Patient-specific bone modelling and remodelling simulation of hypoparathyroidism based on human iliac crest biopsies

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1. Introduction

It is well known that bone is able to adapt its density and micro-architecture to its mechanical environment in such a way that bone is added at highly loaded locations and removed at unloaded locations ('Wolff's law') (Barak et al., 2011; Forwood and Turner, 1995; Frost, 1987a; Goldstein et al., 1991; Rubin and Lanyon, 1987; Schulte et al., 2011; Wolff, 1892). This load-adaptive bone modelling and remodelling process, governed by bone cells, normally leads to bone structures adapted to simple loading conditions.

A second requirement is the assessment of the patient-specific loading history to which the bone micro-architecture was adapted. This is needed to define the starting point for bone modelling/remodelling simulations that are aimed at predicting effects of changes in loading or cell activity. For the latter, we recently

We previously developed a computer model to simulate load-adaptive bone modelling and remodelling also accounting for changes in cell activity (Huiskes et al., 2000; Ruimerman et al., 2005). The model is based on the theory that osteocytes in bone sense mechanical loading and transmit a signal to osteoblasts on the bone surface to form bone accordingly whereas osteoclasts resorb bone in a spatially random fashion to repair micro-damage. This model was capable to predict the effects of altered loading conditions as well as the effects of changes in cell activity on the bone micro-architecture (Huiskes et al., 2000; Ruimerman et al., 2005; van Oers et al., 2008). So far, however, this model was only applied to artificially generated bone structures adapted to simple loading conditions.

To apply load-driven bone modelling/remodelling algorithms to patient data, a first requirement is the measurement of bone micro-architecture in vivo. This is possible now for the peripheral skeleton or from bone biopsies (Boutroy et al., 2005; Boyd, 2008; Majumdar et al., 1997; Muller et al., 2009, 1996, 1994; Rubin et al., 2010a). A second requirement is the assessment of the patient-specific loading history to which the bone micro-architecture was adapted. This is needed to define the starting point for bone modelling/remodelling simulations that are aimed at predicting effects of changes in loading or cell activity. For the latter, we recently
developed a method to estimate in vivo bone loading based on bone morphology (Christen et al., 2012). The approach assumes that due to continuous bone modelling and remodelling, tissue is laid down at high-load locations and removed at low-load locations, which leads to a homogeneous tissue loading distribution. Therefore the loading history can be determined by finding a set of external bone loads that leads to the most uniform loading distribution. By combining the load estimation method and the bone modelling/remodelling algorithm, patient-specific simulations of bone modelling and remodelling are possible. Such simulations would also require patient-specific resorption and formation rates, at least if it is not possible to assume a general average change in these parameters, for example, simulating effects of a certain disease.

In the present study we wanted to test the ability of simulating bone modelling and remodelling in a patient-specific manner using our previously developed bone modelling/remodelling and load estimation algorithms to predict changes in bone micro-architecture during hypoparathyroidism (HypoPT) as an illustrative example. HypoPT is characterized by hypocalcemia in association with a low serum parathyroid hormone (PTH) level (Rubin et al., 2010b) and is mainly associated with a profound suppression of bone turnover by approximately 80% (Rubin et al., 2008) and a substantial increase in bone mass in the range 10–32% (Abugassa et al., 1993; Rubin et al., 2008) or in some cases up to 75% (Rubin et al., 2010a). It is unlikely that such increases in bone mass are the result of the strong suppression of bone turnover alone, since complete suppression of osteoclast activity would increase the bone mass by no more than 5% due to filling of the resorption space (Deng et al., 2005; Heaney, 1994; Khan, 2001; Van Der Linden et al., 2001). The low bone turnover could be explained by down-regulating receptor activator of nuclear factor-κB ligand (RANKL) (Mosekilde, 2008; Wada et al., 2006) and resorption and formation coupling factors such as transforming growth factor-β1 (TGF-β1) (Tang et al., 2009). On the other hand, it is also known that PTH alters the sensitivity of osteocytes to mechanical loading (Miyachi et al., 2000), e.g. due to triggering calcium influx and thus the expression of insulin-like growth factor 1 (IGF-I) which influences bone formation (Lean et al., 1995; Mikuni-Takagaki et al., 1996). We therefore hypothesize that HypoPT must also lead to increased osteocyte mechanosensitivity in order to explain the changes in bone mass seen in patients (Fig. 1).

The goal of this study was to test this hypothesis by combining patient-specific bone modelling/remodelling simulations and experimental data obtained from bone biopsies taken from the iliac crest. Bone loading was estimated for healthy human iliac crest biopsies and applied in computer simulations of bone modelling and remodelling to simulate HypoPT. The resultant bone micro-architectures were then compared to age-matched biopsies of HypoPT patients.

2. Materials and methods

2.1. Bone samples

Human cadaver samples of the iliac crest (Dequeker, 1994) were used as the starting point to simulate HypoPT. Samples of subjects who had a disease that could possibly affect bone structure were excluded. The remaining samples were age-matched to bone biopsies from the iliac crest of HypoPT patients as prepared and documented earlier (Rubin et al., 2010a), leading to a clinically healthy group of 7 samples and a clinically HypoPT group of 13 samples with ages ranging from 59 to 66 years.

2.2. Micro-computed tomography

Biopsy micro-architecture of the clinically healthy and clinically HypoPT groups were assessed using micro-computed tomography (micro-CT) as in the previous studies (Dequeker, 1994; Rubin et al., 2010a). For our simulations, cuboids of 4.23 × 4.23 × 3.96 mm³ with a voxel size of 45 μm³ from the clinically healthy group (n = 7) were transformed into finite element (FE) models by exporting the bone density values using Image Processing Language (IPL, Scanco Medical AG, Brüttisellen, Switzerland). To compare bone morphometric parameters, biopsies of the clinically HypoPT group (n = 13) were used.

2.3. Bone remodelling algorithm

A previously developed and tested load-driven bone modelling/remodelling algorithm (Huiskes et al., 2000; Ruimerman et al., 2005) was used to simulate HypoPT starting with healthy iliac bone biopsies. The algorithm is based on the theory that osteocytes within the bone sense mechanical loading, calculated using FE analysis and represented as strain energy density rate, U, and transmit a signal to the osteoblasts on the bone surface to form bone accordingly. Only osteocytes within the influence region, dinf, contribute to the final osteocyte signal received by the

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**Fig. 1.** Schematic overview of the proposed mechanisms involved in HypoPT. We hypothesized that low PTH suppresses bone resorption via parathyroid hormone receptor 1 (PTH1), e.g. due to down-regulation of RANKL and reduced bone formation via coupling factor TGF-β1. In addition, the change in PTH level increases the osteocyte mechanosensitivity, e.g. due to increased calcium influx leading to increased expression of IGF-I.
osteoblast at the bone surface. The osteocyte signal is assumed to decay with increasing distance $d$ between osteocyte and osteoblast according to an exponential function with a typical decay distance $D$. The total osteocyte signal, $P$, received from a total of $N$ osteocytes at location $x$ of an osteoblast can then be written as:

$$P(x,t) = \sum_{i=1}^{N} \frac{\delta(t-d_{i,\text{ref}})}{\mu} U(x_i, t) \quad \text{if} \quad d(x,x_i) < d_{\text{ref}}$$

(1)

where $\delta$ is the osteocyte mechanosensitivity, $x_i$ the location of the osteoblast and $x_i$ the location of osteocyte $i$. The osteocyte mechanosensitivity is a relative measure quantifying the osteocyte sensitivity to the mechanical loading where a value of 1.0 represents the healthy physiological situation.

If an osteoblast receives a total signal, $P$, that exceeds a certain bone formation threshold, $k$, the volume of bone matrix formed, $V_{\text{incr}}$, over time, $t$, with the formation rate, $\tau$, is determined by

$$\frac{dV_{\text{incr}}}{dt} = \tau(P(x,t) - k) \quad \text{if} \quad P(x,t) > k$$

(2)

Osteoclasts are assumed to resorb bone at a random location on the trabecular surface at a rate that is determined by the osteoclast activation frequency, $f_{\text{act}}$. At each location, the volume of bone resorbed per cavity is determined by $V_{\text{res}}$. Therefore, the total volume of resorbed bone, $V_{\text{res}}$, over time is determined by

$$\frac{dV_{\text{res}}}{dt} = f_{\text{act}} V_{\text{res}}$$

(3)

Model parameters were defined according to bone physiological values and the bone formation rate, $\tau$, and threshold, $k$, were determined empirically based on experience from earlier studies (Table 1) (Huiskes et al., 2000; Ruimerman et al., 2005; van Oers et al., 2008).

2.4. Bone loading estimation method

Since a load-driven bone modelling/remodelling approach was used, physiological in vivo loading conditions had to be found for each biopsy. For that purpose, our recently developed bone loading estimation method was used (Christen et al., 2012). This method is based on the hypothesis that the internal micro-architecture of bone is adapted to its mechanical environment by adding bone at highly loaded sites and removing bone at low loaded sites leading to a homogeneous tissue loading distribution. In order to comply with our bone adaptation hypothesis in this study and because bone cells act according the osteocyte signal which incorporates mechanical tissue loading, we assumed that bone strives for a homogeneous osteocyte signal, $P$, throughout the bone. The loading history is determined by scaling a set of $n$ predefined unit loads with a scaling factor, $s$, until the summed resultant osteocyte signal of each load case, $i$, reaches the most uniform distribution throughout the bone using an optimization technique. With the bone formation threshold, $k$, as a target value, the scaling factors can be determined by minimizing the residual function, $r(s)$:

$$\min_{s} r(s) = \int \left( \sum_{i=1}^{n} s P_{\text{mm}}(s,k) - k \right)^2 dV$$

(4)

Scaling each unit load with its scaling factor finally gives the loading magnitude, $A_i$. In this study the loading was estimated for each biopsy of the clinical healthy group using a total of 6 unit load cases representing distributed normal and shear loads prescribed as stresses, each in the three orthogonal directions (Fig. 2). These unit load cases should be able to represent biopsy-specific loading conditions since combining them results in a wide spectrum of forces. Even after optimizing loading conditions, however, no perfect homogeneous osteocyte signal distribution was obtained. For that reason, an initial bone modelling/remodelling simulation for each model was performed in which the micro-architecture was adapted to the predicted model-specific external loading conditions and bone formation and resorption were balanced. This homeostatic micro-architecture as well as the model-specific loading conditions, further called adapted group, were then used as the starting point for the HypoPT simulations and the healthy reference for all other results.

2.5. Hypoparathyroidism simulation

To simulate HypoPT, osteoclast activation frequency, $f_{\text{act}}$, was reduced by 80% according to clinical measurements (Rubin et al., 2008) and the osteocyte mechanosensitivity, $\mu$, was increased stepwise by 0%, 20%, and 40%, since no data were found in the literature for the amount of increase in osteocyte mechanosensitivity.

2.6. Evaluation of results and statistical analysis

To quantify the change between the micro-architectures of the original and load-adapted healthy biopsies, and compare HypoPT simulation results to the age-matched clinical HypoPT group, common morphometric parameters for trabecular bone were determined using IPL, wherever applicable using direct measurement methods (Hildebrand and Rueegsegger, 1997; Odgaard, 1997; Odgaard and Gundersen, 1993): bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), structure model index (SMI), and connectivity density (Conn.D) (Table 2). The three HypoPT simulations with 100%, 120%, and 140% osteocyte mechanosensitivity were compared to the clinical HypoPT group with a one-way analysis of variance (ANOVA) for each morphometric parameter (Table 3). Dunnett’s post hoc test (2-sided) was then used to compare each simulated HypoPT group against the clinical HypoPT group. The assumption of homogeneity of variances was verified using Levene’s test and if $p < 0.05$, Dunnett T3 post hoc test for comparison with unequal variances was used. For all statistical analysis, IBM SPSS Statistics 19 (SPSS Inc., Chicago II, USA) was used and a value of $p < 0.05$ was considered as significant.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocyte density</td>
<td>$n$</td>
<td>$\text{mm}^3$</td>
<td>$4.4 \times 10^6$</td>
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<tr>
<td>Osteocyte mechanosensitivity</td>
<td>$\mu$</td>
<td>$\text{mm}^{-1}$</td>
<td>1.0</td>
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<tr>
<td>Osteocyte influence distance</td>
<td>$d_{\text{ref}}$</td>
<td>$\mu$m</td>
<td>150</td>
</tr>
<tr>
<td>Osteocyte decay distance</td>
<td>$D$</td>
<td>$\mu$m</td>
<td>100</td>
</tr>
<tr>
<td>Bone formation threshold</td>
<td>$k$</td>
<td>$\text{mm}^{-1}$</td>
<td>$5.0 \times 10^6$</td>
</tr>
<tr>
<td>Bone formation rate</td>
<td>$\tau$</td>
<td>$\text{mm}^{-1}$</td>
<td>$2.0 \times 10^{-7}$</td>
</tr>
<tr>
<td>Resorption amount per cavity</td>
<td>$V_{\text{res}}$</td>
<td>$\text{mm}^3$</td>
<td>$5.6 \times 10^{-5}$</td>
</tr>
<tr>
<td>Osteoclast recruitment frequency</td>
<td>$f_{\text{act}}$</td>
<td>$\text{Hz}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Loading magnitude</td>
<td>$A_i$</td>
<td>$\text{MPa}$</td>
<td>0.91 (0.31)</td>
</tr>
<tr>
<td>developed bone loading estimation</td>
<td>$A_0$</td>
<td>$\text{MPa}$</td>
<td>0.87 (0.23)</td>
</tr>
<tr>
<td>method $^a$</td>
<td>$A_0$</td>
<td>$\text{MPa}$</td>
<td>0.71 (0.21)</td>
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<tr>
<td>developed bone loading estimation</td>
<td>$A_0$</td>
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<td>0.73 (0.20)</td>
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<tr>
<td>method $^a$</td>
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<tr>
<td>Loading frequency</td>
<td>$f$</td>
<td>$\text{Hz}$</td>
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<tr>
<td>Bone stiffness</td>
<td>$E_b$</td>
<td>$\text{GPa}$</td>
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<td>Poisson’s ratio</td>
<td>$\nu$</td>
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<td>0.3 $^b$</td>
</tr>
<tr>
<td>Material constant</td>
<td>$\gamma$</td>
<td></td>
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</tr>
<tr>
<td>Increment</td>
<td>$\text{Incr}$</td>
<td>$\text{Year}$</td>
<td>0.13 $^f$</td>
</tr>
</tbody>
</table>

$^a$ Muller et al., (1996).
$^d$ Mullender et al., (2012).
$^g$ Values refer to as mean (SD).

Fig. 2. Unit load cases that were scaled according to the estimated scaling factors for each biopsy and then used for the bone modelling/remodelling simulations. Stresses represented as distributed forces are applied to the faces of the biopsy in the direction and at the face as indicated by the arrows.
3. Results

The bone loading estimation method predicted loading with a high variation between biopsies for each of the 6 load cases (Table 1). Predicted loading was applied to the initial biopsies to adapt the micro-architecture until bone formation and resorption equilibrium was reached. In that adaptation simulation, mean BV/TV and SMI changed within 1 standard deviation, mean Tb.Th and Tb.Sp within 2 standard deviations, and Tb.N and Conn.D within 2.5 standard deviations. Initial and its adapted micro-architecture of a biopsy were also compared qualitatively (Fig. 3a, b).

With respect to the HypoPT simulations, not increasing the osteocyte mechanosensitivity, thus only suppressing osteoclast activation by 80%, led to an average increase in BV/TV of 6.2%. Reducing the osteoclast activation frequency and increasing the osteocyte mechanosensitivity by 20% and 40% caused an average increase in BV/TV of 21.2% and 35.1% respectively (Table 2). The stepwise increase of the mechanosensitivity also indicates a positive relationship between bone mass and osteocyte mechanosensitivity.

Bone resorption and formation dynamics were different when increasing the osteocyte mechanosensitivity in addition to osteoclast suppression. When only osteoclasts were suppressed, resorption and formation dropped sharply to a new equilibrium and bone formation lagged slightly behind resorption (Fig. 4a). Simulations with more sensitive osteocytes led to a peak bone formation before dropping to a lower level at a new equilibrium of bone formation and resorption (Fig. 4b).

Bone volume fraction (BV/TV) and trabecular thickness (Tb.Th) were greater and trabecular separation (Tb.Sp), structure model index (SMI), and connectivity density (Conn.D) were smaller in the simulated HypoPT groups compared to the adapted healthy group, the only parameters that did differ significantly were Tb.Th, Tb.N, and Conn.D. In the simulation with 120% mechanosensitivity additionally Tb.Sp was significantly different and with 100% mechanosensitivity, all measured morphometric parameters were significantly different from the clinical HypoPT group except BV/TV and Tb.Th (Table 3). Microarchitectures were also compared qualitatively showing the difference in bone mass between the adapted and simulated as well as the clinical HypoPT groups (Fig. 3b–d).

4. Discussion

According to the HypoPT bone modelling/remodelling simulations the two mechanisms, suppressed osteoclast activation combined with increased osteocyte mechanosensitivity, led to a gain in bone mass of 35%, which is in the range of what has been measured in HypoPT patients (Abougassa et al., 1993; Rubin et al., 2010a, 2008). This supports the hypothesis that certain hormones alter the sensitivity of bone cells to mechanical loading as already proposed by Frost (1987b) in his ‘mechanostat’ theory, which states that bone resorption exceeds formation below a certain threshold of mechanical loading and that above this threshold, bone is maintained within a particular range of stimulus. He speculated that hormones might alter this threshold allowing an increase in bone formation at the same mechanical strain level when the threshold is lowered (Ryder and Duncan, 2000). Such a mechanism was required in our HypoPT simulation because if only osteoclast activation was suppressed, bone mass increased by only 6%, a change which is related to the filling of the resorption space of approximately 5% (Deng et al., 2005; Heaney, 1994; Khan, 2001; Van Der Linden et al., 2001).

Increased osteocyte mechanosensitivity indeed resulted in a further gain in bone mass in our HypoPT simulations. Resorption and formation dynamics of the simulation revealed that, if only osteoclasts were suppressed, resorption and formation dropped sharply and were not able to account for the bone mass gained in HypoPT (Fig. 4a). In contrast, if mechanosensitivity was increased, bone formation peaked first before dropping to a lower level at a new equilibrium of bone formation and resorption (Fig. 4b). This peak bone formation, the sharp drop of bone resorption, as well as the new equilibrium at a lower level have been similarly measured in iliac bone biopsies after parathyroidectomy in the first weeks (Yajima et al., 2007, 2003).

Previous assessments of bone morphology showed that trabecular structure is highly abnormal in HypoPT (Rubin et al., 2010a, 2008). In general it was reported that BV/TV and Tb.Th are markedly increased and Tb.Sp is lowered. Further, an unusual dominant plate-like structure was found, indicated by a very low or even negative value for SMI. In one of these studies Tb.N and Conn.D were markedly elevated as well. In our HypoPT simulations with increased mechanosensitivity, BV/TV and Tb.Th increased and Tb.Sp decreased relative to the adapted healthy group. The SMI also decreased, indicating that the micro-architecture in the simulations did change according to the general pattern observed in HypoPT.
and after the initial modelling/remodelling simulations were carried out, other morphometric parameters between the models before and after biopsy of the patient. However, only minor changes in bone mass differences, the initial structure was not identical to original initially adapted structures for the comparison of structural changes, the initial structure was not identical to original.

Conn.D was limited. When using our algorithm, investigation of diseases or treatments depends on the bone modelling/remodelling algorithm. This enabled patient-specific simulations regarding bone micro-architecture when used in patient-specific bone modelling/remodelling simulations and thus enabled to investigate HypoPT regarding BV/TV, Tb.Sp, and SMI, but not Tb.Th, Tb.N, and Conn.D. However, compared to the 100% and 120% mechanosensitivity simulations, the 140% mechanosensitivity HypoPT simulation was closest to the clinical HypoPT group. We thus conclude that the micro-architectural changes during our simulations correspond to the general transitions observed in previous studies and that the HypoPT simulation with 140% mechanosensitivity was the most consistent with the clinical biopsy findings.

A strong point of our study is that we used bone modelling/remodelling simulation in combination with a load estimation algorithm. This enabled patient-specific simulations regarding bone micro-architecture and bone loading conditions, in order to investigate the disease HypoPT. Before simulating HypoPT, each biopsy was adapted to the estimated loading until bone formation and resorption equilibrium was reached. Comparing morphometric parameters from before and after this initial adaption shows that the bone micro-architecture did not change much since mean values changed within less than 2.5 standard deviations (Table 2, Fig. 3a,b). This indicates that the main bone micro-architecture was preserved when loading was applied that was predicted using the load estimation method presented in this study. Especially a sustained bone mass was desired since this is the characteristic parameter that changes drastically in HypoPT. The outcome of this initial simulation also depends on the bone modelling/remodelling algorithm. Conn.D was the only parameter that did change drastically which indicates that when using our algorithm, investigation of diseases or treatments related to Conn.D is limited.

Our study also has some limitations. First, because we used initially adapted structures for the comparison of structural differences, the initial structure was not identical to original biopsy of the patient. However, only minor changes in bone mass and other morphometric parameters between the models before and after the initial modelling/remodelling simulations were found especially with respect to bone mass, which was the main concern in this study. Second, the effect of aging through the simulations was neglected when adapted and HypoPT biopsies were age-matched. We assumed that this would not have major effects on our results since the simulation time of approximately 1 year was rather low. Third, averaged model parameters for resorption/formation rates were used. Since the parameters could be changed according general changes in HypoPT, using patient-specific rates would most likely not have influenced the outcome of this study. Finally, the number of age-matching samples was limited.

In conclusion, the estimated loading successfully preserved the micro-architecture when used in patient-specific bone modelling/remodelling simulations and thus enabled to investigate HypoPT with simulations based on patient data. These HypoPT simulations revealed that osteoclast suppression and increased osteocyte mechanosensitivity could explain the substantial gain in bone mass measured in HypoPT patients and thus corroborate the Frost hypothesis that certain hormones, such as PTH, might trigger the sensitivity of bone cells to mechanical loading (Frost, 1987b). This improves our understanding of HypoPT and also illustrates the ability of the presented simulation approach to use clinical data for bone modelling and remodelling simulations. Ultimately, such tools enable researchers to answer bone related research questions based on clinical data, and help clinicians to improve their prognosis and allow them to test different treatments.

**Conflict of interest**

All authors have no conflicts of interest.

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