High-resolution MRI and micro-FE for the evaluation of changes in bone mechanical properties during longitudinal clinical trials: application to calcaneal bone in postmenopausal women after one year of idoxifene treatment

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Abstract

Objective. To investigate whether recently developed in vivo high-resolution magnetic resonance-imaging and micro-finite element techniques can monitor changes in bone mechanical properties during long-term clinical trials aiming at evaluating the efficacy of new drugs for the treatment of osteoporosis.

Design. Comparison of baseline and follow-up mechanical parameters calculated using micro-finite element analysis of the calcaneus for subjects participating in a study investigating the effect of idoxifene.

Background. Contemporary measurements for the evaluation of bone mechanical properties, based on dual-energy X-ray absorptiometry measurements, are not very accurate and require large trial populations.

Methods. A total of 56 postmenopausal subjects received either a placebo, 5 mg or 10 mg per day of idoxifene. Magnetic resonance-images of the calcaneus were made at baseline and after one year. Mechanical parameters of a trabecular volume of interest in the calcaneus were calculated using micro-finite element analysis.

Results. Although there were no significant differences between the mean changes in the treated groups and the placebo group, there were significant changes from baseline within groups after one year of treatment. Significant changes, however, were found only for mechanical parameters and only in the treated groups.

Conclusions. The present study is the first demonstration that longitudinal changes in bone mechanical properties due to trabecular micro-architectural changes may be quantified in long-term clinical studies. Since significant changes in mechanical parameters were obtained for the treated groups whereas no significant change in bone mass was found we conclude that the application of these techniques may increase the clinical significance of these trials.

Relevance

A precise diagnosis of in vivo bone mechanical properties that accounts for (changes in) trabecular bone architecture is of particular importance for longitudinal clinical trials aiming at evaluating the efficacy of new drugs since it can lead to clinically relevant results from shorter follow-up intervals and may enable a reduction of the number of patients involved in the trial.

Keywords: Osteoporosis; Bone mechanical properties; Magnetic resonance imaging; Finite element analysis; Bone treatments

1. Introduction

The mechanical integrity of the skeleton can be seriously affected by bone diseases, of which osteoporosis is the most common one. An accurate assessment of the mechanical integrity of bones in vivo is essential for the diagnosis of such diseases. Most quantitative diagnostic methods for bone mechanical integrity used nowadays are based on estimates of bone density measured by...
dual-energy X-ray absorptiometry (DEXA); this is the only material property that can now be clinically measured non-invasively. Although such measurements quantify the amount of bone, they do not sufficiently reflect (changes in) bone mechanical integrity [1,2], in particular since they do not account for the mechanical consequences of bone micro-architecture degradation typically seen with osteoporosis. Delmas [3] stated that the prediction of osteoporotic fractures from bone mass measurements is "... as good as that of using blood pressure to predict stroke". Hence, although bone mass correlates significantly with fracture risk, its predictive value is poor, and cannot be used as a reliable diagnostic tool by itself for the purposes implied above. From a mechanical point of view this result is not surprising. The mechanical properties of trabecular bone (e.g., stiffness and strength) depend not only on the amount of bone mass (as quantified by DEXA) but also on the spatial arrangement of the trabeculae. Consequently, changes in bone mechanical properties due to changes in its architecture, in particular those that do not go together with changes in bone mass, might not be detected with these diagnostic methods.

A precise diagnosis of bone mechanical properties that accounts for changes in bone architecture is of particular importance during longitudinal clinical trials aiming at evaluating the efficacy of new drugs for the treatment of osteoporosis. Such drugs could induce subtle changes in bone architecture that can lead to significant changes in its mechanical properties without significant changes in bone mass. An evaluation of the mechanical effects of these structural changes potentially could lead to the detection of significant changes in bone mechanical properties before significant changes in bone mass can be detected or in response to agents in which the relationship between changes in bone mechanical properties and bone mineral density (BMD) was different from currently approved therapies. This, in turn, could lead to clinically relevant results from shorter follow-up intervals. Accounting for changes in bone architecture may also enable a reduction of the number of patients involved in the trial because information on bone mechanical properties may be obtained from every participant instead of the small proportion who may sustain a fracture.

An accurate determination of bone mechanical properties that fully accounts for the structural arrangement of trabeculae is possible with a recently developed micro-finite element (FE) technique [4,5]. With this technique, the morphology of the trabecular bone is measured by a large number of sequential cross-sectional images. These digitized images are stacked in a computer in which the 3-D trabecular structure is reconstructed as a rectangular voxel grid, with voxels representing bone tissue or marrow. By converting voxels representing bone tissue to equally shaped brick elements in a micro-FE model a micro-FE model is generated that can represent the trabecular structure in great detail. By simulating compression tests, this technique enables a complete evaluation of trabecular bone anisotropic elastic properties [6,7], tissue loading [8] and multi-axial strength [9]. The micro-FE approach, however, was developed for use in combination with images obtained from in vitro imaging techniques, such as micro-computed tomography (CT) and serial sectioning, that enable a resolution of 50 μm or better.

Results of several recent studies demonstrated that the micro-FE approach can provide an adequate evaluation of structure-related bone mechanical properties also for bone in vivo. Presently, two imaging techniques exist that can be applied to bone in vivo and provide a resolution adequate to visualize individual trabeculae. With the first of these techniques, a peripheral quantitative computed tomography (pQCT) device is used with an isotropic spatial resolution of 165 μm [10,11]. With the second, a whole-body magnetic resonance (MR) scanner is used, providing a resolution of approximately 150–200 μm in plane at a plane thickness of 300–500 μm [12,13]. The resolution of these imaging techniques thus is considerably less than that of the in vitro imaging techniques. In addition, these imaging techniques cannot provide images with the same level of signal-to-noise-ratio and reproducibility. Nevertheless, when applying proper image processing techniques, adequate 3-D reconstructions of the trabecular architecture can be generated from images at this resolution [14,15]. In a number of earlier validation studies, the feasibility of this micro-FE approach in combination with such pQCT and MR images was investigated [16–18]. In these studies it was found that micro-FE analyses can provide accurate results, in particular when using a special ‘mass preservative’ segmentation technique [16], but that correction factors might be needed to predict accurate values. In a recent study, it was found that micro-FE analyses based on pQCT images of whole bones in situ can provide a prediction of bone strength that is much better than predictions based on DEXA scans [19].

In the present study, we aim to find a rigorous answer to the question whether these recently developed high-resolution MR-imaging and micro-FE techniques can monitor changes in bone mechanical properties during long-term clinical trials aiming at evaluating the efficacy of new drugs for the treatment of osteoporosis. For this study, high-resolution MR-images of a large number of patients were available from a large study investigating the effect of idoxifene, a selective estrogen receptor modulator, on change in bone mineral density in a cohort of postmenopausal women. In the present study we use MR-images of the calcaneus that were available at baseline and after one year of the treatment. A specific purpose of this study was to investigate whether the use
of micro-FE can reveal changes in bone mechanical properties over the one-year interval.

This study is part of a larger project aimed at establishing the feasibility and potential of MR imaging and micro-FE analyses for bone in vivo in longitudinal trials. In an earlier study, we investigated the reproducibility of the MR-imaging procedure and the parameters obtained from these [20]. In that study it was found that a reproducibility of 2–4% can be expected for 2-D structural parameters and from 4–9% for 3-D parameters (including micro-FE calculated elastic moduli). In another earlier study [21], we used MR images from the same idoxifene trial to quantify micro-FE calculated moduli, morphological parameters DEXA measurements, biochemical markers and their interrelationships for subsets of the population at baseline. The present study is the first to investigate changes in bone mechanical properties after one year of treatment.

2. Methods

2.1. Patients and subjects

A subset of 56 subjects participating in a large randomized double blind multicenter study investigating the effect of idoxifene on lumbar spine BMD were recruited for MR imaging of the calcaneus at two institutions, namely University of California, San Francisco (UCSF) and the University of Washington (UW) Seattle, USA, in accordance with the regulations of the Committee of Human Research at the respective institutions. Subjects did not have a history of substance abuse (including alcohol) over the past years, treatment with any investigational drug within the last 30 days, or other medical condition and lifestyle habits, which would affect bone turnover. Subject with anatomical deformities of the spine sufficient to interfere with the assessment of bone mineral density or with fractures of the lumbar (L1–L4) vertebrae were excluded. In addition, for the MR scans, subjects with pacemakers, metallic fragment in the eyes, vascular clips, aneurysm clips, cochlear implants, claustrophobic subjects and those with body mass exceeding 113 kg were excluded.

All subjects in the subset were postmenopausal and received 500 mg/day of elemental calcium and either a placebo (n = 18), 5 mg/day (n = 23) or 10 mg/day (n = 15) of idoxifene.

2.2. BMD measurement

DEXA scans of the calcaneus were performed with a QDR 2000 scanner (Hologic, Waltham, MA, USA) using a procedure described earlier [12]. Subjects were scanned lying on their sides with their forefoot fixed with adhesive tape. Manufacturer-supplied software was used for the data analysis. The average BMD value was determined for a rectangular region of interest, 1.35 cm² in size, located approximately at the center of the posterior calcaneus. Measurements were made at baseline and after one year of treatment.

2.3. Magnetic resonance imaging

All MR images were acquired using a General Electric Signa scanner operating at 1.5 T (General Electric Medical Systems, Milwaukee, WI, USA). The subject lay supine, with the foot held firmly in a custom built holder. The foot holder minimized patient motion during image acquisition and also provided reproducible positioning for successive scans of the same subject. Images were obtained at the calcaneus using special-purpose coils. Sagittal high-resolution images were obtained at a spatial resolution of 195 μm in plane and 500 μm in slice direction using a high-resolution modified 3-D GRE pulse sequence [12]. The flip angle, echo time and repetition time were 30°, 5.6 and 29 ms, respectively. The total imaging time for the high-resolution scan was 12 min. MR images were obtained at baseline and after one-year follow-up.

2.4. Image processing

The MR scans were processed using software developed in house. Correction algorithms were applied to correct for coil inhomogeneities. A rectangular volume of interest (VOI) of 101 × 101 × 20 voxels, corresponding to a side length of 19.7 × 19.7 × 10.0 mm³, was selected at the center of the calcaneus. The exact location of this region was defined by the center of the largest circle that could fit within the posterior part of calcaneus, thus providing a reproducible location based on anatomical features (Fig. 1).

A global threshold criterion based on two reference intensity levels, one for marrow fat and one for bone, was determined as described earlier [12]. A special iterative segmentation algorithm was used to ensure that a well connected structure remains after segmentation with a correct volume fraction. In an earlier study it was found that this segmentation algorithm provides the most accurate results for FE analyses when using images of the resolution considered here [16,17]. With this algorithm the images are segmented using the global criterion and a reference bone volume fraction, as quantified by the amount of bone tissue volume (BV) over the total volume (TV), is calculated as the number of bone voxels over the total number of voxels in the VOI. Following, parts that are poorly connected to the main structure are removed. This is necessary to avoid numerical problems during the FE analyses and would not affect the results of these analyses since the poorly connected parts do not contribute to the stiffness of the...
specimens and do not carry load. Removal of these unconnected structures, however, reduces the bone volume fraction. To compensate for this, a new threshold criterion is determined iteratively such that the volume fraction of the final reconstruction corresponds to the reference value. This procedure ensures that the final FE-models will have a properly connected structure with a correct volume fraction.

2.5. Finite element analysis

The segmented reconstructions of the volumes of interest selected in the baseline and one-year images were converted to micro-FE models by converting the voxels that represent bone tissue to equally shaped 8-node brick elements. This resulted in FE-models with approximately 85,000 elements (Fig. 2). The tissue element properties were chosen linear elastic and isotropic with a Young’s modulus of 10 GPa and a Poisson’s ratio of 0.3 for all models. Using a special-purpose FE-solver [22], six FE-analyses were performed for each specimen, representing three compression tests in the three spatial directions and three shear tests. Total cpu time was on the order of 1 h per VOI when using a fast workstation computer. The anisotropic stiffness matrix of the VOI was calculated from the results of these analyses. An optimization procedure was then used to find a new coordinate system aligned with the best orthotropic symmetry directions of the specimen [6]. The compliance matrix was rotated to this new orthotropic coordinate system, and the three Young’s moduli, three Poisson’s ratios and three shear moduli were calculated in these principal directions. The matrices were sorted such that the Young’s modulus in the primary direction \( E_1 \) represents the largest modulus of the specimen and the third Young’s modulus \( E_3 \) the smallest one: \( E_1 > E_2 > E_3 \). The shear moduli were denoted as \( G_{12} \), \( G_{23} \), and \( G_{13} \). In addition the elastic anisotropy ratios...
$E_1/E_3$, $E_2/E_3$ and $E_1/E_2$ were calculated. The advantage of the applied optimization and rotation procedure is that the actual values found for the elastic parameters are independent of the rotation of the specimen and reflect the values in the bone principal directions.

2.6. Statistical analysis

For all parameters, the mean values and standard deviations of the parameters were calculated at baseline and after one year of treatment. The average change in the parameters after one year of treatment was quantified as:

$$\Delta V = \frac{1}{N} \sum_{i=1}^{N} \left( \frac{V_{i, \text{1 year}} - V_{i, \text{Baseline}}}{V_{i, \text{Baseline}}} \right) 100\%,$$

with $V_{i, \text{1 year}}$ the value of the parameter for patient $i$ after one-year follow-up, $V_{i, \text{Baseline}}$ the value at baseline and $N$ the number of subjects in the group. All calculations were done on the blinded data. Statistical analyses were done using SPSS 10.

3. Results

3.1. Descriptive statistics

An overview of the mean and standard deviations for the investigated parameters at baseline and after one-year follow-up is given in Table 1 for the placebo, the 5 mg and the 10 mg group. The percentage change from baseline for the parameters is represented in a box plot (Fig. 3). This plot revealed that the median value of changes in BV/TV and elastic constants are close to zero for the placebo group and increase with increasing idoxifene dose. The plots also show a number of outliers, however, only for the placebo and the 5 mg group and not for the 10 mg group.

3.2. Significance analysis

Given the limited sample size of this study, we chose not to exclude outliers but to investigate the significance of the observed changes with non-parametric tests. The significance of changes between groups was investigated with a Kruskal–Wallis test. This test revealed that there were no significant differences between the mean changes in the treated groups and the placebo group for any of the investigated parameters.

Using a paired Wilcoxon signed rank test, however, significant changes from baseline within groups were found after one year of treatment, but only for the treated groups (Fig. 4). Values at one year for the placebo group were not significantly different from baseline for any parameter. For the treated groups,

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n=18)</th>
<th>5 mg (n=23)</th>
<th>10 mg (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{i, \text{Baseline}}$</td>
<td>0.390 (0.053)</td>
<td>0.407 (0.065)</td>
<td>0.396 (0.052)</td>
</tr>
<tr>
<td>$V_{i, \text{1 year}}$</td>
<td>0.447 (0.065)</td>
<td>0.512 (0.065)</td>
<td>0.442 (0.052)</td>
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<td>$V_{i, \text{Baseline}}$</td>
<td>2.329 (0.455)</td>
<td>2.433 (0.485)</td>
<td>2.383 (0.465)</td>
</tr>
<tr>
<td>$V_{i, \text{1 year}}$</td>
<td>2.430 (0.485)</td>
<td>2.434 (0.485)</td>
<td>2.446 (0.485)</td>
</tr>
<tr>
<td>$V_{i, \text{Baseline}}$</td>
<td>0.64 (0.15)</td>
<td>0.64 (0.15)</td>
<td>0.63 (0.15)</td>
</tr>
<tr>
<td>$V_{i, \text{1 year}}$</td>
<td>0.70 (0.15)</td>
<td>0.70 (0.15)</td>
<td>0.70 (0.15)</td>
</tr>
</tbody>
</table>

Table 1. Mean and standard deviations for the investigated parameters at baseline and after one-year follow-up per group.
changes significantly different from baseline were found for the highest longitudinal modulus $E_1$ in the 5 mg group and for the lowest modulus $E_3$ in the 5 mg and 10 mg group. Significant changes from baseline were also found for all shear moduli in the 5 mg group and for $G_{23}$ in the 10 mg group. Predominant changes for the treated groups were found for the smallest longitudinal modulus $E_3$ which increased by 20–31% and for the shear moduli in the third direction that increased by 14–24%.

No significant changes were found for the BMD values measured from the DEXA scans of the calcaneus nor for changes in the reference volume fraction (BV/TV) determined from the MR-images on which the micro-FE models were based (Fig. 5).

One-year values for anisotropy ratios were significantly different from baseline for all groups, including the placebo group (Fig. 6). In all groups the anisotropy ratio was significantly reduced after one year of treatment, with the largest reduction for the placebo group.

4. Discussion

In the present study, we asked the question whether recently developed high-resolution MR-imaging and
micro-FE techniques can monitor changes in bone mechanical properties during long-term clinical trials. Based on the results obtained here we conclude that, indeed, the application of these techniques can be justified. Significant changes in mechanical parameters were obtained for the treated groups whereas no significant change in bone mass was found. Consequently, the application of these techniques may increase the clinical significance of these trials. In addition, the methods can provide direct and very detailed information about mechanical parameters, which are the most relevant parameters given the fact that it is the decreased bone strength caused by the pathophysiological changes of osteoporosis that leads to bone fractures.

No significant changes were found, however, between the treated groups and the placebo group for any of the investigated parameters. This is likely due to the sample size, the large heterogeneity in the placebo group, the relatively short follow-up period of one year and the small effect of idoxifene on systemic bone loss after one year (approximately 2% increase from placebo at the lumbar spine [23]). A trend to an increase in bone mass, as represented by an increase in BV/TV, is found in the present study as well for the VOI in the calcaneus (Figs. 3 and 5). However, this trend is not supported by the DEXA measurements that indicate only a minor increase in bone mass for all groups with the lower values for the treated groups. Although differences between DEXA BMD and MR-calculated BV/TV results could exist due to the fact that the former include changes in the cortical bone as well, a likely explanation for this discrepancy is that changes in BV/TV and mechanical parameters were close to the limit of reproducibility of the method. The trend to increased BV/TV thus might reflect an overestimation of bone mass for the one-year images. However, since groups were randomized and all treated in the same way, the results for all groups would be affected to the same extent. Consequently, although reproducibility uncertainties may be responsible for the considerable increase in BV/TV seen after one-year and for some of the scatter seen in the MR data, they would not affect our main conclusion with regard to the improved significance of the results.

Since the calculated elastic constants are largely dependent on the bone volume fraction calculated from the MR-images (BV/TV), spread in the volume fraction due to reproducibility or other issues will result in spread in the calculated elastic constants as well. As a result of the spread in the calculated BV/TV, the dose dependent response that is seen in the median values of Fig. 3 is not seen in the average change of each parameter as shown in Figs. 4 and 5. The elastic anisotropy ratios, however, being dimensionless parameters, are much less dependent on (uncertainties in) the volume fraction. It can be seen that these parameters show a dose dependent response (Fig. 6). The fact that the anisotropy ratios are not much dependent on the bone volume fraction can also explain why significant changes in the elastic anisotropy ratios can exist for groups in which no significant changes in elastic moduli could be detected.

The fact that there was much more spread in the data for the placebo group than for the treated groups could suggest that the idoxifene treatment inhibits drastic changes in the trabecular architecture and thus may have beneficial effects on an individual as well as a population level. This suggestion would be supported by the fact that changes in bone anisotropy were smaller in the treated bone than in the placebo group. Given the limited population size, however, we can only speculate at this point.

While the calcaneus is not usually affected by osteoporotic bone fractures, it is a site that is used in the studies of osteoporosis as calcaneal measures have been shown to relate to the prevalence of osteoporotic hip fractures [24–26]. However, our aim in this study was to determine whether it was feasible to longitudinally monitor changes in bone mechanical properties using non-invasive methods in vivo. The choice for this site was largely determined by limitations of the high-resolution imaging method. Presently, resolutions of 195 μm or better can be obtained only for the peripheral skeleton, such as the wrist and the heel. MR-images of the distal radius were available for many patients in the idoxifene study as well and we are presently investigating if similar changes can be detected for that site.

There are issues of importance that warrant discussion. First, the clinical trial, which was planned for two years, was discontinued after one year due to an unacceptable safety profile of the drug. This also implies that there may have been patient non-compliance due to adverse reactions, which might have contributed to the large scatter in the data. Second, in the present analyses only changes in elastic parameters were calculated. Although micro-FEA can be used for the calculation of bone strength as well [27], albeit probably not with images of this resolution, this was not the goal of the present study. For the site investigated here, a quantification of bone strength was considered not very relevant. Nevertheless, since there is a high correlation between bone elastic properties and strength the results obtained here can be used as a first estimate for changes in strength as well. Third, the resolution of the MR-images is poor for the imaging of trabecular bone structure, in particular in the ML direction, which corresponded to axial direction of the MR-images in which the resolution was only 500 μm. In this direction, results of the FE-analyses can be less accurate. However, with the use of the special iterative segmentation algorithm used in this study it is possible to create meaningful micro-FE models even at this resolution.
The present study is the first demonstration that longitudinal changes in bone mechanical properties due to trabecular micro-architectural changes may be quantified in long-term clinical studies. Important advantages over analysis methods based on BMD are that the approach presented here provides information about changes that are specific to cancellous bone regions and that the parameters detected in the micro-FE analyses provide a direct measure of bone mechanical properties.

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References