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Response of Bone Turnover Markers to Three Oral Bisphosphonate Therapies in Postmenopausal Osteoporosis: the TRIO Study.

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Abstract

Introduction: Biochemical response to bisphosphonate therapy can be assessed using either a decrease in bone turnover marker beyond the least significant change (LSC) or a reduction to within a reference interval (RI). We compared the performance of these target responses and determined whether response was related to the type of bisphosphonate, compliance and baseline bone turnover markers.

Methods: Biochemical responses to three oral bisphosphonates were assessed in an open, controlled trial comprising 172 postmenopausal osteoporotic women (age 53-84 years), randomized to alendronate, ibandronate or risedronate, plus calcium and vitamin D supplementation for two years. The LSC for each marker was derived within the study population whereas RIs were obtained from a control group of healthy premenopausal women (age 35-40 years).

Results: Over 70% of women achieved a target response for serum CTX and PINP, irrespective of the approach used. The percentage decrease at 12 weeks was greater for women with baseline PINP above the RI -63% (difference 13%, 95%CI 0 to 27.1, P=0.049) and good compliance -67% (difference 15.9%, 95%CI 6.3 to 25.5, P=0.001). Responders had a greater increase in spine bone density compared to non-responders; for example 6.2% vs. 2.3%, (difference 3.9%, 95%CI 1.6 to 6.3, p=0.0011) for PINP LSC. The magnitude of change in bone markers was greater with ibandronate and alendronate than risedronate.

Conclusions: Both approaches to response identified similar proportions of women as responders. Non-responders had smaller increases in BMD and we suggest that biochemical assessment of response is a useful tool for the management of women with postmenopausal osteoporosis.

Key words: postmenopausal osteoporosis, bone turnover markers, variability, bisphosphonate

Mini Abstract (50 words)

We used bone turnover markers to identify women who responded to bisphosphonate treatment for osteoporosis. Response was more likely with alendronate and ibandronate, than risedronate. There was a greater decrease in bone markers if baseline bone turnover markers were higher and if the patient took more than 80% of her medication.

Introduction

Oral bisphosphonates are the most commonly used medications for the treatment of osteoporosis [1] . They are an effective treatment for osteoporosis as they reduce bone turnover, increase bone mass and reduce fracture rate [2] . Randomized controlled trials of alendronate, risedronate and ibandronate have reported their effect on fracture risk reduction [3-8] . In the UK, guidance from the National Institute for Health and Care Excellence (NICE) recommends alendronate as the first choice of treatment; if patients are unable to comply or are intolerant of alendronate then risedronate or ibandronate are considered as alternative treatments.

Bone resorption markers decrease earlier and usually by a greater magnitude than bone formation markers in response bisphosphonate treatment [9-11] . There is comparative evidence that alendronate results in a greater reduction in bone turnover markers (BTMs) than risedronate [12] and that ibandronate results in an earlier response, but a reduction of similar magnitude to alendronate [13] .

Pre-treatment levels and changes in BTMs appear to relate to outcomes such as improvement in bone mineral density (BMD) and reductions in fracture risk. For example, higher baseline markers are associated with a greater increase in BMD and greater reduction in non-vertebral fractures with treatment [14, 15] . The evidence relating changes in BTMs to fracture risk has recently been summarised by Vasikaran et al [9] . More than 20 prospective studies of the use of bone markers to predict fracture were reviewed and most reported that at least one BTM was significantly associated with fracture risk, particularly the bone resorption markers. High levels of BTMs may predict fracture risk independently from BMD in postmenopausal women [16] . A subsequent study indicates that change in PINP can account for 60% of the fracture risk reduction with zoledronic acid treatment [17] .

In clinical practice, it is useful to identify patients who fail to respond to treatment so that adherence can be reviewed, causes of non-response can be elucidated and alternative treatments considered. Poor persistence and poor compliance with dosing instructions might underlie a failure of treatment. Patients may be taking the treatment correctly but have poor absorption or unidentified underlying medical conditions that are increasing bone turnover [9, 18] .

The International Osteoporosis Foundation (IOF) has proposed that failure to respond to treatment might be defined as two or more incident fragility fractures or by lack of BTM response and a significant decrease in BMD [19] . The BTM working group of the IOF and International Federation of Clinical Chemistry (IFCC) describe two ways of monitoring response to treatment using BTM [9] . Firstly there is the least significant change approach (LSC) signifying the minimum change in BTM that can be attributed to treatment effect rather than random variation of the marker (usually with 95% certainty). This is most commonly expressed as a percentage change [20, 21] or alternatively as absolute units [22] ; the target for treatment with bisphosphonates being to reduce the bone markers by at least the LSC. The second approach is that the target for treatment is to decrease the BTM to the lower half of the premenopausal reference interval (RI) [9, 23] . However not all women are above the RI mean before starting treatment.

The aims of this study were to 1) to compare two approaches (LSC and RI) for identifying women that reach the target response to oral bisphosphonate therapy, 2) to identify determinants of response and 3) to determine if reaching the target response for bone markers is associated with change in bone mineral density.

Methods

Study Design
The TRIO study comprised a 2-year, open-label, parallel, randomised control intervention trial of three orally administered bisphosphonates. The effects of the treatments on bone density results and fracture history have recently been published [24] . Healthy premenopausal women were also recruited to act as a parallel control and reference group.

Study Population
We recruited postmenopausal women with osteoporosis defined by dual energy x-ray absorptiometry (DXA) bone mineral density (BMD) at the lumbar spine or proximal femur of i) T score $\leq$-2.5 or ii) T score $\leq$-1.0 plus a prevalent non-traumatic fracture. The participants were recruited through a hospital metabolic bone clinic and from general practice registers. Inclusion criteria were that the women were ambulatory, less than 85 years old, more than 5 years postmenopausal and able to give informed consent. Inclusion criteria for the group of healthy premenopausal women included ages 35 to 40 years, regular menstrual cycles and non-use of hormonal contraception. Exclusion criteria for both groups were fracture in the previous 12 months, the use of medications or diagnosis of any disease or medical condition known to affect bone, or a BMI outside the range of 18 to 35 kg/m^2. The study was approved by the Sheffield Research Ethics Committee and the Medicines and Healthcare

Products Regulatory Agency (MHRA) and was carried out in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. Written informed consent was obtained for all individual participants included in the study.

Study interventions

In this open-label study, the women with osteoporosis were randomised to receive one of three oral bisphosphonates at the licensed dose: (i) ibandronate (Bonviva, Roche, 150 mg once a month), (ii) alendronate (Fosamax, Merck, 70 mg once a week), or (iii) risedronate (Actonel, Warner-Chilcott, 35 mg once a week). To minimise bias the drugs were prescribed under a coding system using a stratified block randomisation method. Adherence was assessed using medical events monitoring system (MEMS) bottle caps (AARDEX, Zurich, Switzerland). In keeping with usual clinical practice, participants also received calcium carbonate 3g (1200 mg elemental calcium) and cholecalciferol 20 micrograms (800 IU) per day (Adcal D3, two tablets daily, ProStrakan) which was initiated one week before the bisphosphonate at the baseline 1 visit. The healthy pre-menopausal women were not prescribed any medications throughout the study.

Study Assessments

Anthropometric measurements; height (to the nearest 0.1cm), was measured using a wall-mounted stadiometer (Seca 242, Seca, Birmingham, UK) and weight (to the nearest 0.1kg) using an electronic column scale (Seca, Birmingham UK). Body mass index (BMI) was calculated to the nearest kg/m^2. Bone mineral density (BMD, g/cm^2) of the lumbar spine (LS) and proximal femur were measured by dual energy x-ray absorptiometry (DXA) using a Discovery A densitometer (Hologic Inc, Bedford, MA).

Biochemistry

In the postmenopausal women receiving treatment, samples for biochemistry were collected at baseline 1 (week -1), baseline 2 (week 0) then at 1, 2, 4, 12, 13, 48 and 96 weeks while samples were collected at baseline in the healthy premenopausal control women. Blood was collected after an overnight fast and the sample was left to clot for 30 minutes at room temperature before centrifugation at 2500g for 10 minutes. Second void fasting morning urine samples were collected. Samples were stored at -80°C until analysis and all visits of individual participants were measured in one analytical batch.

The C-telopeptide of type I collagen (CTX), N-mid osteocalcin (OC), intact pro-collagen I N-propeptide (PINP), bone alkaline phosphatase (BoneALP) and 25hydroxyvitamin D (25OHD) were measured using the IDS-iSYS

automated immunoassays (Immunodiagnostic Systems, Boldon, UK). The inter-assay coefficients of variation (CVs) were 6.5%, 5.0%, 7.2%, 3.5% and 6.7% respectively. We excluded subjects with a baseline CTX result below the limit of detection for the assay (0.033 µg/L n=3). The N-telopeptide of type I collagen (NTX) was measured in urine by an automated competitive immunoassay (Vitros ECi, Ortho-Clinical Diagnostics, High Wycombe, UK; inter-assay CV 6%). The NTX was expressed as a ratio to urinary creatinine concentration measured by the dry slide method (Vitros 250, Ortho Clinical Diagnostics, High Wycombe, UK; inter-assay CV 3%).

Statistical Analysis

Baseline demographics were reported as mean and standard deviation (SD) or median with interquartile range for each treatment group. The mean percentage change for each BTM from baseline 1 (week -1) was calculated for all time-points. Repeated measures ANOVA was used to compare change over time with Bonferroni post hoc correction.

Statistical calculations were performed using MedCalc Statistical Software version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) and computing package R (http://R-project.org).

Establishing least significant change: The LSC was calculated using measurements from the 12 and 13 week visits of the treatment group (n=147). This represents the within person variability for measurements a week apart for women on treatment. The difference between week 12 and 13 was only significant for BoneALP in the risedronate group (difference -1.06, 95%CI -1.7 to -0.4, P<0.01).

The distribution of the measurements was positively skewed so a $\log_{10}$ transform was used to give an approximate Normal distribution. LSC was then calculated on the log-transformed data as follows:

$$\text{LSC}_{\text{log}} = Z' \times \sqrt{2} \times \text{SD}_{\text{RMS}}$$

where SD_{RMS} is the root-mean-square standard deviation calculated from the log-transformed data, and Z' is equal to 1.96 for 95% confidence level. The LSC as a percentage change on the original scale is then given by:

$$\text{LSC} = 100 \times \left(10^{\pm \text{LSC}_{\text{log}}} - 1\right)$$

Participants who met the target response (responders) were defined as those in whom the percentage decrease from baseline in the concentration of a specific bone marker was greater than the LSC. Those with a decrease less than the LSC were classified as not reaching the target response (non-responders).

Establishing premenopausal mean: We established RIs from 87 premenopausal women who were vitamin D replete (25OHD >50nmol/L). The reference data for each BTM was presented as the geometric mean and 95%

RI. All data were log_{10} transformed. The 95% RI was calculated for each marker as the mean $\pm$ 1.96 SD. (using the Robust method as recommended for n<120 reference samples (CLSI C28-A3), Medcalc) [25] .

The target for response to treatment using RIs was defined as a result below the mean value for premenopausal women and this was assessed using the available BTM result from the time-points on treatment mentioned above, with the baseline 1 visit BTM used as the pre-treatment value. A Chi-squared test was used to compare the effect of treatment group on the number of participants reaching the target for response.

Results

Study Population

The baseline demographics are presented in Table 1. As expected, the LS-BMD was considerably lower and all the BTMs consistently higher in the postmenopausal women with osteoporosis than in the healthy, premenopausal controls. In the bisphosphonate group, BTM data were available for 172 women at baseline, n=149 at 1 year and n=94 at 2 years. Some participants were lost to follow up beyond year 1 due to a delay in ethical approval to extend the study from one to two years [24] .

Percentage change from baseline

There was a decrease in BTMs in response to treatment with each of the three bisphosphonates over 2 years (Fig 1).

For bone resorption, serum CTX consistently showed greater reductions compared to urinary NTX during treatment with any of the bisphosphonates. At 12 weeks, for the ibandronate group this difference was -18%, (95% CI -29 to -8, P<0.001), alendronate -22%, (95%CI -29 to -14, P<0.001) and risedronate -30%, (95%CI -44 to -16, P<0.001). The magnitude of change was greater in the ibandronate and alendronate groups than in the risedronate group (Table 2). As expected, there was an earlier decrease in bone resorption compared to bone formation in all groups (Table 2) and the ibandronate group had a larger initial decrease in bone resorption at week 1 (CTX-80%) compared to alendronate (difference -31%, 95%CI -42 to -20, P<0.001) and risedronate (difference -45%, 95%CI -25 to -3, P=0.0087).

Within the formation markers, serum PINP consistently showed a greater percentage decrease than either BoneALP or OC. In the ibandronate group at week 12 the decrease in PINP (-63%) was greater than for OC (difference -24%, 95%CI -31to -17, P<0.0001) and BoneALP (difference -26%, 95%CI -33to -20, P<0.001), for alendronate PINP (-56%) was greater than OC (difference -20%, 95%CI -28 to -11, P<0.001) and BoneALP

(difference -24%, 95%CI -33 to -15, P<0.001) and for risedronate PINP (-48%) was greater than OC (difference 16%, 95%CI -26 to -7, P<0.001) and BoneALP (difference 19%, 95%CI -29 to -10, P<0.001). There was no significant difference between the change in OC and BoneALP for any of the three treatments at week 12. At week 48, the decrease in PINP (-71%) in the ibandronate group was still greater than OC (difference -17%, 95%CI -25to -20, P<0.001) and BoneALP (difference -26%, 95%CI -33 to -18, P<0.001) and was also greater for PINP (-66%) in the alendronate group (OC difference -13%, 95%CI -21 to -5, P<0.001, BoneALP difference -24%, 95%CI -32 to -17, P<0.001). For both ibandronate and alendronate the decrease in OC was greater than BoneALP (difference -8%, 95%CI -16 to -1, P=0.019, difference -11%, 95%CI -19 to -4, P=0.0014 respectively). In the risedronate group the change in PINP (-51%) was greater than BoneALP (difference -16%, 95%CI -29 to -2, P=0.02).

Responder analysis - least significant change

The LSC estimates for each BTM are shown in (Table 3) and were used to calculate the number of responders at 12 and 48 weeks (there was no further change in BTM after week 48). A high proportion of women reached the target for treatment for the serum BTMs but fewer subjects reached the target response for the urinary marker NTX.

At 12 weeks CTX LSC classified 127/146 as responders, NTX 50/148, PINP 125/149, OC 96/148, and BoneALP 89/149.

There was a significant difference in the proportion of women reaching the target response between the three treatment groups at 12 weeks and 48 weeks for, CTX (12 weeks P=0.0131, 48 weeks P<0.001), NTX (12 weeks P=0.0042, 48 weeks P=0.0028) and PINP (12 weeks, P=0.0355, 48 weeks P<0.001) and at 12 weeks for Bone ALP (P=0.027) but not at 48 weeks (P=0.216). There was no difference in response between treatment groups for osteocalcin at 12 weeks or 48 weeks (P=0.631, P=0.244 respectively).

The percentage change in BTM at 12 weeks for individual participants and the LSC for each BTM are shown in Fig2.

For bone resorption markers, more women were classified as responders in the alendronate group (CTX 49/50, NTX 23/51) than the risedronate (CTX 37/47, P=0.0075, NTX 9/47, P=0.002) and ibandronate groups (CTX 41/49, P=0.033). For the bone formation markers, more women reached the target for response in the ibandronate group compared to risedronate (PINP 47/50 vs 36/48, P=0.0198, BoneALP 37/50 vs 23/48, P=0.0146).

There was no effect of treatment group on LSC responders for OC. Several women had an increase in bone markers at week 12, however, not all BTM were elevated and most had a subsequent decrease. Poor compliance was identified in one of these women and another had a minor orthopaedic operation which would be expected to increase BTMs, particularly PINP [26, 27] .

Responder analysis - premenopausal mean

The change in bone resorption (CTX) and bone formation (PINP) over 2 years of treatment are shown in Fig3. These two markers were selected for the figure as they had the greatest magnitude of change and are also recommended as the reference bone markers by the IOF-IFCC Bone Marker Standards Working Group [9, 28] . While bone markers decreased to the lower half of the premenopausal RI for most participants during treatment, the number of women defined as responders (BTM below the premenopausal mean) differed between the BTMs; with 129/146 classified as responders by 12 weeks for CTX, 139/148 for NTX, 126/149 for PINP, 93/148 for OC and 28/149 for Bone ALP,. The number of responders for all BTMs in each of the three treatment groups are shown in Table 3. Using the RI criteria, a high proportion of women reached the target for treatment for the BTMs with the exception of BoneALP. For NTX 139/148 (94%) women were classified as responders, however 51/148 women were below the RI mean at baseline 1 (Table 3). When these women were excluded from analysis the proportion of responders was 90/97 (94%).

There was a difference in the proportion of women reaching the target for response between the treatment groups for CTX (48 weeks P=0.025), NTX (12 weeks P=0.031) and PINP (12 weeks P=0.014, 48 weeks P=0.006). When using the RI approach the number classified as responders at 12 weeks was higher in the alendronate group (51/51) compared to the risedronate group (41/47) for NTX (P=0.027) and higher in the ibandronate group (48/50) compared to risedronate (36/48) for PINP (P=0.0073),

There was concordance between the approaches to determine target response. For CTX (week 12), 127/146 women were classified as responders by LSC, 129/146 were responders by RI, with 117 by both criteria. For PINP (week 12), 125 of the 149 women reached the LSC target and 126 reached the RI target, with 115 classified as a responder by both methods. Using LSC, 110 women were classified as responders and by RI, 115 for both PINP and CTX.

To determine if a target response at 12 weeks was concordant over time, the number of responders at 12 weeks and 96 weeks was calculated for those women that participated for the 2 years of the study (n=94). For all three treatments there was no significant difference between the proportions of responders at 12 weeks compared to 96 weeks for CTX or PINP by either approach for target response. The proportion of women reaching the target for response by LSC criteria for CTX was 86% at 12 weeks and 72% at 96 weeks (P=0.0309) and for PINP 84% at 12 weeks and 77% at 96 weeks (P=0.271). . By the RI criteria CTX response was 86% at 12weeks and 74% at 96weeks (P=0.066) and PINP response was 85% at 12 weeks and 79% at 96 weeks (P=0.343). . There were no significant effects of treatment group other than for CTX by LSC in the risedronate group with 77% responders at 12 weeks and 47% at 96 weeks (P=0.031), the RI approach for CTX in the risedronate group had 80% responders at 12 weeks and 60% at 96 weeks (P=0.159).

Baseline BTM

For the two recommended reference markers CTX and PINP, around 10% of women were below the premenopausal mean before commencement of treatment. This proportion was higher for NTX (34%) and OC (22%) but lower (3%) for bone ALP. At the second baseline visit, after calcium and vitamin D supplementation, the proportion of women below the mean increased to 20% for CTX but did not change for PINP.

There was no effect of baseline CTX value on the percentage change in CTX at 12 weeks. Women with baseline CTX above the upper limit of the RI had a change in CTX of mean -74% (95%CI -80 to -68, n=35), compared to -74% (95%CI -78 to -71, n=98) for those with baseline CTX in the upper half of the RI (difference -0.1%, 95%CI-8 to 8 P=1.0), and -74% (95%CI -83 to -64, n=13) for those in the lower half of the RI at baseline (difference 0.2%, 95%CI -13 to 13, P=1.0).

The women with baseline PINP above the upper limit of the RI had the greatest percentage decrease in PINP at 12 weeks, mean -63% (95%CI -69 to -58, n=49) compared to -52% (95%CI -57 to -48, n=84) for those with baseline PINP in the upper half of the RI (difference 11%, 95%CI 2 to 19, P =0.0089) and -50% (95%CI -59 to -40, n=16) for those with baseline PINP in the lower half of the RI, (difference 13%, 95%CI 0 to27, P=0.0496).

Compliance

Drug accountability was recorded using medical events monitoring system (MEMS) bottle caps (AARDEX, Zurich, Switzerland). Good compliance was defined as more than 80% compliance over the first 48 weeks of the

study (because 48 weeks was when the maximum bone marker response was reached) [29, 30] . The percentage decrease in BTM at 48 weeks was significantly greater in the women with good compliance (n=104) compared to those with poorer compliance (n=31). For CTX -79% vs -64% (difference 15%, 95%CI 5.1 to 25.2 P=0.0035), NTX -59% vs -38% (difference 21%, 95%CI 4.1 to 36.9, P=0.0147), PINP -67% vs -51% (difference 16%, 95% CI 6.3 to 25.5, P=0.0013) and OC -52% vs -43% (difference 9%, 95%CI 1.7 to 17.1, P=0.017), A similar trend was observed for bone ALP by compliance, but the difference was not statistically significant, -42% vs -37% (difference 5%, 95%CI -1.8 to 12.5, P=0.139). Three women did not meet the target for treatment by either LSC or RI approach for PINP or CTX. Two of these had poor compliance at 48 weeks (less than 50%) the third did not have compliance data available.

Response and changes in Bone Density
The percentage change in both lumbar spine (LS) and proximal femur BMD at 96 weeks was greater in those who reached the LSC target for CTX (81/89 subjects) compared to those failing to reach the target response, LS 6.0% (SD 4.2) vs 1.3%, (3.7) difference 4.7% (95%CI 1.7 to 7.8) P=0.0028, FN 3.2% (3.4) vs 0.6% (3.1) difference 2.6% (95%CI 0.07 to 5.1) P=0.044, TH 3.2% (3.0) vs 1.0% (2.6) difference 2.2% (95%CI 0.02 to 4.4) P=0.048. However there was no significant difference in the percentage change in BMD at spine or proximal femur for classification by CTX RI; LS difference 2.5% (95%CI -0.5 to 5.5) P=0.100, FN difference 1.7% (95%CI -0.7 to 4.2) P=0.151, TH difference 1.1 (-1.1 to 3.2) P=0.327.

The percentage change in LS BMD at 96 weeks was greater for those who had reached the target response in PINP by 12 weeks defined by LSC, mean 6.2%, (SD 4.1), n=78 compared to those not reaching the target response, mean 2.3%, (SD 3.6), n=14, (difference 3.9%, 95%CI 1.6 to 6.3 P=0.0011). Similar changes were found for RI classification of responder, mean 6.2% (SD 4.1), n=79 compared to non-responder mean 2.5% (SD 4.1), n=13 for PINP (difference 3.7%, 95%CI 1.3 to 6.1, P=0.0033). The changes in femoral neck (FN) and total hip (TH) BMD were not significantly higher in the responders by either LSC or RI method for PINP. There was no relationship between the baseline 1 CTX or PINP and the percentage change in BMD.

Discussion

The proportion of women treated with oral bisphosphonates that reached the target response for BTM was high, regardless of the definition used. For the two bone turnover markers recommended as reference standards by the IOF-IFCC (CTX and PINP), the proportion reaching the target response ranged from 70 to 100%, by LSC and premenopausal RI methods.

Oral bisphosphonate therapy results in an early decrease in bone resorption markers and a later decrease in bone formation markers, as expected, as these processes are coupled [10] . The magnitude of the changes we observed were similar to those reported in other studies [9, 12, 13, 31] . The effects of ibandronate and alendronate on the bone resorption markers are similar to each other [13] and greater than seen with risedronate [12] . The greater, early decrease for ibandronate is likely to be due to the monthly dose regimen, because patients receive a larger first dose in comparison to the other two bisphosphonates.

 The clinical characteristics of bisphosphonates may relate to their individual profiles [32] , the two most important properties being the affinity to bone mineral and the inhibitory effects on osteoclasts. The antiresorptive effects of nitrogen containing bisphosphonates result from their inhibition of farnesyl pyrophosphate synthase (FPPS) a key enzyme for osteoclast function. Risedronate exhibits high enzyme binding which results in strong inhibition of FPPS, and has only a moderate affinity to bone mineral. Although the reduction in BTM is less for risedronate than other bisphosphonates it is effective for the prevention of all fracture types. The lower mineral affinity may enable a wider distribution within bone [32] . Alendronate and ibandronate are less effective inhibitors of FPSS but have stronger mineral binding affinity.

The proportion of women classified as reaching the target for response differed between markers. There were fewer responders with NTX by the LSC method, although there were more responders using the RI approach, this was also true when those in below the premenopausal mean for NTX at baseline were excluded from the analysis. Urine NTX is more variable than serum assays, and the measurement of creatinine adds to the 'noise', and increases the LSC [27, 33] . The LSC values were calculated using a two tailed approach and were comparable with those of Fink et al for PINP CTX and NTX, and Hannon et al for NTX [20, 34] , but some were higher than reported by others [20, 21] . Differences between studies include the participants that the LSC is calculated from, and type of assay. It has also been proposed that for monitoring treatment in clinical practice

a one-sided probability of 0.05 is appropriate as the direction of change in BTM is known, and some consider a probability of 80% to be adequate [9, 35] .

Bone ALP did not identify as many women as reaching the LSC response as other bone formation markers.   It is not known why bisphosphonates result in a greater decrease in PINP and OC than bone ALP, but this is a consistent finding. It might relate to changes in the maturity of the osteoblast population, as bone ALP is expressed early in osteoblast differentiation. It is also notable that using the premenopausal mean RI method, bone ALP identified few responders. This may be because the age-related increase in bone formation markers is greater with bone ALP than with other bone formation markers.

There was a greater change in PINP at 12 weeks for those with higher PINP at baseline. Bauer et al proposed that bisphosphonates may be more effective in women with elevated BTM at baseline. In the fracture intervention trial (FIT) alendronate reduced the risk of non-spine fracture only for those women who were in the highest 2 tertiles for PINP at baseline when compared to placebo treated women [14] . We are not aware of a previous report of baseline PINP predicting PINP response. We don't have any explanation as to why baseline CTX did not predict CTX response. It may be that PINP has a floor that is somewhat higher than zero due to continued modelling of bone, whereas CTX can be supressed to zero. Some of this may be hidden by the use of percentage changes while the absolute changes within the categories are likely to be quite different.

We observed a clear link between compliance with treatment and BTM change. We have previously used the electronic caps monitoring approach to estimate compliance and found it related to response to raloxifene therapy [30] . The IMPACT study found that adherence to risedronate therapy was positively associated with bone turnover marker change and that good bone resorption marker response (CTX or NTX) was associated with lower risk of non-vertebral fracture [36] .
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We observed that those that reached the target for response for PINP had a greater increase in lumbar spine BMD than those that failed to reach the target for treatment, whichever method was used. This finding is in keeping with other publications linking change in BTM to change in BMD, with alendronate and estrogen therapy [37] , as well as with an evaluation of the use of NTX in clinical practice with risedronate and alendronate [38] .

The two methods of assessing BTM response have limitations. The LSC approach requires that a sample be taken before starting therapy. This could be overlooked or the patient may have low turnover due to previous treatment. In these cases, then the RI approach is particularly useful. However, this also has its limitations. Some untreated women were already in the lower half of the RI for some of the bone markers at baseline. These women will likely have a lesser BTM response. If the baseline marker (e.g. CTX) is low then another bone marker (e.g. PINP) could be measured for comparison.

In women with typical postmenopausal baseline turnover, a lack of BTM response is a useful trigger for further evaluation, to review compliance and investigate for additional causes of high turnover. There may be clinical situations where BTM would be expected to increase (such as starting an aromatase inhibitor), where maintaining BTM at baseline levels could represent a reasonable response to bisphosphonate treatment.

Conclusion

Bone turnover markers can be a useful tool to evaluate response to bisphosphonate treatment. Both LSC and premenopausal RI approaches can identify those that reach the target for response and are associated with BMD change. Bone turnover markers reflect different aspects of the bone remodelling cycle and so response to treatment differs between markers, which needs to be considered in their interpretation.

Tables

Table 1. Baseline data for the three treatment groups and premenopausal women. Data shown as mean (SD) and for BTM as median [interquartile range].

Table 2. The percentage change from baseline 1 (week -1) in bone turnover markers for the 3 treatment groups shown as mean with 95% confidence interval (CI), (note: week 12a is the mean value for 12 and 13 weeks). The percentage change at each visit was compared to baseline by repeated measures ANOVA with Bonferroni post hoc correction (*P<0.05, ***P<0.001)

Table 3: Responder analysis for least significant change (LSC) and reference interval (RI) methods. The number of women with a decrease in BTM greater than LSC, data shown as n/total n (%). The number of women with BTM below the premenopausal RI geometric mean (GM), data presented as n/total n (%). The number of women who were below the RI mean at baseline 1 (week -1) for each treatment group are shown. Week 12a is the average for visits at weeks 12 and 13. (Data log transformed. Reference interval Robust method CLSI C28-A3)

Figure legends

Figure 1. The percentage change from baseline (mean and standard error of the mean) for a) bone resorption markers and b) bone formation markers for the three bisphosphonate treatments (ibandronate, alendronate, risedronate) over 2 years.

Figure 2 The percentage change from baseline in bone resorption (CTX, NTX) and bone formation (PINP, OC, BoneALP) markers at 12 weeks for each treatment group [• Ibandronate (Ibn), ○ Alendronate (Ald), x Risedronate (Ris)]. The shaded area represents the LSC threshold for each BTM.

Figure 3. Bone Markers Reference Interval.

Figure 3. Box and whisker plots of the absolute values for bone resorption (CTX) and bone formation (PINP). The box represents the interquartile range, the middle line is the median and the whiskers show the range of the data for the three bisphosphonates (Ibandronate, Alendronate and Risedronate). The premenopausal reference interval is shown by the horizontal dashed lines and geometric mean as solid line,

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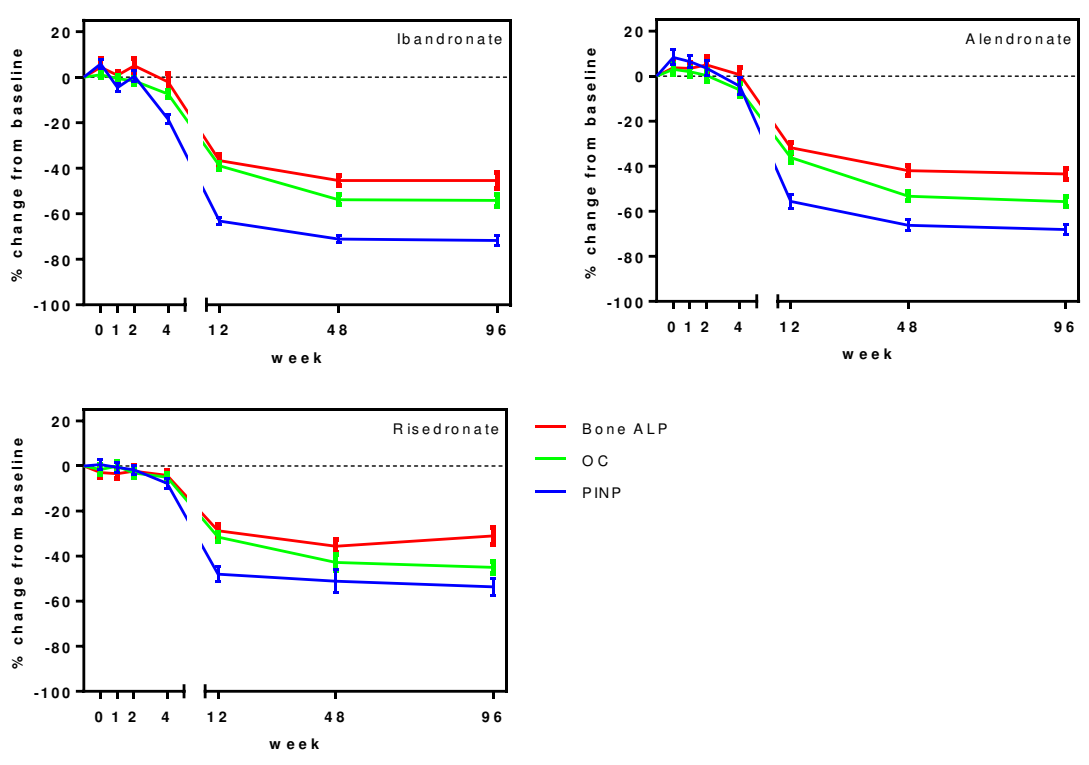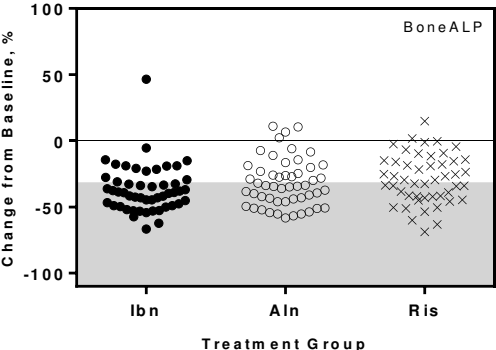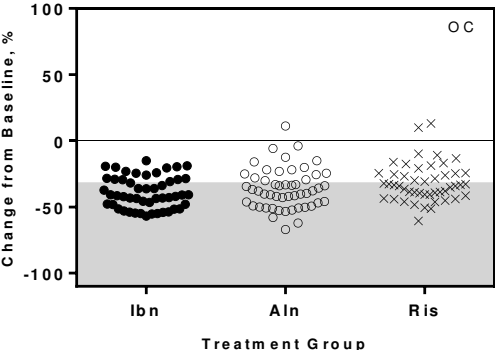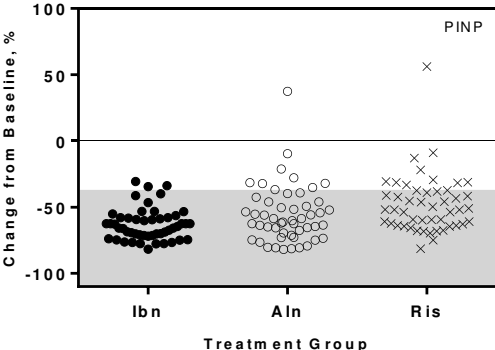
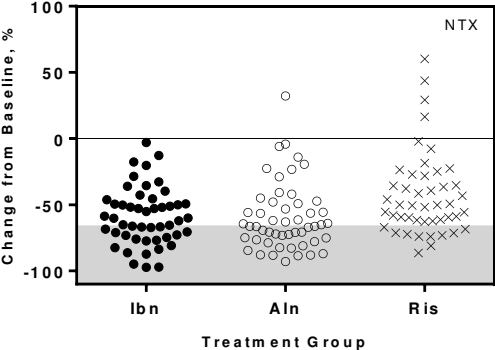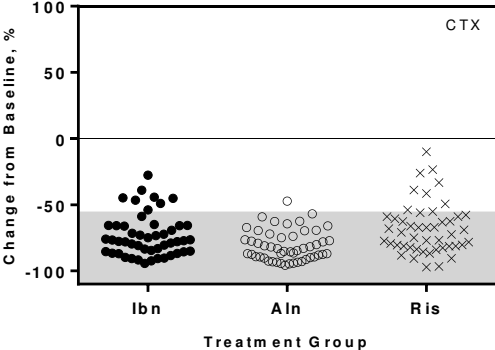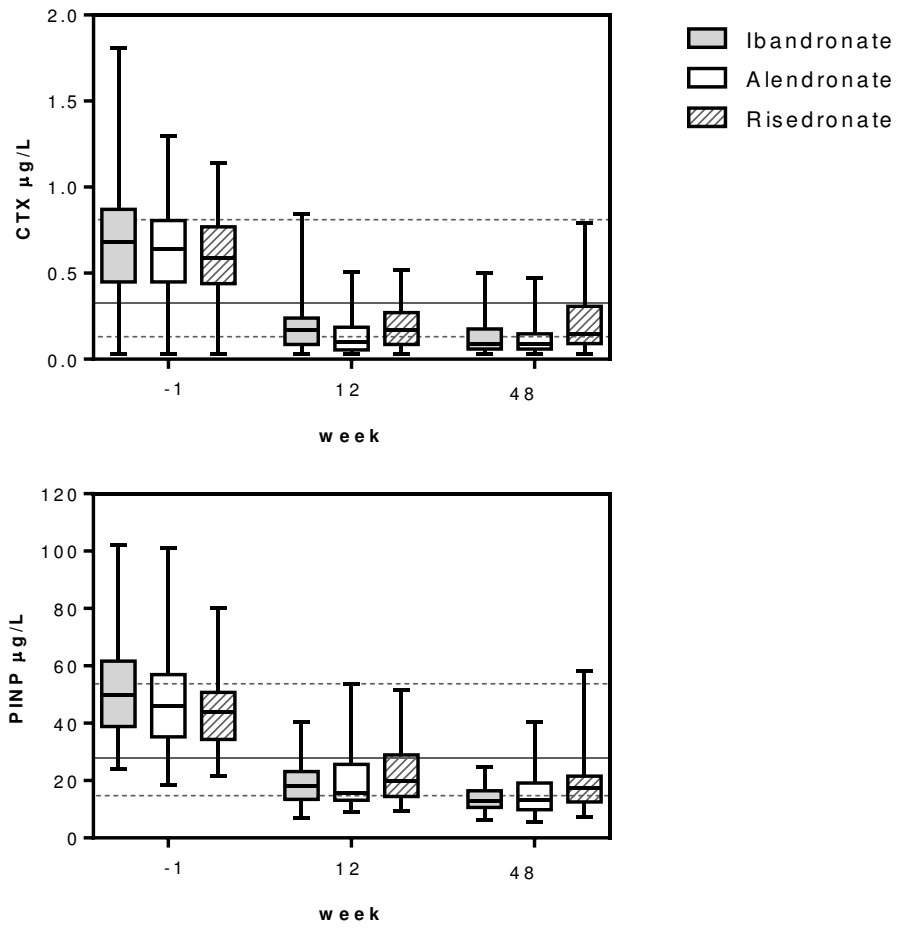**Fig 1**.

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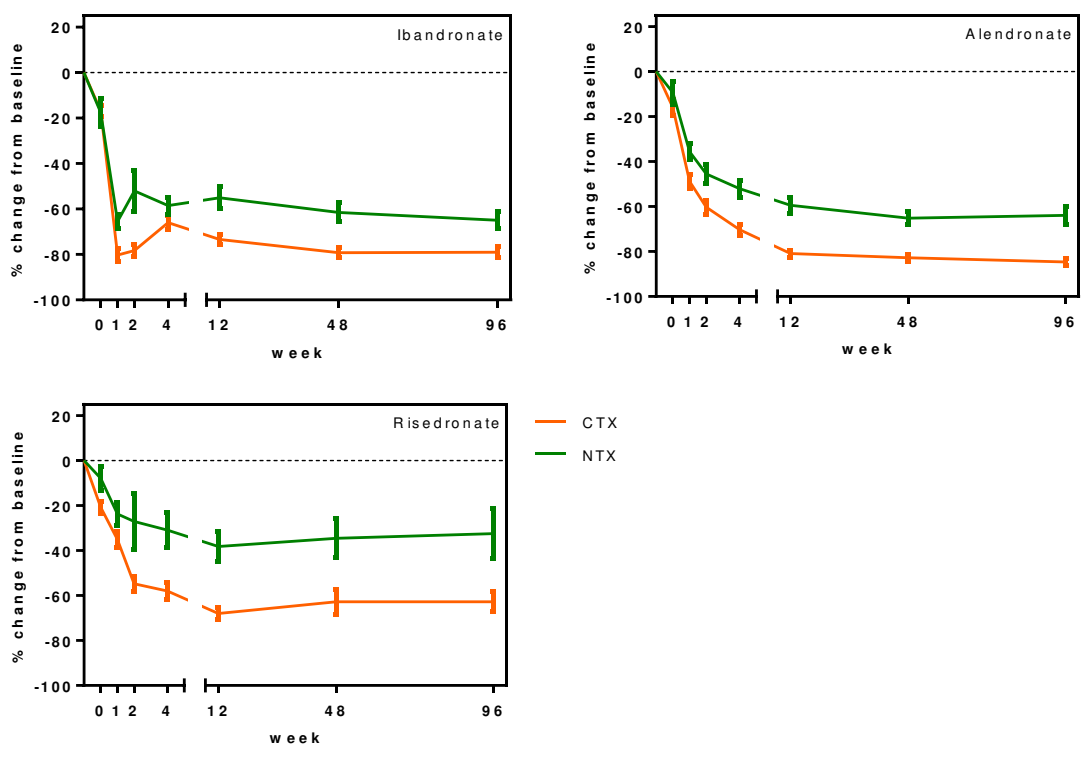
b)

Fig 2.

Fig 3.

TRIO BTM Manuscript Tables:

Table 1. Baseline data for the three treatment groups and premenopausal women. Data shown as mean (SD) and for BTM as median [interquartile range].

	Ibandronate	Alendronate	Risedronate	Premenopausal
n	57	57	58	87
Age	66.9 (7.2)	67.8 (7.8)	66.8 (6.7)	37.9 (1.7)
Height (cm)	159.8 (6.9)	160.1 (5.3)	160.7 (6.0)	164.6 (6.5)
Weight (kg)	67.4 (10.8)	66.3 (10.2)	69.1 (9.4)	67.1 (11.0)
LS BMD g/cm^2	0.796 (0.117)	0.788 (0.104)	0.812 (0.084)	1.092 (0.117)
CTX µg/L	0.68 [0.45-0.87]	0.64 [0.46-0.80]	0.59 [0.44-0.77]	0.32 [0.23-0.41]
NTX nmol/mmol Cr	53.9 [35.8-70.2]	42.9 [31.6-55.6]	39.6 [32.3-55.8]	36.8 [26.8-46.4]
PINP µg/L	49.9 [39.2-61.6]	46.2 [35.3-56.8]	44.0 [34.5-50.5]	29.0 [22.2-34.9]
OC µg/L	25.9 [20.4-32.9]	24.5 [19.3-31.8]	23.3 [17.9-29.4]	18.2 [14.5-22.1]
BoneALP µg/L	18.5 [16.1-27.0]	19.5 [14.7-24.2]	18.8 [16.6-24.1]	9.6 [7.9-11.9]

Table2 The percentage change from baseline 1 (week -1) in bone turnover markers for the 3 treatment groups is shown as the mean with 95% confidence interval (CI), (note: week 12a is the mean value for 12 and 13 weeks). The percentage change at each visit was compared to baseline 1 by repeated measures ANOVA with Bonferroni post hoc correction (*P<0.05, **P<0.001)

	CTX	NTX	PINP	OC	BoneALP
Ibandronate					
week 1	-80 (-86 to -75) ***	-65 (-72 to -58) ***	-5 (-8 to -1)	-1 (-4 to 3)	4 (-3 to 11)
week 4	-66 (-72 to -60) ***	-59 (-66 to -51) ***	-18 (-22 to -14) ***	-7 (-10 to -4) *	-2 (-10 to 6)
week 12a	-73 (-78 to -69) ***	-55 (-65 to -45) ***	-63 (-67 to -60) ***	-39 (-42 to -36) ***	-37 (-42 to -32) ***
week 48	-79 (-84 to -75) ***	-62 (-70 to -53) ***	-71 (-74 to -68) ***	-54 (-58 to -49) ***	-45 (-50 to -40) ***
week 96	-80 (-84 to -74) ***	-65 (-72 to -58) ***	-72 (-76 to -67) ***	-54 (-60 to -48) ***	-45 (-53 to -38) ***
Alendronate					
week 1	-49 (-55 to -42) ***	-36 (-43 to -28) ***	7 (1 to 12)	2 (-2 to 6)	3 (-2 to 9)
week 4	-70 (-75 to -65) ***	-52 (-60 to -45) ***	-5 (-12 to 3)	-6 (-12 to -1)	1 (-5 to 7)
week 12a	-81 (-84 to -78) ***	-59 (-67 to -52) ***	-56 (-62 to -50) ***	-36 (-40 to -32) ***	-32 (-37 to -27) ***
week 48	-83 (-86 to -80) ***	-65 (-71 to -60) ***	-66 (-71 to -61) ***	-53 (-58 to -49) ***	-42 (-47 to -37) ***
week 96	-85 (-88 to -81) ***	-64 (-72 to -56) ***	-68 (-73 to -64) ***	-56 (-61 to -51) ***	-43 (-48 to -39) ***
Risedronate					
week 1	-35 (-42 to -28) ***	-24 (-34 to -13) ***	-1 (-5 to 4)	0 (-4 to 4)	-3 (-8 to 1)
week 4	-58 (-66 to -50) ***	-31 (-47 to -15) ***	-8 (-13 to -3)	-5 (-9 to -1)	-4 (-9 to 0)
week 12a	-68 (-74 to -62) ***	-38 (-51 to -25) ***	-48 (-54 to -42) ***	-32 (-36 to -28) ***	-29 (-34 to -23) ***
week 48	-63 (-74 to -52) ***	-35 (-52 to -17) ***	-51 (-62 to -41) ***	-43 (-50 to -35) ***	-36 (-41 to -30) ***
week 96	-63 (-72 to -54) ***	-32 (-55 to -10) ***	-54 (-61 to -46) ***	-45 (-51 to -39) ***	-31 (-39 to -23) ***

Table 3: Responder analysis for least significant change (LSC) and reference interval (RI) methods. The number of women with a decrease in BTM greater than LSC, data shown as n/total n (%). The number of women with BTM below the premenopausal RI geometric mean (GM), data presented as n/total n (%). The number of women who were below the RI mean at baseline 1 (week -1) for each treatment group are shown. Week 12a is the average for visits at weeks 12 and 13. (Data log transformed. Reference interval Robust method CLSI C28-A3)

BTM	Visit	LSC %	Ibandronate LSC	Alendronate LSC	Risedronate LSC	GM (RI)	Ibandronate RI	Alendronate RI	Risedronate RI
CTX	Week-1	-56	-	-	-	0.32 µg/L (0.13 to 0.81)	5/49(10%)	4/50(8%)	4/47(9%)
	Week 12a		41/49 (84%)	49/50 (98%)	37/47 (78%)		42/49(86%)	48/50(96%)	39/47(83%)
	Week 48		40/45 (89%)	47/48 (98%)	31/44 (70%)		40/45(89%)	46/48(96%)	34/44(77%)
NTX	Week-1	-67	-	-	-	36.1 nmol/mmol Cr (16 to 78)	13/50(26%)	20/51(39%)	18/47(38%)
	Week 12a		18/50 (36%)	23/51 (45%)	9/47 (19%)		47/50(94%)	51/51(100%)	41/47(87%)
	Week 48		23/46 (50%)	27/49 (55%)	10/45 (22%)		43/46(93%)	48/49(98%)	42/45(93%)
PINP	Week -1	-38	-	-	-	28.3 µg/L (15 to 54)	5/50(10%)	7/51 (14%)	4/44 (9%)
	Week 12a		47/50 (94%)	42/51 (82%)	36/48 (75%)		48/50(96%)	42/51(82%)	36/48(75%)
	Week 48		46/46 (100%)	46/49 (94%)	37/45 (82%)		46/46(100%)	46/49(94%)	37/45(82%)
OC	Week -1	-32	-	-	-	18.1 µg/L (10 to 34)	7/50(14%)	11/51(22%)	15/47(32%)
	Week 12a		33/50 (66%)	35/51 (69%)	28/47 (60%)		30/50(60%)	32/51(63%)	31/47(66%)
	Week 48		42/46 (91%)	45/49 (92%)	36/44 (82%)		37/46(80%)	46/49(94%)	35/44(77%)
BoneALP	Week-1	-31	-	-	-	9.5 µg/L (5 to 18)	1/50(2%)	3/51(6%)	0/48(0%)
	Week 12a		37/50 (74%)	29/51 (57%)	23/48 (48%)		11/50(22%)	10/51(20%)	7/48(15%)
	Week 48		38/46 (83%)	36/48 (75%)	30/45 (67%)		15/46(33%)	14/48(29%)	10/45(22%)