THE INFLUENCE OF FUNCTIONAL LOADING ON BONE REMODELLING IN THE HUMAN MANDIBLE

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CHAPTER 1

Overview on microcomputed tomography (Micro-CT)

Microtomography is a technique that uses x-rays to create cross-sections of 3D-objects that are used then to recreate a virtual model without destroying the original one (Fig.1). The term micro is used to indicate that the pixel sizes of the cross-sections, which are generally in the micrometer range or less. Microcomputed tomography (microCT) is a high-resolution type of computerized axial tomography. Typically the spatial resolution of conventional medical CT-scanners is in the range of 1-2.5 mm, which corresponds to 1-10 cubic mm voxel (volume element). With micro-CT, in the most recent releases, resolutions up to few nanometers have been reached. These systems make use of computers or working station, as servers, and are able provide high resolution models, which can be easily assessed in three dimensions. The X-ray tube (open or sealed) produces a conic beam of electron that penetrates the object to be analyzed, and a digital signal is interpreted by the 2D detector as a Digital Radiograph image. The object is positioned on a precision rotational stage and an image is acquired during the rotation at a constant step. The scan usually covers a rotation of 360 degrees, but for specific applications a limited angle scan.

Fig.1 Scheme of computed tomography
can be performed. Microcomputed tomography has been successfully used for different purposes, such as the study of porous or cavity-containing objects (metallic foams, electronics, stones, wood and composite polymers) or for biologic investigations. On the whole, these systems have been widely used for the study of hard tissues because of the high linear attenuation coefficient of the calcified bone and dental matrices. In the recent years, this technique has shown to be promising in the study of trabecular and cortical bone morphology following bone loss in osteoporotic patients or in animal models of osteoporosis. In bone biology, microCTs are widely used for the measurement of the characteristics of the trabecular, cortical, and canalicular network. Indeed, bone histomorphometry and microarchitecture can be easily assessed in order to estimate the mechanical characteristics of bone tissue in different locations. Three dimensional (3D) modelling and analysis reconstruction of specimens can be obtained with several softwares. The ANT software (Skyscan - Aartselaar, Belgium), was successfully used for the studies presented in this thesis. The program allows reconstruction of objects from 2D slices, after thresholding. The reconstructed 3D models are obtained by a surface-rendering algorithm. Four different 3D models can be reconstructed and made visible on the computer screen simultaneously, thus offering the possibility to combine several images. In addition, the program offers facilities for 360° model rotations in all directions, displacements, lightening effects and colouring of the desired structures. A very interesting facility for the study of porous structures was the possibility to make the virtual models semi-transparent. Another interesting possibility was to obtain 2D reslices of the objects across a plane, positioned in a specified direction. Morphometric measurements can be done on 2D images and 3D models with software.

**Micro-CT Specifications**

In the studies presented in this thesis, the following micro-CT system was used.

**Micro-CT 40 SCANCO**

Type Desktop Cone-Beam MicroCT Scanner (Fig.2)

- No specimen preparation required
- Non-destructive
- X-Ray Microfocus X-Ray-source
- 5 µm Spot Size
50-70 kVp, 8 W (160 µA)
Detector 2048x252 elements, 24 µm pitch
Resolution 6 µm nominal, 9 µm (10% MTF @ 12 mm Ø)
Image Matrix variable from 512 x 512 to 2048x20
Specimen Size up to 38 mm Ø
max. Scan Length 80 mm
Computer hp 64bit Itanium IA64 multiprocessor systems
Memory: 48 GB
Disk: 1 TB disk tower
Optional PostScript printers
Software Complete 64-bit imaging solution
- Data acquisition
- Online/Offline Reconstruction
- Sophisticated 2D/3D evaluation
- 3D-visualization/animation
- Archiving
Density Calibration
Database
Browser-access (web-based access)
Electrical 100-240 V/50-60 Hz
300 W
Physical Scanner weight: 130 kg
Scanner dimensions (WxDxH): 85 x 41 x 72 cm
Fig. 2 Scanco MicroCT scanner (MicroCT 40)
CHAPTER 2

Regional variations in mineralization and strain distributions in the cortex of the human mandibular condyle

Abstract

The strain (i.e. deformation) history influences the degree of mineralization of cortical bone (DMB) as well as its osteonal microstructure. This study aimed to examine the relationships of stress and strain distributions with the variations in DMB and the osteonal orientations in the cortical bone of the human mandibular condyle. It was hypothesized that strains are inversely proportional to local DMB and that the principal strains are oriented parallel to the osteons. To test this, ten human mandibular condyles were scanned in a microCT system. Finite element models were created in order to simulate static clenching. Within each condyle, 18 volumes of interest were selected to analyze regional differences in DMB, stress and strains. Subchondral bone showed a lower equivalent strain (2652±612 με) as compared to the anterior (p=0.030) and posterior cortex (p=0.007) and was less mineralized. Contrary to our hypothesis, the results show that strains correlated positively with regional variations in DMB (r=0.750, pb0.001). In the anterior and the posterior cortex, the first principal strain was parallel to the cortical surface and oriented supero-inferiorly with a fan-like shape. In subchondral bone, the first and the second principal strain were parallel to the surface and oriented antero-posteriorly and medio-laterally, respectively. It was concluded that the strain distributions, by themselves, cannot explain the regional differences found in DMB. In agreement with our second hypothesis, the orientation of the osteonal network of the mandibular condyle was closely related to the strain orientations. The results of this study suggest that the subchondral and the cortical bone are structured to ensure an optimal load distribution within the mandibular condyle and have a different mechanical behaviour. Subchondral bone plays a major role in the transmission of the strains to the anterior and posterior cortex, while these
ensure an optimal transmission of the strains within the condylar neck and, eventually, to the mandibular ramus.

INTRODUCTION

Bone is a dynamic tissue capable of adapting its structure to local mechanical stimuli by continuous bone renewal [1–3]. It has been noted that strains occurring within bone affect this turnover and hence bone macrostructure (i.e. shape and size of bones) and microstructure (i.e. osteons, plates, rods etc.) by initiating cell-mediated remodelling [4,5]. Consequently, bone morphology and internal architecture strongly depend on the deformation history [6–8]. Except for the bone structure, the deformation history also influences the mineralization of bone since the average age of bone tissue is inversely proportional to the remodelling rate, and the mineralization is proportional to the bone age. Therefore, bone mineral content is commonly believed to be inversely proportional to the rate of remodelling [9]. For cortical bone, the relationship between mechanical loading and mineralization has been investigated in several bones. Regional variations in cortical bone mineralization have been related to the amount or to the mode (i.e. tensile/compressive) of locally occurring strains [10–12]. A significant lower ash mineral content was found in the highly strained caudal compression cortex of the sheep radius [12]. Differences in mineral content between tension/compression cortices were found also in the mule deer calcaneus [10]. In the human femur, the mineralization of cortical bone was found to vary among sites subjected to different loading conditions [11]. Recently, in the human mandibular condyle, differences in DMB have been found between cortical regions (i.e. anterior, posterior and sub-chondral) [13]. These variations were also attributed to the remodelling process. Although several studies have investigated the stress and strain distributions in the mandible [14–18], the relationship between strains and bone mineralization in the cortex of the human mandibular condyle has not been described. Hence, it is not certain to what extent the observed regional variations in DMB are dependent on the locally occurring strains. Previous studies [19–21] have shown that the orientation of the cortical canals is parallel to locally occurring strains. In human mandibular condyle, the orientation of the canals, which is medio-lateral in subchondral bone and supero-inferior in the anterior and the posterior cortex, suggests an accordingly oriented principal direction of the
strains [22]. However, the data available regarding the strain orientations in this location [14,15,17] are not sufficient to detect the relation between cortical canal orientations and strain directions. The aim of the current study was to analyze the potential relationship between regional variations in DMB with stress and strain distributions in the cortical bone of the human mandibular condyle and to determine the orientation of the strains occurring during functional loading. It was hypothesized that strains correlate inversely to DMB and that the principal strains are parallel to the cortical canals. For this purpose, ten mandibular condyles were analyzed with a microCT scanner. The reconstructed bone tissue was used to generate finite element (FE) models which include the DMB and its inhomogenous distribution. These models were used to analyze regional differences in DMB, von Mises stress, equivalent strain, strain orientations and strain energy density in various cortical bone locations and to calculate the condylar displacements.

Materials and methods

Condyles

Ten left mandibular condyles were obtained from ten embalmed human male cadavers (mean age SD: 69.8±14.4 years, range: 43 to 92 years). On average, the subjects had 10.2±4.5 teeth in the upper jaw and 11.5±2.6 in the lower jaw. No signs of relevant malocclusion or condylar erosions were found. The condyles were separated from the mandible at the transition from the neck to the ramus with a hand saw; condylar bone marrow was left in situ. Specimens were fixated in 60% formalin. The use of the condyles conforms 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.

MicroCT

Three-dimensional reconstructions of the cortical bone of the condyles were obtained using a high-resolution microCT system (µCT 40, ScancoMedical AG, Bassersdorf, Switzerland). Each condyle was positioned to be scanned in frontal slices. The scan resolution was 15 µm, the beam energy 45 kV, which corresponds to an effective energy of approximately 24 keV [23]. To minimize noise, an
integration time of 1250 ms was used. To minimize beam-hardening artefacts, the system was equipped with a specially developed algorithm (Scanco Medical AG, Bassersdorf, Switzerland) and an aluminium filter (0.5 mm) to remove the softest rays. To assess the amount of noise and the effect of beam hardening quantitatively, homogeneous K2HPO4 solutions with different concentrations were scanned [23] using the same settings as described above. Noise level and the effect of beam hardening were maximally 6% and 7%, respectively, which was well below natural variations in mineralization.

The degree of mineralization (DMB) of each volume element (voxel) was computed from the attenuation coefficient using a linear relation, which was calibrated with a phantom containing hydroxyapatite densities of 0, 100, 200, 400 and 800 mg/cm3.

**Finite element analysis**

With a voxel conversion technique [24], finite element meshes were created with 30-µm brick elements. This size was sufficiently less than the requirement of one-fourth of the mean trabecular thickness [25]. On average, the meshes consisted of 38 million elements. The procedure used to define the joint load was described previously [26]. A custom-made program was used to define a joint load resembling static clenching. A result of this procedure is shown in Fig. 1. All joint loads were scaled to obtain a total force of 300 N for every condyle [27]. Finally, the nodes in the saw-plane were fixed. The tissue modulus (Et) was approximated from the DMB value of the corresponding microCT voxel according to $\log Et = -8.58 + 4.05 \times \log [Ca]$ [28]. The concentration of [Ca] was recalculated to the concentration of hydroxyapatite [HA] or DMB by multiplying [Ca] by a factor 2.5 (approximately 40% of hydroxyapatite consists of calcium) and subsequently by multiplying this by 2 g/cm3 [29], which is the bone tissue density. The finite element models were solved using 32 parallel processors on an SGI Origin 3800 and specific software [24].

**Volumes of interest**

Three regions were defined within each condyle, i.e. anterior cortex, posterior cortex and subchondral bone. To define these regions, each condyle was first divided into four supero-inferior parts. In the upper part, the bone between the
apical points of lateral and medial poles was selected as the subchondral region. The 15% most medial and 15% most lateral parts of the condyle were discarded. In the third part from the top, the cortex was divided in an anterior and a posterior region (Fig. 1). To analyze medio-lateral differences, each of the three defined regions was divided into six equal sub-regions from the lateral to the medial border (Fig. 1). In each sub-region a volume of interest (VOI) containing only cortical bone was selected. In total, 18 VOIs were selected in each condyle. In order to check whether the distance from the surface had an effect on the results, in the sub-regions B and D (Fig. 1) deep and superficial layers were distinguished. Two VOIs were finally selected at the medial and lateral pole to calculate the displacements occurring in antero-posterior, supero-inferior and medio-lateral direction.

Fig. 1. Finite element model of the human mandibular condyle. The selection of the sub-regions for both subchondral and cortical bone was limited within the black lines. The letters indicate the six cortical and subchondral sub-regions (A to F; A, most lateral sub-region, F, most medial). The saw plane is indicated by the light blue arrow. The color bar indicates the relative magnitude of load.
Bone surface orientation

In order to relate strain orientation to the direction of the local surface of the cortex, the latter was determined manually. For this purpose, in each sub-region a plane was fitted through three selected points of the bone surface. The normal vector of these planes was considered the local normal vector of the cortex. Angles between the local normal vectors and the strain vectors were determined for all anterior, posterior and subchondral sub-regions.

Statistical analysis

Means and standard errors of mean of DMB, principal strains, von Mises stress, equivalent strain and strain energy density (SED) were calculated for each sub-region. These parameters were also determined for the three regions by combining the six sub-regional results. Grand means and standard deviations were then calculated over all ten condyles. A general linear model (repeated measures) was used to identify regional and sub-regional differences and to test whether the distance from the surface had an effect on the results. Regression analyses were performed to identify correlations between local variations in DMB of cortical bone and local variations in stresses and strains. Tests were performed using SPSS statistical software package (SPSS Inc., Chicago, IL, USA, version 12.0.1).

Results

General results

The total bone volume was (mean±standard error of mean) 748.4±34.2 mm3. The average Young's modulus was 8.3± 0.8 GPa, as calculated from Currey's model [28]. The cortical and the trabecular volume were 77% and 23%, respectively. During simulated static clenching, the condyle was mainly bent in posterior direction. In addition, a larger compliance occurred medially, resulting in torsion around the condylar neck. At the medial apex, the antero-posterior displacement was 0.23± 0.04 mm (laterally: 0.12±0.02 mm) and the supero-inferior displacement was 0.09±0.03 mm (laterally: 0.01±0.02 mm).
Regional differences

The DMB was lower in the subchondral (1023±25 mg/cm³) than in the posterior region (1087±30 mg/cm³, p=0.044). No significant differences were found between the anterior (1102±38 mg/cm³) and the posterior cortex and between the anterior and the subchondral regions (Fig. 3). In the posterior cortex, the von Mises stress (26.5±2.5 MPa) was higher than in the anterior (19.7±2.6 MPa, p=0.035) and subchondral (14.9±1.0 MPa, p=0.002) regions (Fig. 3). No significant differences were found between the anterior and the subchondral region. The strain energy density in the subchondral region was lower (0.020±0.003 J/m³, p=0.008) than in the posterior cortex (0.035±0.007 J/m³). No significant differences were found between the anterior (0.024±0.002 J/m³) and the posterior regions (Fig. 3). The first principal strain was oriented supero-inferiorly and was tensile (positive) in the anterior cortex (3083±463 µε) and compressive (negative) in the posterior cortex (−3698±539 µε). In the subchondral region, it was oriented antero-posteriorly and
compressive (-1550±381 με). The magnitudes of the second principal strain were
-2250±376 με, 2000±313 με and 1070± 251 με for the anterior, posterior and
subchondral region respectively. Finally, the third principal strain was −372±80 με
(anterior region), 680±162 με (posterior region) and 208± 110 με (subchondral
region). The equivalent strain was lower (p=0.030) in the subchondral region
(2652±612 με) than in the anterior (5187±790 με) and the posterior cortex
(5561±805 με, p=0.007). No significant differences were found between the
anterior and the posterior regions (Fig. 3).

Fig. 3. Regional differences in the degree of mineralization (DMB), stress and strain and strain energy
density (SED) of the anterior (dark gray), posterior (light gray) and subchondral (white) regions. Significant
differences between regions are indicated by the P values above the bars. The error lines above the bars
indicate the standard error of mean.
Sub-regional differences

The stress and the strains, as well as the DMB, were not influenced by the depth of the volumes of interest selected (i.e. the DMB, the stress and the strains did not vary significantly within the cortical thickness). The sub-region B in the anterior cortex corresponding to the posterior part of the mandibular notch (Fig. 1) had the highest DMB, but the sub-regional differences were not significant (Fig. 4). Also the von Mises stress, the equivalent strain and the strain energy density did not show significant sub-regional differences for all the three regions. In all 18 sub-regions, the orientation of the first principal strain was parallel to the surface of the cortical bone. In the anterior cortex (Fig. 5), this orientation was fan-shaped (i.e. the strain vectors were oriented significantly more laterally in the lateral sub-regions A and B and medially in the medial sub-regions D, E and F). In the medial sub-regions, the orientation showed the least interindividual variation (sub-regions D, E, F). Similarly to the anterior region, in the posterior cortex the first principal strain was also fan-shaped with very low interindividual variation. The second and the third components had varying orientations in both the regions. In the subchondral region both the first and the second principal strain were parallel to the bone surface. The first principal strain was oriented antero-posteriorly in each of the sub-regions. The second component was oriented medio-laterally (Fig. 5). Seen from the top, both components were slightly rotated clockwise. The negligible third component was oriented perpendicular to the articular surface for all sub-regions.

Regression analysis

Regional variation in DMB was correlated to local variation in the equivalent strain \( r=0.750, \ p<0.001, \) von Mises stress \( r=0.593, \ p=0.009 \), strain energy density \( r=0.625, \ p=0.006 \) and principal strains (1st component \( r=0.690, \ p=0.002 \); 2nd component \( r=0.765, \ p=0.001 \)). No significant correlations were found between histomorphometric parameters of trabecular bone (e.g. trabecular separation, trabecular thickness, bone volume fraction) and the stresses or strains occurring in cortical bone regions for regional as well as sub-regional values. No age-effect on DMB was found.
Fig. 4. Sub-regional differences in the degree of mineralization (DMB), stress and strain and strain energy density (SED) of the anterior (dark gray), posterior (light gray) and subchondral (white) regions. The error lines above the bars indicate the standard error of mean.

Fig. 5. Orientation of the first principal strain in the condyle. The projections of the first principal strains on the frontal plane are depicted for both the anterior and the posterior sub-regions. For the subchondral region, the projections on the horizontal plane of both the 1st (compression) and the 2nd principal strain (tension) are shown. L: lateral pole, M: medial pole. Significant differences between sub-regions are indicated by the P values. A three-dimensional motion representation of the 1st, 2nd and 3rd principal strains is available at http://www.ortodonzia.unina.it/3d.htm.
Discussion
To our knowledge, this is the first study that analyses the regional mechanical behaviour of the cortical bone in the human mandibular condyle by means of FE models which include the mineral distribution of the specimens as scanned with a microCT. As described earlier [15,26], the displacements at the medial and lateral poles indicated that the condyle was slightly bent posteriorly and inferiorly during simulated static clenching. The largest compliance occurred medially, suggesting torsion around a vertical axis through the neck. This is likely due to the condylar morphology as its lateral pole is mostly supported by the mandibular ramus and the condylar neck. The DMB values fall within the ranges found recently in human mandibular condyles [13] although the DMB in the anterior region was found to be lower. This difference results probably from a slightly different location definition. Indeed, in that study the anterior region included a part of the pterygoid fovea, which probably has different bone material properties in comparison with surrounding bone. In accordance with previous studies [30], a significantly lower DMB was found in the subchondral bone than in the posterior cortical region.

The distribution of stresses and strains in the mandibular condylar cortex had topographic characteristics. Once loaded, higher stress occurred in the posterior region. Similar to earlier studies, where strains were measured in vitro [15,17], tension was largely observed anteriorly and compression posteriorly. As compared to previous studies in which strain gauges were used [15,31], the relatively high strains found in our study might be due to the different measurement technique [15,31,32], but also to the relatively low Young's modulus of the FE models [33]. A positive correlation was found between DMB and both stress and strain. This is contrary to the hypothesis that a lower DMB corresponds to higher strain. A positive correlation between DMB and von Mises stress might be explained by the fact that voxels with high DMB have a high Young's modulus and consequently support the larger stresses in the tissue. These voxels, however, should also have a lower strain. Hence, the positive correlation between both stress and strain and DMB cannot be explained by the differences in Young's modulus only. Since the subchondral bone is less mineralized and has significant lower strains (Figs. 3, 6), it is likely to be responsible for the positive correlation found between the DMB and the equivalent strain within all the condyle. Therefore, the strains cannot explain the regional variations found in bone mineral content [13,14]. This suggests that there are other factors, not included in our analysis, that may keep subchondral...
bone less mineralized and stiff although lower stresses and strains occur. In accordance to previous analyses in which strains were found to be parallel to the vertical axis of the condyle [14,15], the first principal strain was parallel to the cortical surface in both the anterior and posterior regions, and oriented supero-inferiorly with a fan-like shape (Fig. 5). This matches exactly the orientation found for the canal network [22]. The least variation occurred in the antero-medial (D, E, F) and in the posterior sub-regions (Fig. 5). This might be explained by the condylar surface, which is relatively flat at these sites. Conversely, the higher variation in the antero-lateral sub-regions (A, B, C) is presumably caused by the mandibular notch. The fan-shaped orientation of the principal strains in the anterior and the posterior cortex, together with the absence of variation from the exterior to the inner layers of the tissue, denotes that cortical bone may play an important role in the transmission of the joint forces to the mandibular ramus. In subchondral bone, the first principal strain (compression) was antero-posteriorly oriented and slightly angled in medial direction. This is in contrast to the medio-lateral orientation of canals, which resemble exactly the orientation of the second (tension) principal strain (Fig. 5). It is important to note that the shear stress that occurred at the cartilaginous/subchondral bone interface may have caused a tension in the underlying bone in the antero-posterior direction [34]. Therefore, the absence of the cartilage in the model likely resulted in an overestimation of the compression in the antero-posterior direction in this region. Furthermore, the tilted orientation of the strains occurring in the subchondral region might be due to the higher displacement occurring in medial sub-regions. Surprisingly, with a load oriented perpendicular to the subchondral region, both the first and the second principal strains in this location were parallel to the surface. Presumably, as in a gothic arch, most of the joint load is transferred through the convex shape of the subchondral bone to the anterior and the posterior cortex. The support from the underlying trabecular bone seems to be less relevant since the third principal strain, which pointed out of the surface, was negligible. These findings support the hypothesis that subchondral bone is primarily involved in the transmission of joint force from the articular cartilage to the cortical bone in the condyle [35,36] and suggest that subchondral bone is more important in the distribution of the load within the mandibular condylar cortex than the underlying cancellous bone. The orientation of the principal strains clearly indicates that both
cortical and subchondral bone are primarily involved in the distribution of the strains within the condyle. It is important to note that, for this study, attempts were made to exclude all factors, other than the applied load, which might increase the amount of variation in the analyzed parameters. Firstly, only specimens from male subjects were used to exclude any effects that might have resulted from postmenopausal hormonal changes [9]. Only subjects with full or almost full dentition were selected. In order to reduce the partial volume effect, voxels of the bone surface were neglected. The selection of the anterior and posterior VOIs was limited to the lower three-quarters of the condyles to be certain of including cortical bone. The lower quarter was discarded as the boundary condition imposed on the sawing plane is not realistic. The selection of subchondral VOIs was limited to the upper third of the condyles although local boundaries might cause a partial effect in this region. However, these boundaries resemble natural conditions. The 15% most medial and 15% most lateral parts were also excluded because at these sites the selection of the VOIs was difficult because of the very thin cortical shell. It is important to remember that the compression in antero-posterior direction in subchondral region was likely overestimated in this study because of the absence of the cartilage in the model. Finally, the absence of interindividual correlations, as well as the low interindividual variation of the DMB and the trabecular morphometric parameters (bone volume fraction: 18.9±1.1%; trabecular thickness: 0.21±0.01 mm; trabecular separation: 0.81±0.02 mm; trabecular number: 0.89±0.03 mm⁻¹), suggests that interindividual variations were minimal. In conclusion, this study has provided a high-detailed description of the strains occurring within different locations of the cortical bone of the mandibular condyle during functional loading, revealing that stresses, strains and DMB have topographic characteristics. Contrary to the hypothesis, the strains correlated positively with the regional variation in DMB. This correlation was largely due to the DMB and the equivalent strain of subchondral bone. However, the strains cannot explain the differences in DMB found between cortical locations. The distribution and the orientation of the strains, together with the orientation of the cortical canals, suggest that the subchondral and the cortical bone have a different mechanical behaviour and are structured to ensure an optimal load distribution within the human mandibular condyle. In particular, subchondral bone, which is more elastic, through the convex shape of the condyle, seems to be largely involved in the
transmission of the strains to the anterior and posterior cortex, while these ensure an optimal transmission of the strains within the condylar neck and, eventually, to the mandibular ramus.
CHAPTER 3

The influence of muscular activity on bone remodelling in the human mandible

Abstract

Bone remodelling, as well as, its degree of mineralization, is affected by muscular activity. In this study bone remodelling at the attachment site of lateral pterygoid muscles was assessed, by measuring the degree of mineralization of bone (DMB), in order to test to which extent muscular activity might influence bone turnover in a certain location. Ten left mandibular condyles were obtained from ten embalmed human male cadavers (mean age ± SD: 69.8±14.4 years, range: 43 to 92 years). A high-resolution microCT system was used to obtain three-dimensional reconstructions of the condyles. For each condyle the attachment site of the lateral pterygoid muscle was identified, and the degree of mineralization measured in that location and compared to a control region where no muscle was attached. At the attachment site the DMB was lower (1036.5±70.3 mg HA/cm$^3$) than in the posterior control region (1079.3±62.3 mg HA/cm$^3$; $p=0.003$). The mineralization in the lateral subregions of the attachment (1052.2±74 mg HA/cm$^3$) was significantly higher ($p=0.016$) than in the medial subregions (1004±66.8 mg HA/cm$^3$).

The result of this study show that bone remodelling is higher at the attachment site of the lateral pterygoid muscle. Hence, muscular activity sensibly affect bone turnover.

INTRODUCTION

Bone is a dynamic tissue capable of adapting its structure to local mechanical stimuli and repairing micro damage [3]. The multi-cellular mechanism responsible for the adaptation, known as bone remodelling, allows for an optimal protection against failure.
Bone remodelling determines the mineral properties of bones. The Degree of Mineralization of Bone (DMB) is inversely proportional to the remodelling rate [9]. In fact, as a consequence of higher remodelling rates, the life span of osteogenic cells is lower, as well as the deposit of mineral content. Therefore, variations in DMB are considered valid indicators of the amount of remodelling. Changes in strain frequencies, magnitudes, and types are related to regional differences in remodelling rates [9]. For instance, it has been shown that the side of a bone, which is loaded compressively has a higher mineral density than the side which is subjected to tensile loading [10]. Also, regional differences in mineralization of long bones have been related to topographical differences in mechanical loading [11].

The characteristics of bone are affected by muscular activity. Indeed, regional variation in bone architecture and mineralization have been related to different sport and daily activities. [37,38].

Recently the DMB of the human mandibular condyle has been reported to be heterogeneous, and the differences in DMB between the anterior and posterior cortices have been suggested to be related to differences in bone remodelling between condylar surfaces [39]. This, in turn, was attributed to the activity of the lateral pterygoid muscle, which is attached at the anterior surface of the condyle. In this study we aimed to measure the DMB at the attachment site of the lateral pterygoid muscle in order to test to which extent muscular attachment might influence bone remodelling. Since the activity of lateral pterygoid muscle has been suggested to be involved in mandibular growth, the relation between muscular activity and bone remodelling at this site might be of importance in view of clinical orthopaedic correction of mandibular deficiency.

The DMB of cortical bone at the attachment site of ten lateral pterygoid muscles of ten mandibular condyles was measured by means of a micro-CT device. The sites of muscular attachment were identified and contoured and three-dimensional distribution of mineralization within each condyle was measured. It was hypothesized that DMB at the attachment site of the lateral pterygoid muscle was lower than in the control region. Since the enthesis of the human lateral pterygoid muscle is heterogeneous in its histological structures (references), differences in DMB within the attachment sites were expected.
Materials and Methods

Condyles

Ten left mandibular condyles were obtained from ten embalmed human male cadavers (mean age ± SD: 69.8±14.4 years, range: 43 to 92 years). These specimens have been used previously [39]. On average, the subjects had 10.2±4.5 teeth in the upper jaw and 11.5±2.6 in the lower jaw. No signs of relevant malocclusion or condylar erosions were found. The condyles were separated from the mandible at the transition from the neck to the ramus with a hand saw; condylar bone marrow was left in situ. The specimens were fixated in 60% formalin.

Bony attachments of upper and lower heads of the lateral pterygoid muscle were identified and dissected (fig.1). The bone was preserved and muscle tissue, as well as the articular capsule, was left in situ. Using cyanoacrylate (Histoacryl blue, Braun Melsungen AG Melsungen, Germany), slices of radiopaque markers (hand rolled gutta-percha points for dental use, size #30, Demedis, Dusseldorf, Germany) were glued to the external face of the muscle close to the bone surface at the medial, lateral and inferior boundaries of the attachment zone.

The use of the condyles conforms 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.
A high-resolution microCT system was used to obtain three-dimensional reconstructions of the condyles (µCT 40, Scanco Medical AG, Bassersdorf, Switzerland). Each condyle was positioned to be scanned in frontal slices. The scan resolution was 30 µm, the beam energy 55 kV. To minimize noise, an integration time of 1250 ms was used. To minimize beam-hardening artefacts, the system was equipped with a specially developed algorithm (Scanco Medical AG, Bassersdorf, Switzerland) and an aluminium filter (0.5 mm) was used to remove the softest rays. To assess the amount of noise and the effect of beam hardening quantitatively, homogeneous K$_2$HPO$_4$ solutions with different concentrations were scanned [23] using the same settings as described above. Noise level and the effect of beam hardening were maximally 6% and 7%, respectively, which was well below natural variations in mineralization.
The degree of mineralization (DMB) of each volume element (voxel) was computed from the attenuation coefficient using a linear relation, which was calibrated with a phantom containing hydroxyapatite densities of 0, 50, 200, 800 and 1200 mg/cm³.

Volumes of interest

In each condyle two regions were defined, namely the attachment region of the lateral pterygoid muscle, and a control region at the posterior site. To define the attachment site, each condyle was first divided into an anterior and posterior part. A plane passing through the most posterior radiopaque marker was used to define the two regions (see Fig 2).

In the anterior part, the muscle attachment zone was delimited inferiorly, medially and laterally by the radiopaque markers. The upper limit of the anterior surface was considered as the superior boundary of the attachment site. To analyze sub-regional differences, the attachment area was further divided into eight sub-regions as shown in fig. 2b.

For the control region a part of the posterior cortex was used, where no muscles or ligaments are attached to the cortex. To define this region, each condyle was divided into four supero-inferior zones. In the third one from the top, the control region was selected.

In each sub-region a volume of interest (VOI) containing only cortical bone was selected. To avoid surface artefacts, the two most superficial layers of voxels in each VOI were discarded.
Means and standard deviations of DMB were calculated for each VOI. The same parameters were also determined for the two regions by combining the subregional results. Grand means and standard deviations were then calculated over all ten condyles. One tailed paired Student T-test was used to compare the DMB of the attachment sites with the control regions. A general linear model (repeated measures) was used to identify mediolateral and superoinferior differences within the attachment site. Statistical analysis was performed using SPSS Software (version 12.0.1Inc., Chichago, IL, USA.).

Results

The regional differences in DMB are shown in fig.3. At the attachment site the DMB was lower (1036.5±70.3 mg HA/cm³) than in the posterior control region (1079.3±62.3 mg HA/cm³, p=0.003). A representation of the three-dimensional distribution of mineralization is given in Fig. 3

On average, the DMB increased in medio-lateral and in supero-inferior directions.
The mineralization in the lateral subregions C-F (1052.2±74 mg HA/cm³) was significantly higher (p=0.016) than in the medial subregions A-D (1004±66.8 mg HA/cm³). The sub-regions G-H showed a higher mineralization (1062±27.7 mg HA/cm³; p=0.049) as compared to the sub-regions D-E-F (1027.5±28.3 mg HA/cm³). No significant differences were found between the sub-regions A-B-C (1028.6±18.5 mg HA/cm³) and the lower subregions (Fig. 4). Inter-regional differences explain 7.4% of the total variation. Inter-individual differences explain 63.3% of the total variation.

Fig. 3 DMB at the attachment site and in control region
Fig. 4 Mediolateral and super-inferior differences in DMB within the attachment site

**Discussion**

To our knowledge this is the first study that analyzed the DMB at the attachment site of the lateral pterygoid muscle in human mandibles. The results of the current study suggest that remodelling rate, as assessed by DMB, at the attachment site of the lateral pterygoid is higher than in the posterior control region, where no muscle or tendon is attached. This finding is in accordance to previous studies which showed a relationship between muscular activity and bone remodelling. However, the novelty of our study is that it is the first one that related directly and anatomically bone remodelling and muscular attachment site. Different explanations might be given for the results found. First, the pulling of the lateral pterygoid muscle at this site might determine increased bone turnover, hence lower mineralization. Another possible explanation for this result might be related to the anterior-inferiorly directed load that during function is exerted in mandibular condyle. This, in turn, might cause anterior bulging of the condyle,
which may determine differences in bone turnover between the two sites investigated.

Differences in cortical bone mineralization were found between the sub-regions of the attachment site. In particular, a slightly increase in DMB in medio-lateral direction has been found. The histological characteristics of the enthesis of the lateral pterygoid muscle might explain the differences found in bone mineralization. Indeed, within the attachment zone of the lateral pterygoid muscle at the pterygoid fovea of the neck of the mandible a transition from a chondral to a periosteal structure in the tendon enthesis has been identified in four steps in a cranial-caudal direction. In particular, long tendon fibers of the cranialmost muscle fibers insert into a layer of fibrocartilaginous tissue immediately below the attachment of the mandibular joint capsule. Moreover, few tendon fibers insert immediately to the bone, while fibers below this area insert immediately to the bone and in part they interweave with the collagen fibrils of the periosteum. The caudalmost tendon fibers are completely interwoven with collagen fibrils orientated in parallel to the bone surface and elastic fibers of the periosteum [40]. Differences were also found within the structure of the proximal patellar enthesis [41]. These differences have been related to unequal force transmission from bone to tendon.

It is important to note that, for this study, attempts were made to exclude all factors which might increase the amount of variation in the analyzed parameters. Firstly, only specimens from male subjects were used to exclude any effects that might have resulted from postmenopausal hormonal changes [9]. Only subjects with full or almost full dentition were selected. In order to reduce the partial volume effect, voxels of the bone surface were neglected. The selection of the anterior and posterior VOIs was limited only to cortical bone.

In conclusion, our study has shown that the cortical bone at the attachment site of the lateral pterygoid muscle is less mineralized than a control region where no muscle or tendon is attached. Finally topographic changes in bone mineralization between the sub-regions of the attachment site were found. The results of the present study might be of particular interest in view of possible application of functional therapies in orthopaedic correction of mandibular deficiency. Indeed, our results show that muscles might considerably effect bone remodelling at a certain site.
Fig. 5 Representation of the three-dimensional distribution of DMB in ten condyles.


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ORAL PRESENTATIONS AND POSTERS

  ORAL PRESENTATION

- Farella M, Michelotti A, Cioffi I. The use of a new web-based interface to carry out multicentre RCTs using RDC/TMD criteria
  ORAL PRESENTATION
  International RDC/TMD Consortium 6° Annual Meeting - Brisbane, Australia June 28, 2006

- Fragnito R, Farella M, Cioffi I, Michelotti A, Martina R. Test diagnostici in odontoiatria e loro ruolo nelle decisioni cliniche

- Tagliaferri, R, Cioffi, I, Martina, R. The relationship between mandibular side shift and unilateral posterior cross-bite
  POSTER European Orthodontic Society Congress. Wien 2006

- Cioffi I. Sviluppo di un’interfaccia web per la realizzazione di una sperimentazione clinica multicentrica
  ORAL PRESENTATION
  XII Simposio delle Scuole Ortodontiche. Firenze, 30 Marzo 2007
  Awarded as best graduation thesis in orthodontics in 2006


• Cioffi I. Basi Biologiche delle terapie funzionali. I Summit Orthodontic school, Gaeta, Italy- 18-20 September 2008. ORAL PRESENTATION.


PUBLICATIONS in journals indexed in SCI


OTHER SCIENTIFIC PUBLICATIONS

INDICATORS OF ESTEEM
• Referee for the Journal of Biomechanics (IF 2.8)
• Referee for the Journal of Oral Sciences (IF2.07)
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