pigogram per milligram of protein.

Statistical Analyses

Statistics were performed using the SAS statistical software package (26). Initially, descriptive statistics were performed for all the variables followed by the t-test to compare the two categories of premenopausal and postmenopausal women. A p-value < .05 was used to reject the null hypothesis. Whenever possible, the “all-possible-regressions” procedure as described by Kleinbaum et al. (27) is to be preferred. Consequently, a general linear model (Proc GLM) was applied to all the variables to determine relationships among the oral variables and to determine if there were relationships between the oral and the second metacarpal variables. The adjusted R² criteria were used for selecting the best model (27).

RESULTS

Twenty-eight women were evaluated in this study. Their ages ranged from 23 to 78 years. With respect to menopausal status, the premenopausal group consisted of 17 women, and the postmenopausal group consisted of 11 individuals.

The average age of the women in the premenopausal group was 32.6 years, with an age range of 20–48 years. Three of the premenopausal women reported using birth control pills.

The average age of the women in the postmenopausal group was 59.6 years, with an age range of 49–78 years. The women in the postmenopausal group reported being postmenopausal for an average of 4.5 years. Five of the postmenopausal women reported partial hysterectomies. There was no oophorectomy present in this cohort. All the postmenopausal women were on estrogen replacement therapy (0.625 mg sodium estrone sulfate and 10 mg proges- terone per day) and had been on this regimen for at least 5 years. The mean BMI was nearly the same for each group (Table 1).

The oral examination of the subjects revealed a population in generally good dental health, receiving periodic routine care. Considering that missing dentition can affect ABD as described by von Wowern (28), the majority of the women examined were completely dentate. Only six individuals had missing dentition. Only one of the premenopausal women was missing teeth, whereas five postmenopausal women revealed missing dentition. The edentulous
ALVEOLAR BONE LOSS AND DENSITY

Table 1. Descriptive Statistics and t-Tests Results, Oral Bone Variables

<table>
<thead>
<tr>
<th>Source</th>
<th>Premenopausal* (n = 17)</th>
<th>Postmenopausal* (n = 11)</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.6 ± 9.9</td>
<td>59.6 ± 8.5</td>
<td>27</td>
<td>-89</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>25.0 ± 3.94</td>
<td>20.4 ± 3.88</td>
<td></td>
<td>-01</td>
<td>.01</td>
</tr>
<tr>
<td>Missing teeth (MT)</td>
<td>3.5 ± 1.46</td>
<td>2.55 ± 3.42</td>
<td></td>
<td>01</td>
<td>.02</td>
</tr>
<tr>
<td>Total alveolar bone loss (ABL) (mm)</td>
<td>1.43 ± .50</td>
<td>2.30 ± .80</td>
<td></td>
<td>1.42</td>
<td>.02</td>
</tr>
<tr>
<td>Maxillary ABL (mm)</td>
<td>1.42 ± .47</td>
<td>2.26 ± .90</td>
<td></td>
<td>1.39</td>
<td>.0001</td>
</tr>
<tr>
<td>Mandibular ABL (mm)</td>
<td>1.39 ± .58</td>
<td>2.25 ± .98</td>
<td></td>
<td>.95</td>
<td>.0001</td>
</tr>
<tr>
<td>Total alveolar bone density (ABD) (mm/Al)</td>
<td>1.0 ± .09</td>
<td>.75 ± .08</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Maxillary ABD (mm/Al)</td>
<td>1.01 ± .12</td>
<td>.83 ± .08</td>
<td></td>
<td>4.52</td>
<td>.0001</td>
</tr>
<tr>
<td>Mandibular ABD (mm/Al)</td>
<td>1.01 ± .12</td>
<td>.83 ± .08</td>
<td></td>
<td>4.52</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Note: NS = not significant. *Data given as mean ± SD.

Table 2. Descriptive Statistics and t-Tests Results, Oral Biochemistries

<table>
<thead>
<tr>
<th>Source</th>
<th>Premenopausal* (n = 17)</th>
<th>Postmenopausal* (n = 11)</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulated whole saliva (ml/min)</td>
<td>2.53 ± 1.09</td>
<td>2.93 ± 1.96</td>
<td>27</td>
<td>-62</td>
<td>NS</td>
</tr>
<tr>
<td>Salivary total protein (mg/ml)</td>
<td>1.87 ± .81</td>
<td>1.70 ± 1.34</td>
<td></td>
<td>.39</td>
<td>NS</td>
</tr>
<tr>
<td>Salivary IL-6 (pg/mg of protein)</td>
<td>22.0 ± 12.4</td>
<td>30.8 ± 8.21</td>
<td></td>
<td>-2.13</td>
<td>.05</td>
</tr>
<tr>
<td>Salivary IL-8 (pg/mg of protein)</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Maxillary GCF</td>
<td>22.8 ± 7.78</td>
<td>19.1 ± 8.51</td>
<td></td>
<td>1.16</td>
<td>NS</td>
</tr>
<tr>
<td>Protein concentration (mg/ml)</td>
<td>.33 ± .16</td>
<td>.38 ± .22</td>
<td></td>
<td>.63</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 concentration (pg/mg of protein)</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Volume (µl)</td>
<td>.56 ± .27</td>
<td>.62 ± .37</td>
<td></td>
<td>.56</td>
<td>NS</td>
</tr>
<tr>
<td>Mandibular GCF</td>
<td>22.1 ± 6.52</td>
<td>18.6 ± 5.92</td>
<td></td>
<td>1.44</td>
<td>NS</td>
</tr>
<tr>
<td>Protein concentration (mg/ml)</td>
<td>.45 ± .14</td>
<td>.35 ± .15</td>
<td></td>
<td>1.69</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 concentration (pg/mg of protein)</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Volume (µl)</td>
<td>.61 ± .24</td>
<td>.53 ± .28</td>
<td></td>
<td>.56</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: ND, not detectable; NS, not significant; GCF = maxillary gingival crevicular fluid. *Data are given as mean ± SD.

areas of the postmenopausal women had either fixed or removable protheses to replace the missing dentition.

With respect to the soft tissue examination, there was no evidence of either oral mucosal lesions or clinical evidence of active periodontitis. There was no evidence of bleeding upon probing, or the presence of calculus, and all pocket measurements were under 3 mm. To control for the presence of any occult or asymptomatic periodontal disease, IL-8 (a pro-inflammatory cytokine present in periodontal inflammation) was assayed in the stimulated whole saliva and GCF samples. The assays revealed no detectable IL-8 in either the saliva or GCF specimens (Table 2).

The analyses of the oral variables revealed no statistical differences between the premenopausal and postmenopausal groups with respect to SWS, salivary total protein (S_T), maxillary and mandibular GCF volumes and constituents, PCA, and CMI (Tables 2 and 3). The postmenopausal group had a higher average of missing teeth (MT) than the premenopausal group; however, the difference was not statistically significant (Table 1).

The postmenopausal group had significantly lower TDEN, MDEN, MBDEN, UCBDEN, and RCBDEN. In addition, the postmenopausal women had significantly higher SalivaryIL-6 (SIL-6) levels and more TABL, MXABL, and JVW (Tables 1–3).

The general linear model analyses concerning the relationship between the alveolar bone variables and metacarpal densities with cytokine concentrations, age, and menopausal status are shown in Table 4. The table shows only the statistically significant regressions and the best model with respect to the relationship between the dependent and independent variables. The results of the linear regression analyses with respect to the ABL variables showed a strong linear relationship for TABL, MXABL, and JVW with age (Figure 3). In contrast, the oral bone densities (TDEN, MDEN, MBDEN) showed a stronger association with menopausal status (Figure 4) and SIL-6. The changes in the densities of the second metacarpal were best explained by a linear regression model with respect to IL-6 concentrations in the gingival crevicular fluid and SIL-6 (Figure 5).

Table 5 shows the regression analyses for the association of oral radiographic variables with those of the second metacarpal. The oral bone loss variables showed no rela-
Table 3. Descriptive Statistics and t-Tests Results, Skeletal Bone Variables

<table>
<thead>
<tr>
<th>Source</th>
<th>Premenopausal (n = 17)</th>
<th>Postmenopausal (n = 11)</th>
<th>df</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent cortical area (%)</td>
<td>.89 ± .06</td>
<td>.85 ± .11</td>
<td>27</td>
<td>1.10</td>
<td>NS</td>
</tr>
<tr>
<td>Medullary bone density (mm/AI)</td>
<td>.96 ± .07</td>
<td>.82 ± .09</td>
<td></td>
<td>4.19</td>
<td>.001</td>
</tr>
<tr>
<td>Ulna cortical bone density (mm/AI)</td>
<td>.99 ± .08</td>
<td>.90 ± .09</td>
<td></td>
<td>2.47</td>
<td>.02</td>
</tr>
<tr>
<td>Radial cortical bone density (mm/AI)</td>
<td>1.02 ± .08</td>
<td>.90 ± .08</td>
<td></td>
<td>3.36</td>
<td>.004</td>
</tr>
<tr>
<td>Cross-sectional moment of inertia (mm²)</td>
<td>13.8 ± 8.43</td>
<td>15.8 ± 8.61</td>
<td></td>
<td>-.19</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: NS, not significant.

Table 4. Significant “All-Possible-Regressions” Procedure for Alveolar Bone Loss, Alveolar Bone Density, and Second Metacarpal Bone Densities with Respect to Salivary IL-6, Age and Menopausal Status

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Predictor Variables</th>
<th>r² (explained variability)</th>
<th>Adjusted r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ABL</td>
<td>Age</td>
<td>.43</td>
<td>.45</td>
</tr>
<tr>
<td>Maxillary ABL</td>
<td>Age</td>
<td>.30</td>
<td>.36</td>
</tr>
<tr>
<td>Mandibular ABL</td>
<td>Age</td>
<td>.28</td>
<td>.31</td>
</tr>
<tr>
<td>Maxillary ABD</td>
<td>Mstat, IL-6</td>
<td>.45</td>
<td>.49</td>
</tr>
<tr>
<td>Mandibular ABD</td>
<td>Mstat, IL-6</td>
<td>.46</td>
<td>.51</td>
</tr>
<tr>
<td>Total ABD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary ABD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular ABD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second Metacarpal Variables</td>
<td>Mstat</td>
<td>.38</td>
<td>.41</td>
</tr>
<tr>
<td>Medullary density</td>
<td>Mstat</td>
<td>.38</td>
<td>.41</td>
</tr>
<tr>
<td>Ulna cortical density</td>
<td>Mstat</td>
<td>.22</td>
<td>.22</td>
</tr>
<tr>
<td>Radial cortical density</td>
<td>Mstat</td>
<td>.32</td>
<td>.35</td>
</tr>
</tbody>
</table>

Note: All regression p-values are greater than p < .01.

Figure 3. Age versus total, maxillary, and mandibular alveolar bone loss. • age vs total bone loss; □ age vs maxillary bone loss; △ age vs mandibular bone loss; —— regression.

Figure 4. Scatter plot for oral bone densities in relation to menopausal status.

Figure 5. Scatter plot for second metacarpal bone densities in relation to menopausal status.

Table 6 demonstrates the association between the oral variables with the densities of the second metacarpal. S_AL and MdDEN were the best oral predictors of changes in second metacarpal densities (Table 6).

DISCUSSION

The results of this study suggest that postmenopausal women exhibit increased ABL and reduced ABD, which may be secondary to aging or to changes in menopausal status. In addition, the changes in alveolar bone secondary to menopause may directly influence the longevity of the dentition in postmenopausal women (8,9). There are few studies in the literature that investigate the relationship...
between the maxillary and mandibular alveolar processes, the number of missing teeth, salivary cytokines, and skeletal bones with respect to changes in menopausal status; however, below we endeavor to compare the results of this study with the available literature.

The results of this study suggest that postmenopausal women had more missing teeth than the premenopausal women (Table 1). The differences were not statistically significant due to the lack of power resulting from the small sample size. This association, however, tends to be supported by the findings of Krall (7) and Kribs et al. (8,9), who found a greater incidence of tooth loss among postmenopausal women.

With respect to ABL, the results of this study showed significantly more ABL in postmenopausal women than in the premenopausal group (Table 1), but no significant differences between premenopausal and postmenopausal women with respect to PCA and CMI (Table 3). In addition, there were no associations between ABL and any of the traditional histomorphometric measurements (PCA and CMI) of the second metacarpal. The lack of an association between ABL and PCA and CMI agrees with those of Ward et al. (22) and Elders et al. (6), who reported no relationship between measurements of the second metacarpal and ABL. One possible reason for the lack of an association between ABL and PCA and CMI may be due to the lack of precision of the PCA and CMI indices. The PCA and the later derived CMI indices were initially developed by Barnett and Nordin (29) and refined by Garn et al. (30) to provide physicians with an easy, cost-effective method for assessing hand radiographs in the clinical diagnosis of osteoporosis. This methodology is not as precise as radiographic densitometry and may not be sensitive enough to detect changes in metacarpal bone structures (17). In addition, the indicators appear to be age related. The linear regression models for ABL demonstrated that 31–45% of the variability could be explained by the model. Likewise, PCA in this and other studies has been shown to be more directly associated with age than with menopausal status (second degree polynomial).

Using radiographic densitometry to assess the alveolar bone and the second metacarpal, the results showed significant differences in density with respect to menopausal status (Tables 1 and 4). These findings agree with von Wowern (31), who found a decrease in mandibular bone density in edentulous postmenopausal women as compared to an edentulous and dentate premenopausal group. In addition, he found a corresponding decrease in radial and ulna bone densities (29). A correlation between the mandible and the bones of the forearm was reported in the von Wowern study. The correlation between the alveolar processes and the second metacarpal, albeit different skeletal bones, is in agreement with the findings of the von Wowern study.

In addition to the association between densities of the alveolar processes and the second metacarpal, the cytokine IL-6, and menopausal status explained 35–45% of the variability in the regression models, with menopausal status being the dominant predictor variable explaining the majority (35–40%) of the changes in ABD. It is worth noting that S_{IL-6} explained a portion of the model (5%) in a very healthy population of pre- and postmenopausal women. Further research is needed to determine if S_{IL-6} is a stronger predictor for changes of alveolar bone among osteopenic and osteoporotic women as compared to healthy cohorts. Unlike S_{IL-6}, the investigators found no relationship between IL-6 concentrations in GCF and the ABD or any correlation between S_{IL-6} and gingival concentrations of IL-6. One explanation may be that a large portion of the IL-6 is tissue bound and only a portion of the total IL-6 is being observed (11). In patients with ABL, GCF IL-6 levels are further reduced (11).

Reductions in second metacarpal densities were strongly associated with changes in menopausal status (Tables 3 and 5). These findings are similar to those reported by Matsumoto et al. (17), in which a 20% reduction in metacarpal density reduction occurred between premenopausal and postmenopausal Japanese women. The women in our cohort (Caucasians) displayed a 15% reduction in meta-

Table 5. Regression Analyses for the Association Between Oral Radiographic Variables with Those of the Second Metacarpal

<table>
<thead>
<tr>
<th>Bone Density Association</th>
<th>Adjusted r²</th>
<th>r² (explained variability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total oral bone density</td>
<td>.25</td>
<td>.28</td>
</tr>
<tr>
<td>with medullary density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total oral bone density</td>
<td>.20</td>
<td>.24</td>
</tr>
<tr>
<td>with ulna cortical density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total oral bone density</td>
<td>.28</td>
<td>.31</td>
</tr>
<tr>
<td>with radial cortical density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary bone density</td>
<td>.19</td>
<td>.22</td>
</tr>
<tr>
<td>with medullary density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary bone density</td>
<td>.15</td>
<td>.19</td>
</tr>
<tr>
<td>with ulna cortical density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary bone density</td>
<td>.20</td>
<td>.24</td>
</tr>
<tr>
<td>with radial cortical density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular bone density</td>
<td>.35</td>
<td>.37</td>
</tr>
<tr>
<td>with medullary density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular bone density</td>
<td>.24</td>
<td>.27</td>
</tr>
<tr>
<td>with ulna cortical density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular bone density</td>
<td>.37</td>
<td>.40</td>
</tr>
<tr>
<td>with radial cortical density</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** All regression p-values are greater than p < .01.

Table 6. Significant “All-Possible-Regressions” Procedure for Predicting Second Metacarpal Densities Using Salivary IL-6 and Alveolar Bone Densities

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Predictor Variables</th>
<th>Adjusted r²</th>
<th>r² (explained variability)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary density</td>
<td>IL-6, mandibular</td>
<td>.40</td>
<td>.45</td>
</tr>
<tr>
<td></td>
<td>bone density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulna cortical density</td>
<td>IL-6, mandibular</td>
<td>.20</td>
<td>.28</td>
</tr>
<tr>
<td></td>
<td>bone density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radial cortical density</td>
<td>IL-6, mandibular</td>
<td>.41</td>
<td>.46</td>
</tr>
<tr>
<td></td>
<td>bone density</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** All regression p-values are greater than p < .01.
carpal density using a densiometric methodology similar to
Matsumoto et al. (17).

The results of this study found a strong association
between bone densitometries of the oral cavity with those
of the second metacarpal (Table 5). Both maxillary and
mandibular ABD showed strong associations with the
metacarpal densities; however, the strongest relationship
was with the mandibular and the metacarpal densitometries.
One possible explanation for the relationship is that the
mamulde may flex or become "loaded" during function
similar to the second metacarpal.

One objective of this study was to determine if measures
of the oral cavity (ABL, ABD, salivary cytokines, etc.)
could predict changes in skeletal bones. Tables 5 and 6
show that this may be possible. Mandibular bone density
showed a strong correlation with the density determinations
of the second metacarpal, which could account for as much
as 40% of the variability in the regression model. In addition,
when $S_{a4}$ was added to the regression model with mandibular
density, the combination of the two variables could account for
as much as 45% of the variability. The relationship between estrogen depletion, decreases in ABD, and increases in $S_{a4}$ has also been observed in animal studies
performed by the investigators of this study (32). Ovariectomized sheep displayed decreased ABD and increased $S_{a4}$ as compared to control animals. $S_{a4}$ levels
were increased 33% in this study. This increase is similar to
the 36% increase observed in this study.

Serum was not available in this study to determine the
relationship between serum IL-6, $S_{a4}$, and the oral and
skeletal bone density measurements. Because serum IL-6 is
predictive of metabolic bone disease, more investigation is
required concerning the validity of $S_{a4}$ and its relationship
to ABL. In addition, more research is required to determine
the relationship between the alveolar bone, $S_{a4}$, and other
skeletal components in healthy and diagnosed osteoporotic
women of varying races.

In conclusion, the results of this study suggest that
changes in alveolar bone in healthy women are influenced
by age and menopausal status. ABL appears to be more
associated with age, where as reduced ABD is more associated
with estrogen depletion. In addition, the changes in
ABD are correlated with changes in the second metacarpal
density. This correlation suggests that metabolic bone dis-
ases have a direct impact on the oral condition and may
place women at risk for the end point sequela; that is, miss-
ing teeth.

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Address correspondence to Dr. Charles F. Streckfus, Department
of Diagnostic Studies, University of Mississippi Medical Center, 2500 North
State Street, Jackson, MS 39216-4505.

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