Mapping bone changes at the proximal femoral cortex of postmenopausal women in response to alendronate and teriparatide alone, combined or sequentially

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Abstract

Combining anti-resorptive and anabolic drugs for osteoporosis may be a useful strategy to prevent hip fractures. Previous studies comparing the effects of alendronate and teriparatide alone, combined or sequentially using Quantitative Computed Tomography (QCT) in postmenopausal women have not distinguished cortical bone mineral density (CBMD) from cortical thickness (CTh) effects, nor assessed the distribution and extent of more localised changes. In this study a validated bone mapping technique was used to examine the cortical and endocortical trabecular changes in the proximal femur resulting from an 18 month course of alendronate or teriparatide. Using QCT data from a different clinical trial, the global and localised changes seen following a switch to teriparatide after an 18 month alendronate treatment or adding teriparatide to the alendronate treatment were compared.
CTh increased (4.8%, $p<0.01$) and CBMD decreased (-4.5%, $p<0.01$) in the teriparatide group compared to no significant change in the alendronate group. A large CTh increase could be seen for the switch group (2.8%, $p<0.01$) compared to a significantly smaller increase for the add group (1.5%, $p<0.01$). CBMD decreased significantly for the switch group (-3.9%, $p<0.01$) and was significantly different from no significant change in the add group. CTh increases were shown to be significantly greater for the switch group compared to the add group at the load bearing regions.
This study provides new insights into the effects of alendronate and teriparatide combination therapies on the cortex of the proximal femur and supports the hypothesis of an increased bone remodelling by teriparatide being mitigated by alendronate.

Keywords: Osteoporosis, Alendronate, Teriparatide, QCT, Cortical thickness, BMD.

1. Introduction

A consensus statement by The National Institutes of Health (NIH) on the prevention, diagnosis, and therapy of osteoporosis defines osteoporosis as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture [1]. Several drugs have been introduced for the prevention of osteoporotic fragility fractures. These include bisphosphonates such as alendronate (ALN) and the selective estrogen receptor modulator raloxifene (RLX). Bisphosphonates are antiresorptive agents that inhibit the osteoclastic bone destruction, allowing for the continued mineralisation of existing bone matrix. Antiresorptive therapies, however, reduce bone remodelling and do not restore or regenerate bone architecture.
Parathyroid hormone (PTH(1-84)), or its 1-34 amino acid terminal recombinant form teriparatide (TPTD), are anabolic agents which, when administered once daily, promote the formation of new bone and improve its micro-architecture [2, 3, 4]. TPTD increases bone remodelling by stimulating both osteoblastic and osteoclastic activity. While remodelling is preceded by a prior bone resorption phase, modelling acts without bone resorption and results in an increase in bone mass. Modelling occurs throughout life but is less frequent after skeletal maturity. The net increase in bone mass by TPTD from DXA studies indicates the presence of some degree of modelling [5]. Indeed histomorphological analyses indicate significantly more modelling on teriparatide treated patients compared to placebo [6]. Other studies indicate that the heterogeneity of mineralization in cortical bone increases in response to an increased remodelling by TPTD [7].
Conversely ALN has been shown to reduce the rate of bone remodeling by a decreased osteoclastic activity [8]. Secondary calcification of the matrix, however, is now prolonged. This increases the uniformity and average degree of matrix mineralisation [9, 10]. As

suggested in [9] this does not exclude a continued positive balance or mild augmentation of bone matrix volume, hence a thickness increase.
Both antiresorptive drugs and PTH analogues result in an increase in Bone Mineral Density (BMD) to varying extents [11, 12, 13]. Due to the development of osteosarcoma in Wistar rats treated with extremely high dose PTH 1-34 [14, 15], regulatory agencies limit the use of PTH analogues in humans to a 2 year period. While TPTD has proven effects on reducing the incidence of vertebral fractures, particularly so for moderate and severe collapse [2], there is also interest in the effects the drug has on hip bone structure. However, it is important to bear in mind that TPTD has not been shown to prevent hip fracture in adequately powered clinical trials. Based on cost and clinical effectiveness, the National Institute for Health and Care Excellence (NICE) in the UK recommends ALN as a first treatment option for fracture prevention, with RLX as an alternative [16]. Hence, TPTD is only routinely recommended by NICE when the osteoporotic patient with multiple (usually vertebral) fractures has not responded well to treatment with an antiresorptive agent, or cannot tolerate the alternatives. At that point in the treatment pathway, the physician needs to decide whether to either switch to or add TPTD to the patients' current treatment. In clinical practice, the anti-resorptive is almost always stopped, but the rationale for this is not entirely clear. Conversely, the effects of combining TPTD with an antiresorptive agent are not obvious and need careful study.

The effects of adding versus switching to TPTD after prior treatment with ALN have previously been studied by examining aBMD changes from DXA [17] and volumetric Bone Mineral Density (vBMD) changes from QCT [18]. These and other studies comparing combination therapy with ALN/PTH sequential therapy have yielded subtly different results. One clinical consideration is the transient decline in total hip aBMD during the first 6 months of single agent TPTD treatment, which occurred in patients pre-treated with ALN [19, 20] and was more noticeable in those with a poor treatment response [21]. These studies indicate that continuing ALN during the TPTD treatment might mitigate the initial BMD reduction which would presumably benefit the patient already at high risk of hip fracture.

While combination PTH 1-84 and ALN treatment resulted in significantly greater hip aBMD than single agent PTH 1-84, the single agent strategy increased the volume of the cortical compartment, which was not seen in the combination group [22]. However, vBMD decreased with the single PTH 1-84 approach, implying by inference that the increase in cortical volume was associated with increased porosity. Volumetric BMD remained unchanged with combination treatment. Conversely, TPTD in combination with ALN had less efficacy in enhancing aBMD compared with single agent TPTD in the study of Finkelstein et al. [11]. The study of Ettinger et al. [19] suggested that such TPTD density gains might be blunted by prior anti-resorptive treatment; in that study, patients were pre-treated with ALN for 18-36 months and there was no significant increase in total hip aBMD after switching to TPTD for 18 months. Cosman et al. found (with a similar drug regime) that aBMD at the hip *did* increase slightly when patients pre-treated with ALN instead continued ALN as a *combination* ALN/TPTD treatment [23], although the difference with a single ALN treatment was not significant.

Most of the previous research analysing the cortical bone mineral density, use methods based on thresholding or a fixed region from the outer cortex. Changes in the density from such a predefined region can then be due to changes in density, thickness or a combination of both. The previously reported changes in cortical BMD [18] thus relate more closely to changes in the mass of the cortex. In the proposed method, both the thickness and cortical density are accurately measured and distinguished from each other, which has been validated from high

resolution CT [24, 25, 26]. Furthermore, other studies examined the changes only globally and have not examined the location and distribution of the added bone mass, something we have now addressed with the present study.

In previous work, a method was presented to map the cortical thickness over the proximal femoral surface, using ordinary clinical Computer Tomography (CT) scan data, which was shown to be accurate down to a thickness of 0.3 mm [24]. In addition to thickness, also the cortical bone mineral density, endocortical trabecular density and cortical mass surface density can be measured over the femoral bone surface, and differences highlighted by parametric mapping techniques [25, 26]. In this work, we use these techniques to compare the effects of an 18 month treatment by ALN versus TPTD by analysing the global cortical changes from a previously published clinical trial [27]. We follow that analysis with a study on a separate previously published dataset [18] where all the osteoporotic research participants had been established on ALN therapy, as per usual clinical practice. In these subjects we compare the global and local changes in cortical parameters 18 months after switching treatment from ALN to TPTD, versus 18 months after combining ALN with TPTD.

2. Materials and Methods

2.1. Data

In this study the Quantitative Computed Tomography (QCT) scans from two distinct clinical trials were analysed. All QCT scans were segmented while blinded to the treatment group but not the time point. The subsequent cortical measurements are acquired in an automated process requiring no further manual processing of the individual scans.

2.1.1 Alendronate vs Teriparatide

For examining the effects of an 18 month treatment of ALN versus TPTD, a dataset used in a previously published study [27] was collected. All participants were part of a randomized, parallel, double-blind clinical trial and received either teriparatide (20 μg/day) or oral alendronate sodium (10 mg/day) for a period of 18 months. All participants were ambulatory, 5 years or more past menopause, had a BMD T-score between –2.5 and –4.0 at either the lumbar spine or femoral neck, and had normal or clinically insignificant abnormal laboratory values, including serum calcium, PTH 1-84, 25-hydroxyvitamin D, and alkaline phosphatase. The ALN cohort contains 18 subjects with a mean (± standard deviation) age of 62.3 ± 8.3 years and the TPTD cohort 19 subjects with a mean age of 66.9 ± 7.6 years. The baseline characteristics of this dataset are presented in Table 1 which shows no significant differences between the two cohorts for any variables.
Women were excluded if they had prior treatment with PTH or a PTH analogue; treatment with bisphosphonates within 12 months, anabolic corticosteroids or calcitriol or vitamin D analogues or agonists within 6 months, estrogens or selective estrogen receptor modulators within 3 months, or calcitonin within 2 months; therapeutic doses of fluoride; systemic corticosteroid use within 1 month or for more than 30 days in the prior year; use of anticoagulants within 1 month; history of diseases other than postmenopausal osteoporosis that affect bone metabolism; malignant neoplasms within 5 years; carcinoma in situ of the uterine cervix within 1 year; nephrolithiasis or urolithiasis within 2 years; abnormal uncorrected thyroid function; liver disease or clinical jaundice; impaired renal function; alcohol or other drug abuse; or poor medical or psychiatric risk for treatment. Patients with an

increased risk of osteosarcoma (i.e., patients with Paget disease of bone, previous skeletal exposure to external beam radiotherapy, or previous malignant neoplasm involving the skeleton) were also excluded [27].
The institutional review board at each clinical site approved the study protocol, which followed the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant before the start of screening tests.

2.1.2 Switch vs Add

A different dataset was used to examine the effects of adding versus switching to TPTD after a previous treatment with ALN. This data resulted from a trial at 11 centres in the United States. Patients were treated with ALN (total of 70 mg/week) for at least 18 months. Each treatment arm was then randomly divided in two subgroups whereby patients were either switched to or given an added treatment of TPTD (20 μg/day) by daily subcutaneous injection. QCT scans were taken at the therapy change point (after 18 months ALN) and again 18 months later. During that 18 months, one group had continued with ALN and TPTD and the other received TPTD alone. For a complete study description we refer the reader to [18].
The dataset analysed in this work includes the subjects given at least 18 months ALN followed by 18 months of TPTD treatment (either alone or in combination). QCT scans were analysed at the therapy change point, following the ALN prior treatment, and after the 18 months TPTD follow-up treatment. Of the 83 subjects where scans were available at both the therapy change point and after the follow-up treatment, one subject was rejected due to excessive osteophytes and one due to large image artifacts resulting from the presence of large metallic implants. This resulted in a dataset which includes 40 subjects in the switch group with a mean age of 69.2 ± 8.2 years and 41 subjects in the add group with a mean age of 67.4 ± 9.9 years. It includes women of at least 50 years with a prior diagnosis of osteoporosis based on areal BMD or fracture history. The full set of baseline characteristics of this study is presented in Table 2. The differences between the switch and add groups were not significant for any variables.
Women were excluded if they had a history of hypercalcemia (except for surgically corrected hyperparathyroidism) or metabolic bone diseases other than osteoporosis; secondary causes of osteoporosis or malignant neoplasms within the past 5 years; active urolithiasis within the past 2 years or at high risk for urolithiasis in the opinion of the investigator; active liver disease; substantially impaired renal function; history of excessive alcohol consumption; or treatment with other bone-active drugs [18].
All patients provided written informed consent and the study was approved by the institutional review board at each centre.

2.2. Cortical measurements

The cortical measurements were performed using Stradwin[1]. The methods incorporated in this software have been shown to be able to measure cortical thickness down to 0.3 mm with a mean (± SD) error of 0.01 ± 0.58 mm [24]. These methods were later evaluated for their ability to measure also the density and mass of the cortex [25, 26]. All volumes are first calibrated using a Mindways calibration phantom (Mindways Software Inc. Austin, TX, United States) which converts the CT Hounsfield units to corresponding K_2HPO_4 equivalent

[1] http://mi.eng.cam.ac.uk/~gmt11/software/software.html

density values expressed in mg/cm^3. The bones are segmented using a user interface provided in Stradwin to generate a mesh of the outer bone surface. For each point on the mesh, samples are subsequently taken in the CT volume along the surface normal thereby extracting a profile of the bone density. A blurred cortical model is then fitted to these samples which results in an estimation of several cortical parameters. In Figure 1, we see a schematic representation of the model fitting process. The blue line represents the real data as sampled from the CT image. The red line depicts the model of the cortex described by the cortical parameters and the dashed red line is the blurred model. The cortical bone mapping (CBM) technique finds the parameters of the model as well as the blur value such that the blurred model best fits the real data. This results in a measurement for the cortical thickness (CTh, mm), the cortical bone mineral density (CBMD, mg/cm^3) as well as a measure for the endocortical trabecular density (ECTD, mg/cm^3), which is the trabecular density directly adjacent to the cortex. Finally, from the CTh and CBMD, we compute the cortical mass surface density (CMSD, mg/cm^2) as CMSD = 0.1 × CTh × CBMD which represents the mass per unit surface area. This measure can be thought of as the total amount of cortical bone (in mg) contained within the calculated endosteal and periosteal cortical boundaries beneath a given area (in cm^2) of bone surface.
By extracting these measurements at every point of the surface mesh, a colour coded map of these values can be shown over the entire bone surface. For a detailed description of this method we refer the reader to previously published work [24, 25, 26]. A previous study already used this technique to assess the cortical thickness and mass changes in response to denosumab [28]. That article provides further descriptions on the cortical parameter mapping technique in a population based study which is described briefly in the following section.

2.3. Statistical analysis

To allow for a longitudinal and population based analysis of the cortical changes, a point correspondence has to be found between the surface maps of all bones. Towards this end a canonical proximal femur mesh is first constructed and deformably registered onto all surface meshes using the in-house developed software tool wxRegSurf[2]. For left femurs, the canonical mesh, which is represented as a right femur, was mirrored to allow for the correct correspondences. After the registration, for each vertex in the canonical model the closest point on the target mesh is found and the corresponding cortical measurement is copied onto the canonical shape. In this way, for each new femur the measurements can be related to the measurements on corresponding locations of all previous femurs. For 3 subjects in the ALN versus TPTD study, one proximal femur was not fully contained in the CT scan and in the add versus switch study 9 subjects had an orthopaedic implant. For these subjects only the contra-lateral femur was used. For the remaining subjects, the cortical maps were averaged over the two to generate a single cortical parameter map for each subject. By examining corresponding points between baseline and follow-up we can map the cortical changes over the surface of the proximal femur. Since the same canonical femur shape is used for all subjects, we can now also derive statistics about the changes throughout the individual cohorts, as well as the differences between them.
For the ALN versus TPTD cohort, the percentage changes of CTh, CBMD, ECTD and CMSD with respect to baseline are measured over the proximal femur surface. The global changes (average of every point on the canonical surface) are subsequently calculated for both cohorts and a two-sample T-test assesses the significance of the difference between the

[2] http://mi.eng.cam.ac.uk/~ahg/wxRegSurf/

two.
Similarly, in the add versus switch trial, for each of the two cohorts, the cortical changes are measured and averaged over the entire surface to assess the global changes for each trial arm. T-tests are performed to assess the significances between the add versus switch trial arms of all global cortical parameters.
Besides an analysis of the mean changes globally, the cortical maps allow us to analyse the localised changes as well as the significance of the changes for each point on the surface. In addition to this analysis of the changes from baseline, we assess the localised average differences and the significances of the differences between the add and switch trial arm. These statistical analyses on the surface maps are performed using SurfStat [29], a Matlab based toolbox for the statistical analysis of univariate and multivariate surface data using linear mixed effects models and random field theory.

3. Results

3.1 Alendronate vs Teriparatide

The cortical thickness changes for treatment with ALN and TPTD and the significances of the differences between them are presented in Table 3. These results show a significant increase in CTh (4.8%, $p<0.01$), decrease in CBMD (-4.5%, $p<0.01$) and increased ECTD (3.7%, $p<0.05$) for the TPTD group, while ALN does not result in any significant changes. Only the changes in CTh and CBMD were statistically significantly different between ALN and TPTD, with $p=0.026$ and $p=0.035$ respectively. CMSD did not change significantly for either ALN or TPTD treatment, nor did the changes differ significantly between the two (Table 3). Cortical maps of the ALN and TPTD single treatment are not included. Only few significant patches were seen for the ALN group whereas the TPTD single treatment group shows largely the same patterns as the TPTD switch group (Figure 2).

3.2 Switch vs Add

The changes of the cortex in response to switching to or adding TPTD after prior treatment with ALN are presented in Table 4. We can see significant differences between the switch and add group for the CTh, CBMD and CMSD ($p=0.025$, $p<0.001$ and $p=0.003$ respectively). While the switch group shows a greater thickening (2.8%, $p<0.01$) of the cortex compared to the add group (1.5%, $p<0.01$), for the switch group we can see a large and significant decrease for the CBMD (-3.9%, $p<0.01$) and no significant change in the add group. The ECTD, increased significantly for both the switch and add group (4.1% and 4.7% respectively, $p<0.01$) with the differences having no statistical significance ($p=0.469$). The switch group, finally, shows a decrease in CMSD (-1.3%, $p<0.05$) compared to an increase for the add group (1.1%, $p<0.05$).
Maps of the cortical changes are shown for the switch group in Figure 2 and the add group in Figure 3. These indicate large regions with a significant increased CTh for the switch group. For the add group, several patches of significant increases are visible which are of combined smaller sized region than the switch group. Looking at the differences in CTh change between the switch and add groups (Figure 4a), two patches of significant differences can be seen. One at the infero-medial junction of the cortex and one at the calcar femorale. These are regions subjected to large compressive forces during stance and normal locomotion. In these regions, the switch group produced significantly greater increases in CTh than the add group.
The colour coded maps of the CBMD changes show large significant localised decreases for

the switch group only, the differences with the add group being significant over large regions (Figure 4b). In contrast, the ECTD maps show large increases for both switch and add group, with no statistically significant differences (Figure 4c).
The cortical maps of the CMSD changes for the switch group show only a region of significant decrease at the lateral side of the trochanter (Figure 2d). For the add groups, however, several patches of significant CMSD increases can be seen at the attachment sites of the psoas major muscle at the lesser trochanter and the quadratus femoris muscle on the inter-trochanteric crest, which are the muscles applying force in normal locomotion (Figure 3d). The map of the significances of the differences between the CMSD changes indicates a large region at the lateral side of the trochanter where the CMSD increase of the add group is significantly different from a CMSD decrease in the switch group (Figure 4d). This is likely due to the large decrease in the switch group at this location (Figure 2d).

4. Discussion

The comparison of the cortical changes between ALN and TPTD is broadly in agreement with previous studies [11, 27] as well as studies comparing the effects of ALN and TPTD on patients with glucocorticoid-induced osteoporosis [12, 13]. In [27], which analysed data resulting from the same clinical trial as reported here, femoral neck aBMD produced significant increases for both an 18 month TPTD and ALN treatment (3.9% and 3.5% respectively) while in contrast to [11] the differences were not significant. In [11], however, the treatment period was 30 months with a TPTD dose twice that of [27]. In [27] a greater but not significant increase in trabecular BMD was observed with TPTD than with ALN in the femoral neck. This is consistent with the global ECTD changes in our analysis (Table 3). Furthermore, in the cortical vBMD of the femoral neck, a significant difference between a decrease in the TPTD cohort (-1.2%) and an increase in the ALN cohort (7.7%) was observed. While this significant difference is consistent with our results, we show a significant decrease in CBMD for the TPTD cohort (-4.5%) which was significantly different from no significant change in the ALN cohort. A similar trend was seen for the CMSD with a 0.3% decrease for TPTD and a 0.1% increase for ALN, although these changes were not statistically significant.
The reduction in the CBMD together with an increase in the ECTD by TPTD (Table 2) has also been seen previously in different studies. In [30] a TPTD treatment was shown to result in a reduced cortical vBMD and increased trabecular and total vBMD. Similarly, in [31] significant decreases in cortical vBMD at the femoral neck and inter-trochanter (-1.2% and -1.5% respectively) were reported after a 72 week once-weekly 56.5 μg TPTD treatment with slight, although not significant, increases in total vBMD. Furthermore, CTh was shown to increase compared to a decrease with placebo with the differences being statistically significant for the femoral neck and shaft region [31]. Care, however, has to be taken in comparing the cortical parameters between studies due to the differences in the techniques used to extract these measurements.

Partial volume effects and kernel dependent blur inherent to CT scans can make the cortex appear either thinner or thicker than it actually is, with converse effects for the CBMD measurements. By the deconvolution method implemented in Stradwin, we are able to accurately separate the thickness of the cortex from the cortical density. We are thus able to measure longitudinal changes of the CTh and CBMD independently as well as the ECTD and derived CMSD measurements. This gives us more insight into the bone remodelling effects caused by specific treatment types. In particular, the increased CTh and increased ECTD we

see in the TPTD group together with a decreased CBMD gives a strong indication of an elevated level of bone remodelling. It supports the hypothesis that increased remodelling by TPTD increases CTh and trabecular BMD by new bone deposits which is associated with an increased porosity as reflected by the decreased CBMD.
Moreover, by using a deconvolution on the density profiles we are able to measure cortices reliably at a sub-voxel level even for cortices as thin a 0.3mm, which is not possible by thresholding methods. This allows us to include measurements in our study over the entire proximal femur, including regions with a very thin cortex such as the femoral head.

Regarding differences in response to switching to TPTD after a previous ALN treatment versus adding TPTD to a continuing ALN treatment, this has previously been evaluated with respect to the vBMD changes at the integral, trabecular and peripheral regions by Cosman et al. [18]. That study indicated a significant increase of the trabecular vBMD, which was larger for the add group than the switch group. In the analysis of Cosman et al., a cortical "peripheral" bone compartment is defined by a threshold on the calibrated density values set at 1.0 g/cm^3 as well as including all bone within 3 mm of the periosteal surface. A reported "peripheral" BMD increase can then, in the case of a thin cortex, be caused by an increased CBMD as well as an increased cortical thickness. For the "peripheral" BMD, the add group resulted in a significant increase (1.9%) which was significantly different ($p < 0.0001$) from a decrease in peripheral BMD of the switch group (-2.2%). In our analysis, which uses data from the same clinical trial, we separate the CTh from CBMD and from this provide measurements of the CMSD which describe the amount of new bone deposits. Reported changes in "peripheral" BMD are therefore expected to relate more closely to the CMSD changes than the CBMD reported in our study. This is indeed reflected in the results where we can see a significant increase in the CMSD for the add group (1.1%) which is significantly different from a significant decrease in the switch group (-1.3%).
While the CMSD is largely in correspondence with the findings for "peripheral" BMD described in [18], in the current analysis of the cortex we find a significantly greater increase of the CTh for the switch group compared to the add group (Table 4). The reduced CBMD and increased CTh of the switch group are in line with the increased remodelling by a treatment of TPTD alone which we saw previously in the TPTD single treatment study (Table 3). Furthermore, the locations of the CTh increases in the switch group correspond to previous findings where the increased thickness in response to TPTD was located at the highly loaded regions of the proximal femur [32].
TPTD has been shown to increase remodeling-based bone formation, and possibly modeling-based bone formation [33, 6], which in our work is reflected as an increased cortical thickness and ECTD and a reduction in CBMD. This effect is greatly reduced when ALN is continued with the TPTD treatment. This supports the hypothesis that ALN mitigates the increased rate of remodelling induced by TPTD. The underlying mechanism of action, however, can only be determined from histology and from this study we can make no conclusive statements about this.

Previous studies on cortical bone microarchitecture changes in women treated with TPTD measured from HR-pQCT have shown no changes in measured cortical thickness (from which pores are disregarded) with either a decreased cortical BMD [34], or an increased cortical porosity [35]. In striking contrast, micro-CT and histomorphometric analyses indicate no change in cortical porosity, while increasing cortical width in osteoporotic women treated with Teriparatide [36, 37], though it must be noted that sampling issues relating to intra-site variability predominate when trying to make assumptions about longitudinal changes on pieces of bone removed from patients. These discrepancies in definitions of porosity and

width/thickness could be explained by the resolution of the modalities and highlight the importance of interpreting the cortical changes in the context of the modality in use.

The relative low resolution of clinical CT scans limits us from measuring detailed changes in endocortical structures and in some situations makes it impossible to distinguish trabecular bone from cortical bone. The endocortex is a compartment where new bone formation, sometimes incorporating new endocortical pores, is commonly seen histologically with teriparatide. This effect inevitably changes (even at the histological level) where one would draw an endocortical boundary. Figure 5 depicts this at a simulated histological resolution (top panel, where new bone formed by teriparatide is shown in green). The lower two panels indicate what this process looks like to our cortical parameter measurements, with the first row of images before treatment (Baseline) and second row after teriparatide (Follow-up). At baseline, our sampling profile passes through a thin cortex at A, but by follow-up a nearby trabecula has been joined to the endocortex, in the process creating a pore. The model in profile A shows how these effects lead to a decrease in measured cortical BMD at this sampling point and an increase in cortical thickness, but without any change in the endocortical trabecular density. The model in profile B shows a slightly different effect where new trabecular bone now meets the cortex and has been sampled straight through; including cortex, newly incorporated pores and the new trabecular tissue. Here the thickness increases modestly, the density is unchanged, and the endocortical trabecular density value has increased. Although speculative, we suspect that a mixture of both these types of changes may account for our finding an increased cortial thickness and endocortical trabecular density as well as the decreased cortical BMD in the proximal femora.

Histomorphometry has shown that TPTD treatment results in an increased hetrogeniety [7], also when preceded by an antiresorptive [38]. Low mineralised endocortical or preosteal bone matrix might affect the cortical measurements in a way that is difficult to predict. Similar to the effects resulting from corticalised trabecular bone described above, these changes might be measured as either an increased thickness with a decreased CBMD, or the change is predominantly described by an increased ECTD. Again, both these two effects are seen in the TPTD treatment groups.

Histological studies describe an increased mean degree of mineralisation [9] and decreased porosity [10] in ALN treated patients, which can be explained by a continued secondary mineralisation or the infilling of the haversian canals. In our study, CBMD with alendronate did not increase (instead trending downwards), but a subset of only 18 subjects may not provide sufficient statistical power to examine this in detail.

Some limitations of this study should be noted. Although the follow-up QCT scans were taken after an 18 month TPTD treatment, improvements in BMD at the proximal femur by TPTD, however, occur predominantly beyond the 18 month period [11]. In [39], TPTD treatment after ALN treatment resulted in a large and significant decrease in total hip aBMD and femoral neck aBMD after the first 6 months (-1.2% and -1.8% respectively). Areal BMD measurements subsequently steadily increased towards the 18 month time point, although these increases were not significant. In the following 6 months, aBMD increased even more rapidly, whereby at the 24 month time point total hip aBMD was increased by 2.1% and the femoral neck by 3.4% compared to baseline (both $p<0.001$).

The model used in this analysis is a simplified model of the cortex. It included only the periosteal bone location, the cortical thickness and a cortical, trabecular and soft tissue

density. At this resolution, our technique is limited by any subtle changes in the transitional zone at the endocortex, as demonstrated in Figure 1. A low density matrix at the endocortical surface might affect the measurements somewhat. However, the changes were measured in an 18 month time frame while primary mineralisation occurs in only a few months. The trends seen in this study therefore include many cycles of remodelling. There might still be newly laid matrix from more recent deposits but that only slightly affects the general trend in cortical changes seen in this 18 month study.
However, recent studies in ewes, which have a remodeling activity close to humans, show that secondary mineralization is complete after a period of 30 months [40]. Thus, the secondary mineralisation of newly formed osteoid can still increase the cortical parameters, especially upon a continuing treatment with an antiresorptive agent.
A study continuing the clinical trial described in [22] has shown that ALN treatment results in a significant increase in trabecular BMD at the hip compared to a decrease with placebo when administered after PTH(1-84) [41]. Stopping the treatment reduces gains made by PTH(1-84) which suggests that ALN therapy may sustain gains in BMD from a PTH(1-84) treatment. Furthermore, in [42] Continuing ALN after an ALN and TPTD combination therapy resulted in continued aBMD increases at both the femoral neck ($4.2 \pm 1.6\%$, $p<0.001$) and total hip ($4 \pm 1.6\%$, $p<0.001$).

It should also be noted that the clinical trials from which our data resulted, did not include a control group. While we can make a comparison between the treatment types, we cannot make any conclusions about the effects of an individual treatment type.

Both ALN and TPTD treatment have been shown to reduce the risk of osteoporotic fractures [43, 2]. However, the fracture risk reduction by TPTD on the proximal femur specifically has not yet been established in a large enough clinical trial. Such study on the effects of TPTD as well as the various proposed combination therapies on the risk of hip fracture is difficult due to the large number of participants required. We can, however, relate the secondary effects of the pharmaceuticals, such as the cortical measurements presented in this study, to hip fracture incidence. This has been briefly investigated in [44] to identify critical regions of the femoral cortex in fracture patients but further research is necessary towards this end.

In conclusion, this study analysed the QCT scans acquired in previous clinical trials to examine more closely the comparative effects of ALN and TPTD as single treatments or in a switch versus add configuration by measuring the cortical changes over the entire bone surface of the proximal femur. With new paradigms emerging in the therapeutics of bone anabolism, it is important to establish the precise effects of current anabolic strategies with teriparatide. Here, we have gained an insight into precisely where cortical parameter changes occur in the adult hip, with a suggestion that some of the remodelling-induced thickness increases with teriparatide (which might be beneficial at high load bearing areas) are mitigated when patients keep taking ALN. How precisely these cortical changes affect the risk of hip fracture remains to be examined in more detail.

Disclosures

KESP and GMT are inventors on a related patent WO 2011/042738, "Image data processing systems." This does not alter the authors' adherence to all the JBMR policies on sharing data and materials.
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Figure legends

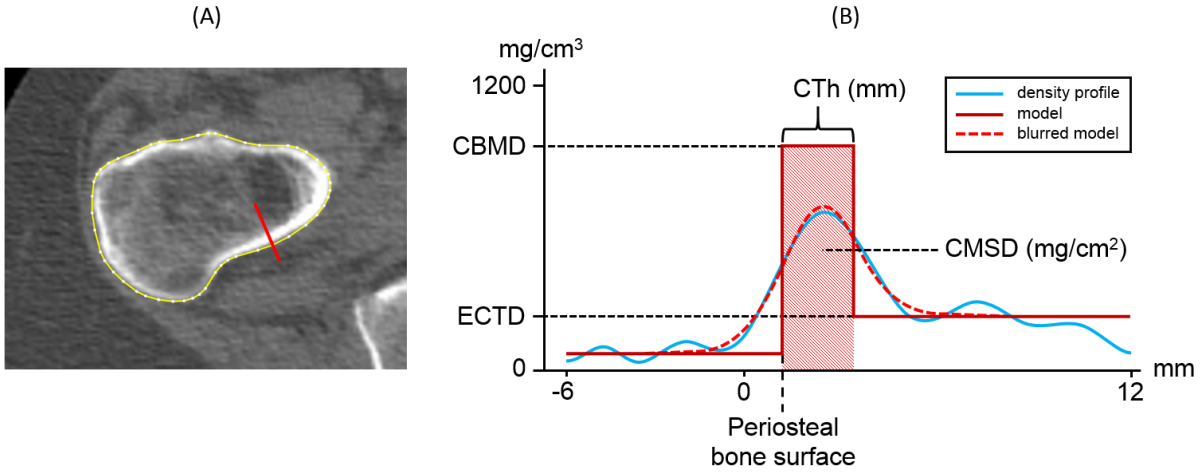

Fig. 1. Schematic representation of the cortical measurements. Left shows the line along which the QCT values are sampled to generate a density profile. Right depicts the cortical model fitted to the density profile with the various cortical parameters.

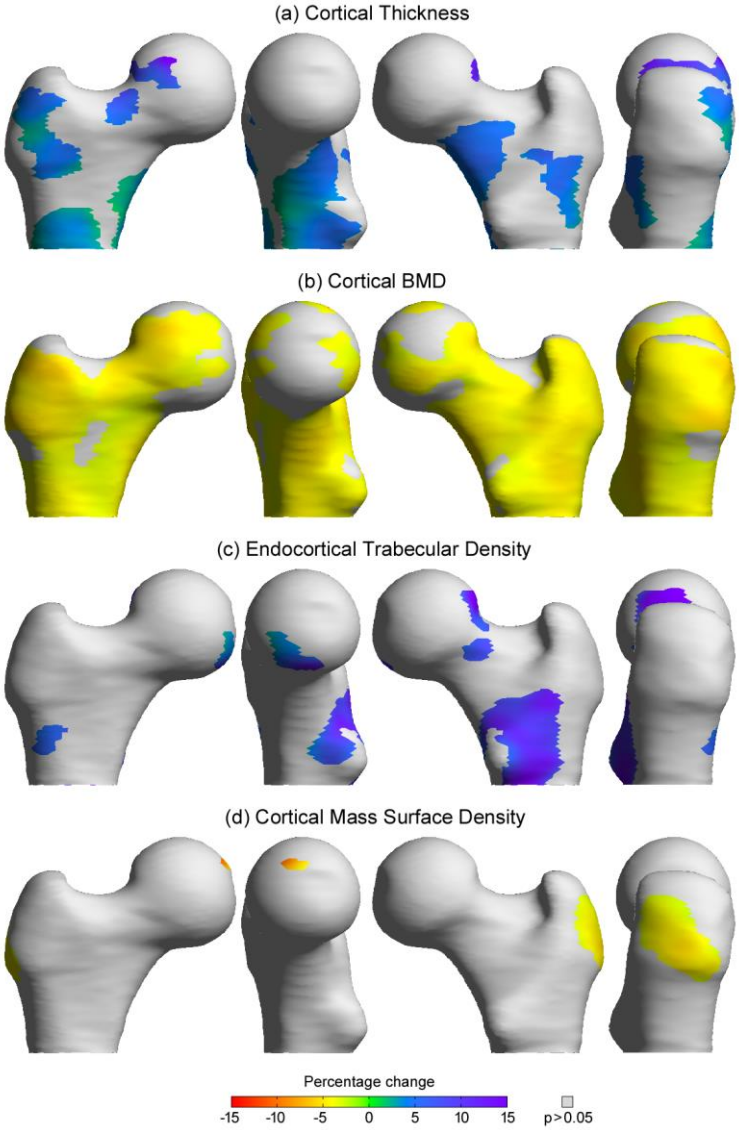

Fig. 2. Cortical changes in response to switching to 18 months of TPTD after a previous ≥18 months ALN treatment. The grey regions indicate non-significant effects (p>0.05).

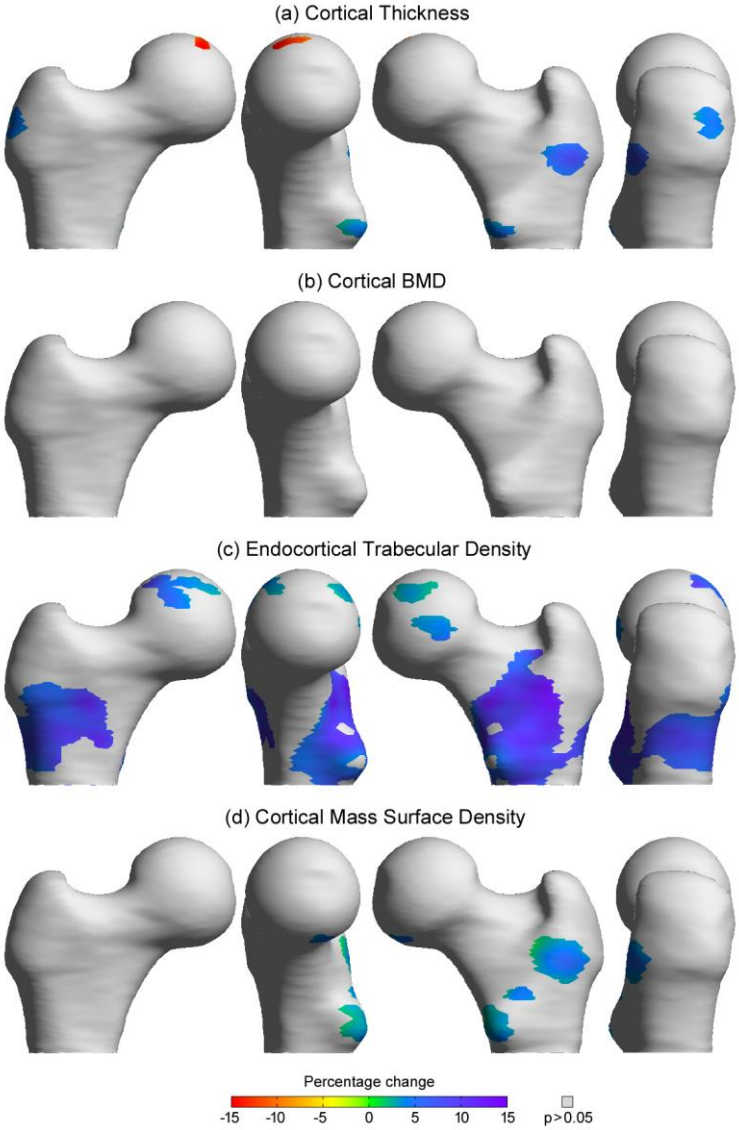

Fig. 3. Cortical changes in response to adding 18 months of TPTD to a continuing ≥18 months ALN treatment. The grey regions indicate non-significant effects ($p>0.05$).

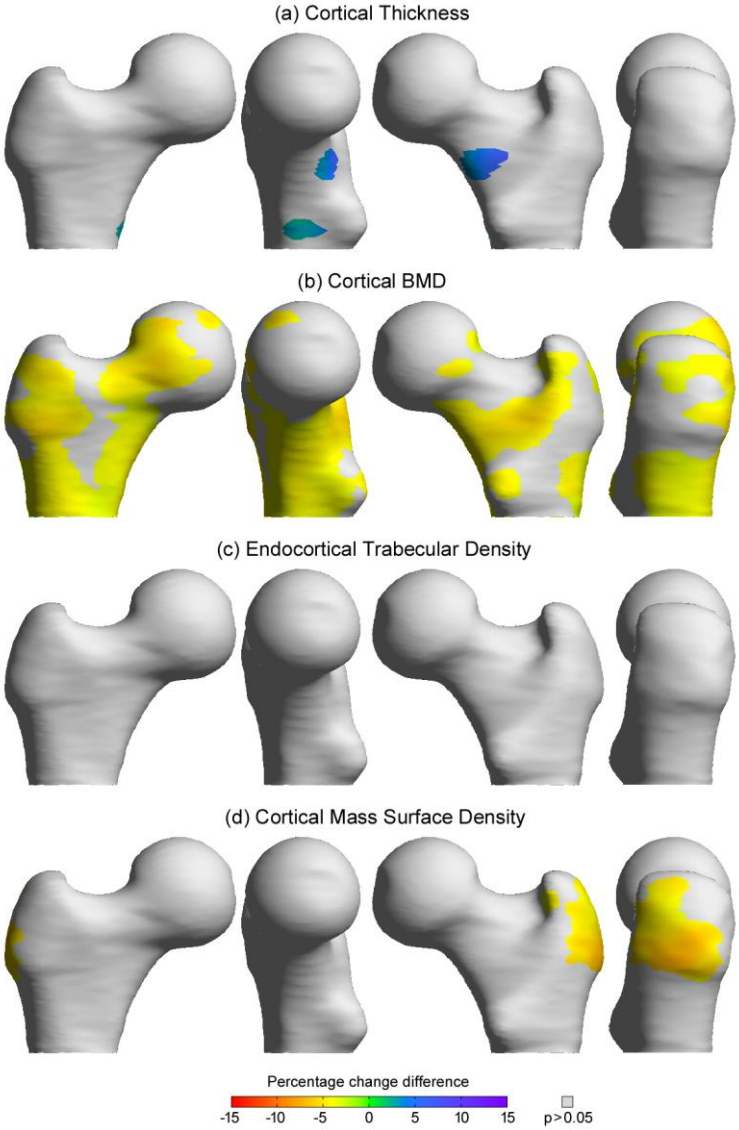

Fig. 4. Cortical maps of the differences between adding or switching to TPTD after a prior treatment with ALN. The colours express the differences as the mean percentage changes of the add group subtracted from the mean percentage changes of the switch group. A positive percentage (blue) indicates significantly greater effects of switching compared to adding. A negative percentage (yellow) indicates significantly greater effects of adding compared to switching. The grey regions indicate non-significant differences ($p>0.05$).

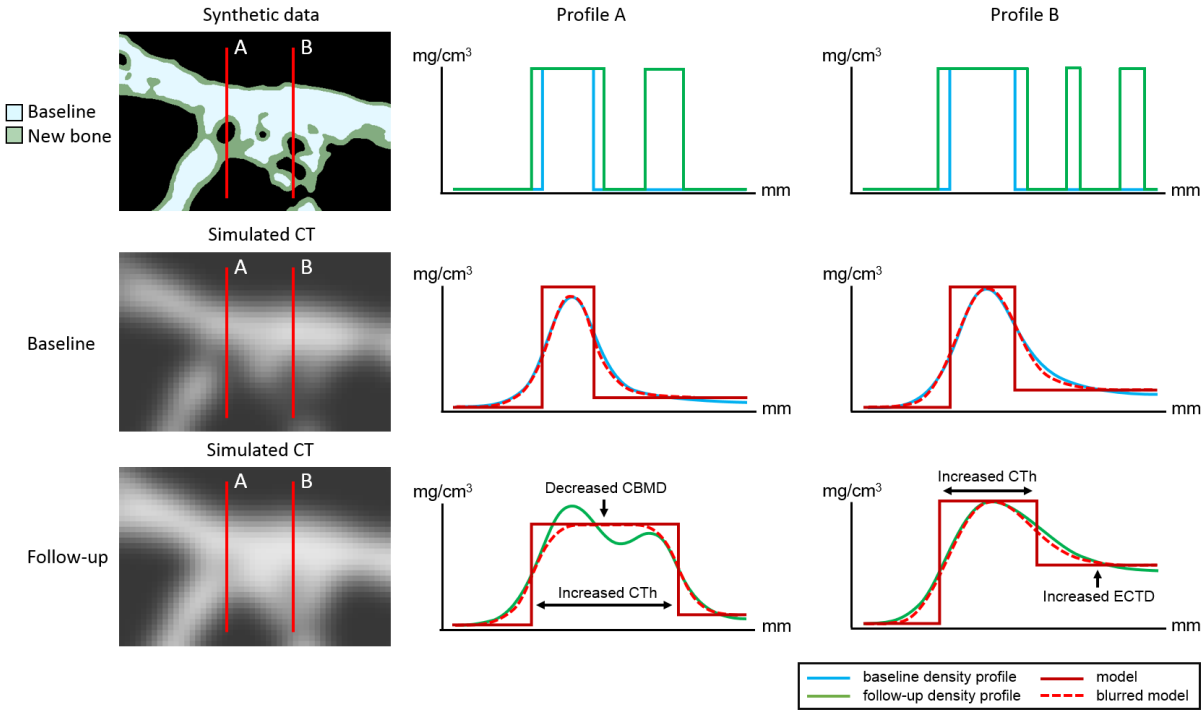

Fig. 5. An illustration of endocortical trabecular bone formation with porosity and their possible effects on the cortical measurements at two sampling locations. Synthetic data represents baseline cortical bone (light blue) and simulated new bone deposits in response to TPTD (green). In this illustration all bone is assumed to contain the same mineral density. Baseline and follow-up simulated CT scans represent the smooth and low resolution images consistent with CT or HR-pQCT. Profile A shows the incomplete corticalization of trabecular bone which leaves a large pore. Here the added trabecular bone is identified as part of the cortex, resulting in an increased thickness, while the pore decreases the CBMD measure. Profile B indicates a larger region of trabecular bone which leaves several pores. This slightly increases cortical thickness and predominantly increases ECTD in the fitted cortical model.

Table 1. Baseline characteristics (mean ± standard deviation) of the ALN vs. TPTD study.

	Alendronate (n=18)	Teriparatide (n=19)
Age (years)	62.3 ± 8.3	66.9 ± 7.6
Weight (kg)	63.0 ± 12.8	63.0 ± 11.1
Height (cm)	156.1 ± 6.2	155.8 ± 6.6
T-score femoral neck	-2.0 ± 1.0*	-2.2 ± 0.7
T-score total hip	-1.9 ± 1.0*	-1.6 ± 0.6
Mean CTh (mm)	1.3 ± 0.1	1.3 ± 0.1
Mean CBMD (mg/cm^3)	928.8 ± 57.6	941.7 ± 28.0
Mean ECTD (mg/cm^3)	120.0 ± 29.7	116.1 ± 26.0
Mean CMSD (mg/cm^2)	121.8 ± 13.7	125.5 ± 12.1

* Mean, standard deviation and significances of differences were derived from n=16 subjects in the alendronate cohort since for two subjects BMD T-score data was not available.

Table 2. Baseline characteristics (mean ± standard deviation) of the switch versus add study.

	Switch (n=40)	Add (n=41)
Age (years)	69.2 ± 8.2	67.4 ± 9.9
Weight (kg)	62.1 ± 9.1	63.3 ± 12.1
Height (cm)	159.9 ± 5.9	159.3 ± 5.9
Prior treatment duration (months)	54.1 ± 23.8	47.7 ± 22.7
T-score femoral neck	-2.4 ± 0.7	-2.3 ± 0.8
T-score total hip	-2.1 ± 0.8	-2.0 ± 0.9
Mean CTh (mm)	1.3 ± 0.1	1.3 ± 0.1
Mean CBMD (mg/cm^3)	916.1 ± 44.9	929.7 ± 46.6
Mean ECTD (mg/cm^3)	101.2 ± 23.2	108.1 ± 29.6
Mean CMSD (mg/cm^2)	123.4 ± 15.4	125.0 ± 16.9

Table 3: Changes in the cortex in response to ALN or TPTD treatment expressed in mean (95% CI) percentage changes from baseline as well as the significances of the differences between the ALN and TPTD groups (p-value).

	alendronate (ALN)	teriparatide (TPTD)	p-value
CTh	1.4 (-0.5 to 3.4)	4.8 (2.7 to 6.8)‡	0.026
CBMD	-1.2 (-3.1 to 0.6)	-4.5 (-6.7 to -2.2)‡	0.035
ECTD	-0.2 (-3.6 to 3.1)	3.7 (0.3 to 7.1)†	0.117
CMSD	0.1 (-1.7 to 1.9)	-0.3 (-2.1 to 1.5)	0.763

†significant change from baseline where $p<0.05$; ‡significant change from baseline where $p<0.01$.

Table 4: Changes of the cortex in response to switching to or adding TPTD after prior treatment with ALN expressed in mean (95% CI) percentage changes from baseline as well as the significances of the differences between the switch and add groups (p-value).

	switch	add	p-value
CTh	2.8 (2.0 to 3.6)‡	1.5 (0.7 to 2.3)‡	0.025
CBMD	-3.9 (-5.1 to -2.7)‡	-0.3 (-1.0 to 0.5)	<0.001
ECTD	4.1 (2.2 to 6.0)‡	4.7 (3.1 to 6.3)‡	0.469
CMSD	-1.3 (-2.6 to -0.1)†	1.1 (0.1 to 2.0)†	0.003

†significant change from baseline where $p<0.05$; ‡significant change from baseline where $p<0.01$.