The effect of bone loss on rod-like and plate-like trabeculae in the cancellous bone of the mandibular condyle

L.J. van Ruijvena,*, E.B.W. Giesenb, L. Muldera, M. Farella, T.M.G.J. van Eijdena

*Department of Functional Anatomy, Academic Centre for Dentistry Amsterdam (ACTA), Universiteit van Amsterdam and Vrije Universiteit, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

bDepartment of Orthodontics and Oral Biology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

cDepartment of Orthodontics, University of Naples Federico II, 80131 Naples, Italy

Received 6 December 2004; revised 21 February 2005; accepted 25 February 2005

Abstract

Bone loss may affect the structure of cancellous bone. But its effect on trabeculae with different characteristics, like rods and plates, is not accurately known. This study analyzes the effect of bone loss on individual rod-like and plate-like trabeculae. 94 specimens were obtained from mandibular condyles from both dentate and edentate humans and scanned with a micro-CT scanner. The bone volume fraction (BV/TV) of these specimens ranged from 7% to 30%. Next, the rod-like and plate-like trabeculae were identified with an especially developed algorithm. Plate volume fraction (PV/TV), rod volume fraction (RV/TV), plate thickness, rod thickness, number of plates, and number of rods were determined. In individual specimens, the thickness of the rods ranged from 40 μm to 180 μm, while the thickness of the plates ranged from 40 μm to 300 μm. In every specimen, the thickness of the plates was larger than the thickness of the rods. Statistical analysis revealed that PV/TV was proportional (r = 0.98, P < 0.001) and RV/TV inversely proportional (r = −0.45, P < 0.001) to BV/TV. Also the thickness of the plates correlated with BV/TV (r = 0.62, P < 0.001), while the thickness of the rods (mean = 90 μm, SD = 7 μm) remained constant (r = −0.09, P = 0.378). A four-fold reduction of the bone volume fraction was accompanied by a three-fold reduction of the number of plates and a 40% reduction of their thickness, but also by a three-fold increase in the number of rods and RV/TV. It was concluded that the effect of bone loss on plate-like trabeculae was opposite to its effect on rod-like trabeculae. Remarkably, the thickness of the rods (90 μm) was independent of the bone volume fraction. This suggests that there is a minimal thickness for trabeculae.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Cancellous bone; Mandible; Rod-like; Plate-like trabeculae

Introduction

Bone loss increases the risk of bone fracture due to a decrease of bone strength [1–3]. The strength of cancellous bone depends on the mechanical properties of the rod-like and plate-like trabeculae and on their three-dimensional structure. Structural changes related to cancellous bone loss include a decrease of bone density [4], a transformation of plate-like trabeculae into rod-like trabeculae [1,5], an increase of anisotropy [6], and a decrease of trabecular thickness [1].

Generally, changes in these parameters have been quantified as average values for relatively large pieces of cancellous bone, disregarding the distinction between rod-like and plate-like trabeculae. A number of studies have indicated that bone loss may have a different effect on different types of trabeculae and that this may be reflected in the mechanical properties of the cancellous bone. For example, in vertebrae, bone loss has been associated with the disappearance of predominantly horizontally oriented trabeculae, causing an increase in mechanical anisotropy [7]. In addition, the thickness of horizontal trabeculae in vertebrae has been reported to decrease with age, while the thickness of vertical trabeculae remained constant [8]. In both studies, information regarding the shape of the
was not considered. Consequently, they rather distinguished horizontal from vertical trabeculae than rod-like from plate-like trabeculae.

In the present study, the effect of bone loss on rod-like and plate-like trabeculae was analyzed, where only information regarding the shape was used to identify individual trabeculae. In 94 cancellous bone specimens obtained from the condyles of 49 human mandibles, numbers, thicknesses, and volume fractions of the rods and plates were determined. A statistical analysis was used to determine whether the architectural parameters for rods and plates correlated with age, bone volume fraction, or structure model index of the specimens. Furthermore, it was analyzed whether these relationships differed between rods and plates. The cancellous bone from the mandibular condyle was chosen, as it consists of both plate-like (oriented parallel to the sagittal plane) and rod-like trabeculae (oriented medio-laterally, perpendicular to the plates). To have a large variation in bone volume fractions, dentate as well as edentate subjects were used, as the latter subjects have less cancellous bone in their condyles [9].

Materials and methods

Specimen preparation

Ninety four cylindrical specimens of cancellous bone (diameter: 3.64 ± 0.14 mm, length: 4.87 ± 0.06 mm (means ± SD)) were obtained from the mandibular condyles of 49 embalmed human cadavers using a custom-made hollow bur. The specimens were produced in the axial and transverse directions. Their position within the condyle, i.e., medial or lateral, was randomized; they were stored in embalming fluid [9]. Twenty-five subjects were edentate (14 female, 11 male, 85.2 ± 8.5 years). Twenty-four subjects were dentate or partially dentate (19 female, 5 male, 74.8 ± 11.7 years) with an average 8.5 dental elements in the upper jaw and 10.7 in the lower jaw. None of the subjects had a known history of temporomandibular joint disorders. The use of the condyles conformed to a written protocol that was reviewed and approved by the Department of Anatomy and Embryology of the Academic Medical Center of the University of Amsterdam.

Micro-CT

To obtain the three-dimensional cancellous bone structure, the specimens were scanned in a micro-CT system [10] (µCT20, Scanco Medical AG, Zürich, Switzerland). Briefly, the specimens were submerged in a fluid to avoid dehydration and scanned at a resolution of 18 μm. The integration time was 60 ms, and the number of slices 270. Bone was identified using a fixed threshold. This threshold was obtained experimentally by matching the bone volume fraction determined from the CT-scans with the volume fraction determined according to Archimedes’ method. Four specimens were excluded from further analysis, because they appeared to contain cortical bone.

Archimedes’ method

After the scanning, the fatty marrow was removed from the bone by an air jet, after which the specimens were soaked in an alcohol/acetone (1/1) mixture for 5 days. Subsequently, the dry weight ($\mu_d$) and the submerged weight ($\mu_s$) were recorded on a balance (Mettler AG204 Deltarange, Mettler Instruments AG, Greifensee, Switzerland). The volume of the bone tissue $V_{tissue}$ was calculated as $(\mu_d - \mu_s) / \rho_L$, where $\rho_L$ is the density of the submersion liquid. Bone volume fraction was calculated as $V_{tissue} / V_{cyl}$, where $V_{cyl}$ is the volume of the cylinder.

Rod-like and plate-like trabeculae

Identification of a trabecula was performed by counting the number of disjunct connections it has with surrounding bone and the number of disjunct bone-marrow surfaces. A rod-like trabecula has two connections and one bone-marrow surface (Fig. 1). A plate-like trabecula has one connection and two surfaces. A junction between a rod and
a plate has two connections and two disjunct bone-marrow surfaces. Other structures that can be identified are plate boundaries (one connection and one bone-marrow surface), junctions between two plates (one connection, three bone-marrow surfaces), and junctions between three rods (three connections, one bone-marrow surface).

The procedure was implemented in a program written in C++ (GCC, Free Software Foundation, Boston, USA). For every bone volume element (voxel), a small spherical volume (with the voxel at its centre) was separated from the CT-scan. Next, the connections of the voxels were identified as the set C of all facial elements that had become surface by the separation and the bone-marrow surface was identified as the set M of all other surface elements that belong to the surface of the fragment. Now, the voxel in the center of the volume was identified by counting the number of disjunct surfaces in the sets M and C. To minimize the number of unidentified voxels, the diameter of the spherical volume was increased iteratively from 320 to 500 μm in steps of 20 μm, until two successive iterations resulted in the same identification.

Histomorphometry

For every specimen, average trabecular thickness (Thall), bone volume fraction (BV/TV), and structure model index (SMI) were calculated [5]. The SMI quantifies the characteristic form of the cancellous bone in terms of plate-like to rod-like. For ideal plate- or rod-like structures, this index is 0 or 3, respectively. This procedure was repeated twice for reconstructions containing only the trabeculae that had been identified as rod-like or plate-like, respectively (Figs. 2B and C). From these two reconstructions, average trabecular thicknesses (Throds, Thplates) and volume fractions (rods: RV/TV, plates: PV/TV) were calculated. Since all rod-like trabeculae were separated from each other by either junctions or plate-like trabeculae, the number of rods was determined by counting the number of fragments. The number of plates was calculated with the parallel plate model [11] as (PV/TV) / Thplates. For these calculations, the software program μCT Evaluation v4.5a (Scanco Medical AG, Zürich, Switzerland) and custom-made software were used.

Statistical analysis

Since there are large regional differences in the cancellous structure of one condyle [12], all 90 specimens were treated as independent samples of cancellous bone. The mean values and standard deviations were calculated for all variables, and Pearson correlation coefficients were calculated for all combinations. For several variables, relationships with the bone volume fraction were estimated by linear regression analysis. For all statistical calculations, SPSS v11.5.1 (SPSS Inc. Chicago, Illinois, USA) was used.

Results

Four typical examples of the identification of the trabeculae are shown in Fig. 3. In these specimens, the bone volume fractions were 8%, 11%, 15%, and 17%, respectively. The visually estimated orientation of the rods ranged from parallel to the plates (0°) to perpendicular to the plates (90°), with a preference for the angles 0°, 45°, and 90°.

The descriptive statistics of the results are shown in Table 1. A paired samples t test indicated that the difference between the thicknesses of the rods and plates was significant (P < 0.001). The algorithm identified 81% of the bone voxels as plate-like, 11% as rod-like, and 7% as one of the junctions mentioned. The remaining voxels (1%) were more complex fragments mainly caused by the coring procedure and complex junctions (e.g. junctions with four rods or a plate with two rods). The average number of rods in the specimens was 82.

Table 2 gives the Pearson correlation coefficients between the various parameters. Bone with a high volume fraction (BV/TV) had thicker plates, a larger number of plates, and, consequently, a larger plate volume fraction (PV/TV) than bone with a low BV/TV; on the other hand, the number of rods and the rod
volume fraction (RV/TV) were less. Bone with a high RV/TV had a lower PV/TV, thinner plates, and a larger number of rods than bone with a low RV/TV. The thickness of the rods did not depend on any of the volume fractions. Bone with thick plates had a larger PV/TV, less but thicker rods, and a lower RV/TV than bone with thin plates. Older subjects (last column) had no significant decrease of BV/TV, but they did show a significant increase of RV/TV and the number of rods. The structure model index (SMI) was proportional to RV/TV and the number of rods, but inversely proportional to BV/TV, PV/TV, and the number of plates.

A scatter plot with RV/TV and PV/TV as a function of BV/TV is shown in Fig. 4. It clearly illustrates the difference in the relationship between the total amount of bone and rod-like and plate-like trabeculae. Subjects with a high BV/TV also have a high PV/TV, but a low RV/TV. The reduction of the bone volume fraction is almost entirely caused by the reduction of the plate volume fraction.

Fig. 5 shows the thicknesses of the rods and plates as a function of BV/TV. The thickness of the rods did not depend significantly on BV/TV. The thickness of the plates decreased from 160 μm at a bone volume fraction of 0.30 to 100 μm at a bone volume fraction of 0.07. At low BV/TV, the thickness of the plates became almost identical to the thickness of the rods. In every specimen, the thickness of the plates was larger than the thickness of the rods. In individual specimens, the thickness of the rods ranged from 40 μm to 180 μm, while the thickness of the plates ranged from 40 μm to 300 μm.

Fig. 6 shows the number of plates as a function of BV/TV. Combined with Figs. 4 and 5, this figure shows that the decrease of the bone volume fraction was caused mainly by a reduction of the number of plates and to a lesser extent by a reduction of their thickness.

Discussion

The method developed to identify different trabecular structures has proven to be stable and able to identify rod-like and plate-like trabeculae in the mandibular condyle.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone volume fraction [%]</td>
<td>90</td>
<td>15.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Thickness bone [μm]</td>
<td>90</td>
<td>124</td>
<td>19</td>
</tr>
<tr>
<td>Structure model index</td>
<td>90</td>
<td>1.05</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Plates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate volume fraction [%]</td>
<td>90</td>
<td>12.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Thickness plates [μm]</td>
<td>90</td>
<td>126 decreases</td>
<td>21</td>
</tr>
<tr>
<td>Number of plates [mm⁻¹]</td>
<td>90</td>
<td>0.98</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Rods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rod volume fraction [%]</td>
<td>90</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Thickness rods [μm]</td>
<td>90</td>
<td>91</td>
<td>7</td>
</tr>
<tr>
<td>Number of rods [mm⁻³]</td>
<td>90</td>
<td>1.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

α The number of specimens with valid test results is indicated.

b A paired t test indicated that rods and plates had different thicknesses (P = 0.000).
Further analysis is required to determine the applicability of this method in other bones than the mandibular condyle. In combination with other methods, it can also help to study properties for rod-like and plate-like structures separately. For instance, it can be used to analyze whether inhibitors of bone resorption affect rods and plates differently, or with large-scale finite element analysis to analyze how loads are distributed over the plate-like and rod-like trabeculae.

A few remarks with respect to the accuracy of the method should be made. As it is not certain at what thickness plates become rods, there is no gold standard with which the results can be compared. Therefore, we verified the results visually by inspection of all cross-sections along the three axes of the CT-scans and three-dimensional reconstructions. The main error came from rod-like trabeculae at the boundaries of the specimens, which were created by the coring (see also Fig. 3). On average, the number of artificially introduced rods was approximately five (6%). This error is less in specimens with a low bone volume fraction because of the lower number of plates.

Additionally, a number of voxels were not identified as one of the mentioned structures. This number depended strongly on the diameter selected for the spherical volume. By definition, this diameter determines the difference between rod-like and plate-like trabeculae. Since it is not certain at what diameter the distinction between rod-like and plate-like trabeculae should be made, we used the diameter of the spherical volume to minimize the number of unidentified voxels. We have checked the accuracy of the method for diameters ranging from 180 to 600 μm. At a diameter of 350 μm, the number of unidentified voxels reached a minimum of approximately 5%. Therefore, the interval to iteratively increment the radius was taken from 320 to 500 μm. To keep the computation time within limits and to avoid trivial iterations, a step-size of 20 μm was

### Table 2
Correlation matrix between the parameters

<table>
<thead>
<tr>
<th></th>
<th>RV/TV</th>
<th>PV/TV</th>
<th>Number of rods</th>
<th>Number of plates</th>
<th>Th_all</th>
<th>Th_rods</th>
<th>Th_plates</th>
<th>SMI</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone volume fraction</td>
<td>-0.453</td>
<td>0.981</td>
<td>-0.407</td>
<td>0.895</td>
<td>0.619</td>
<td>-0.094</td>
<td>0.618</td>
<td>-0.690</td>
<td>-0.120</td>
</tr>
<tr>
<td>Rod volume fraction</td>
<td>-0.591</td>
<td>0.965</td>
<td>-0.482</td>
<td>-0.475</td>
<td>-0.104</td>
<td>-0.503</td>
<td>0.780</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td>Plate volume fraction</td>
<td>0.555</td>
<td>0.689</td>
<td>0.679</td>
<td>0.006</td>
<td>0.679</td>
<td>-0.727</td>
<td>-0.152</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rods</td>
<td></td>
<td></td>
<td>-0.405</td>
<td>-0.547</td>
<td>-0.286</td>
<td>-0.569</td>
<td>0.500</td>
<td>0.234</td>
<td></td>
</tr>
<tr>
<td>Number of plates</td>
<td></td>
<td></td>
<td>0.289</td>
<td>0.440</td>
<td>0.996</td>
<td>-0.131</td>
<td>-0.098</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness bone</td>
<td></td>
<td></td>
<td>0.411</td>
<td>0.111</td>
<td>0.065</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness rods</td>
<td></td>
<td></td>
<td></td>
<td>0.130</td>
<td>-0.130</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness plates</td>
<td></td>
<td></td>
<td></td>
<td>0.098</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structure model index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.173</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.01.

b P < 0.05.

Fig. 4. Scatter plot of the volume fractions of the plate-like (r = 0.98, P = 0.000, N = 90) and rod-like (r = -0.45, P = 0.000, N = 90) trabeculae versus the bone volume fraction.
chosen. By checking the repeatability of successive results obtained after stepwise incrementing the diameter, the number of unidentified voxels was further reduced to 1%.

Finally, in the current version, broken rods are identified as plate boundaries (one connection and one bone-marrow surface) and consequently marked as plate-like trabeculae. This error is estimated to be less than 1%.

It might be argued whether the bone volume fraction is a good indicator for bone loss, as variation in bone volume fraction is not only caused by bone loss, but also by normal inter-individual variation. An alternative indicator might be the absence of teeth. When specimens from dentate and edentate subjects are compared, and the absence of teeth is taken as a measure for bone loss, we also find that bone loss results in a lower bone volume fraction ($P = 0.001$), a lower plate volume fraction ($P < 0.001$), a lower number of plates ($P < 0.001$), a higher rod volume fraction ($P = 0.006$), and a higher number of rods ($P = 0.029$).

Cancellous bone loss is generally ascribed to thinning and perforation or disappearance of the trabecular structure [13–15]. The present study shows that the reduction of the bone volume fraction is mainly caused by the reduction of the

![Fig. 5. Scatter plot of the thickness of the plates ($r = 0.62$, $P = 0.000$, $N = 90$) and rods ($r = -0.094$, $P = 0.378$, $N = 90$) versus the bone volume fraction.](image)

![Fig. 6. Scatter plot of the number of plates ($r = 0.90$, $P = 0.000$, $N = 90$) and the number of rods ($r = -0.41$, $P = 0.000$, $N = 90$) versus the bone volume fraction.](image)
number of plates. A four-fold reduction of the bone volume fraction (from 0.30 to 0.07) is accompanied by a three-fold reduction of the number of plates (Fig. 6), and only a 40% reduction of the thickness of the plates (Fig. 5). At the same time, the thickness of the rods remained constant, while the number of rods and their total volume show a three-fold increase. Consequently, at a high bone volume fraction, the volume fraction is mainly determined by the plate-like trabeculae, but at a low bone volume fraction, the volume of the rod-like trabeculae also becomes relevant. For example, at a bone volume fraction of 7% (i.e. two standard deviations below the mean bone volume fraction [12]), the rod-like trabeculae contain 30% of the bone volume, compared to only 4% at a bone volume fraction of 25%.

A decrease of the bone volume fraction results in a larger volume fraction of the rods. Although rods in the mandibular condyle are mainly oriented medio-laterally, this does not necessarily imply an increase of the number of rods in this direction. It appeared from the visual inspection that in specimens with a low bone volume fraction a larger fraction of the rods was oriented parallel to the plates. Most likely, the increase of the number of rods was the result of perforation of plate-like trabeculae and therefore these rods are not oriented medio-laterally.

The structure model index is supposed to be a measure for the relative number of rod- and plate-like trabeculae. Therefore, it was expected to be proportional to the rod volume fraction. It was, however, surprising that their mutual correlation coefficient was only 0.58 (for a non-linear relationship 0.66). This suggests that the rod volume fraction contains additional information, which is not present in the structure model index. This information might be related to the thickness of the plates, since the rod volume fraction correlated with the thickness of the plates, while the structure model index did not.

It is surprising that for low bone volume fractions the thickness of the plates converges to the thickness of the rods. Probably, the bone multicellular units [16] impose a minimum thickness to the trabecular structure. During remodeling, the osteoclasts in these units create lacunae in the trabeculae, which are refilled with new bone by the osteoblasts of the unit. This process is probably driven by the strains in the trabeculae [17]. Since these lacunae are approximately 55 μm [18] deep, rods with a diameter of 50 μm or less will be separated in two parts, after which the strain will drop to zero. This means that the osteoblasts will not repair the created fissure, but instead the whole rod will be resorbed by the osteoclasts. This finding is in agreement with earlier findings [19,20].

To our knowledge, there has been only one other study in which the thicknesses of individual rod-like trabeculae have been determined. In that study, rod-like trabeculae were identified visually, and the thicknesses were measured by analyzing anaglyphs. This resulted in a thickness of 123 μm ± 36 μm (mean ± SD) for rod-like trabeculae of the human vertebrae and a decrease of this thickness with age and bone volume fraction [21].

In summary, in the present study, a distinction could be made between rod-like and plate-like trabeculae in the mandibular condyle. It was shown quantitatively to what extent the thicknesses and volume fractions of rod-like and plate-like trabeculae are affected by a decrease of the bone volume fraction. The applied algorithm can, for instance, also be used to analyze whether inhibitors of bone resorption affect rods or plates differently or to analyze in combination with finite element analysis the distribution of stresses and strains in rods and plates.

Acknowledgments

This work was sponsored by the National Computing Facilities Foundation (NCF) for the use of supercomputing facilities. This research was institutionally supported by the Inter-University Research School of Dentistry through the Academic Centre for Dentistry Amsterdam. We would like to thank the Academic Computer Services Amsterdam for the use of their technical support. We are grateful to Geerling Langenbach and Jan Harm Koolstra for their comments on the manuscript.

References


