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Original Article

Effects of Discontinuing Oral Bisphosphonate Treatments for Postmenopausal Osteoporosis on Bone Turnover Markers and Bone Density.

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Abstract

Introduction: Bisphosphonates (BPs) continue to suppress bone turnover markers (BTMs) after treatment has stopped, leading to the suggestion that a pause in treatment could be considered for low-risk patients. Indirect comparisons suggest that after cessation of treatment, the effects on bone may differ between drugs. We investigated the effects of stopping oral BP treatments for postmenopausal osteoporosis on BTMs and bone mineral density (BMD).

Methods: We studied postmenopausal osteoporotic women who had previously taken part in a two-year randomized study of three oral BPs (ibandronate, alendronate, or risedronate). At the end of the study, women with hip BMD T-score >-2.5 and considered clinically appropriate to discontinue treatment, were invited to participate in a further two-year observational study. Biochemical response was assessed using BTMs and BMD was measured by dual-energy X-ray absorptiometry.

Results: All BTMs increased after treatment withdrawal but remained below the pre-treatment baseline with less suppression of BTMs for the risedronate group compared to alendronate and ibandronate up to 48 weeks. There was no difference between the BP groups 96 weeks after stopping treatment. The change in BMD during the 96 weeks after stopping treatment was -1.6% (95% CI -1.9 to -1.2, P<0.001) for the total hip and -0.6% (95% CI -1.1 to -0.2, P=0.17) at the lumbar spine with no difference between the three BP groups (P=0.85 and P=0.48 respectively).

Conclusion: For all treatment groups, there was an increase in BTMs and a decrease in hip BMD after stopping BPs for 2 years; however none returned to pre-treatment baseline values.

Key words: bone markers, bone density, ibandronate, alendronate, risedronate

Mini abstract:

The antiresorptive potency varies between different bisphosphonates. We investigated the effect of stopping oral bisphosphonate treatment for postmenopausal osteoporosis (ibandronate, alendronate, risedronate) on BTMs and BMD. After stopping treatment, all three groups showed an increase in BTMs and a decrease in hip BMD; however, none returned to pre-treatment baseline values.

Introduction

Bisphosphonates (BPs) are the most commonly-used treatment for osteoporosis with demonstrated efficacy in reduction of fracture risk over three to five years of treatment [1-3]. Although BPs are usually safe and well tolerated, it is common practice to consider a pause in treatment after five years due to concerns about rare adverse effects [3-5]. However, several large clinical trials report the risk of osteonecrosis of the jaw and atypical femur fracture to be low in long-term treatment [6, 7].

The BPs licensed in the UK for the treatment of osteoporosis differ in their affinity to bind to the bone surface and are distributed through the bone at different rates [8, 9]. The unique profile of binding affinity and antiresorptive potency of each of the bisphosphonates may influence the speed and magnitude of both the onset and the offset of treatment effect [3, 10]. Alendronate has a higher affinity for calcium hydroxyapatite than risedronate; it binds to the bone surface more avidly but is dispersed through the bone more slowly and less extensively. In the first two years after stopping oral BPs, alendronate can be detected in about 40% of patients' urine, but risedronate cannot be detected, suggesting a longer retention of alendronate [11].

Osteoporotic women treated with alendronate have a decrease in bone turnover markers (BTMs) into the premenopausal range and an increase in bone mineral density (BMD); this effect is sustained for ten years with continued treatment [12]. The persistence of low BTMs after treatment may be associated with continued beneficial effects [13]. Bisphosphonates accumulate in bone over time and are released into the circulation during bone turnover after treatment is discontinued [14]. It is proposed that the BPs released during bone resorption would be available to inhibit bone resorption at other sites [15]. As the drug is still present in bone after discontinuation of treatment, the anti-fracture effect may persist, and it might be reasonable to periodically consider a pause in treatment for patients who are not at high current risk of fracture [3, 16-18].

There have been several studies of the effects of stopping BP treatment [19-21]. Discontinuation of alendronate treatment is associated with an increase in bone turnover and a decrease in bone density but not full return to pre-treatment levels [12, 19, 22]. Black *et al*. found that women who discontinued alendronate after five years of treatment had no increase in the risk of non-vertebral or morphometric vertebral fracture, but did have a higher risk of clinical vertebral fracture compared with those who continued alendronate for ten years [19]. The incidence of fracture after stopping treatment was higher in those with lower baseline BMD or prevalent vertebral fracture. Bauer *et al*. reported that among the women who discontinued alendronate after four to five

years, age and hip BMD at the time of stopping treatment predicted clinical fractures during the next five years, and bone loss of greater than 3% at the total hip two years after cessation of therapy was associated with fracture risk [23]. Discontinuation of risedronate after three years of treatment resulted in complete resolution of effects on BTM and partial resolution of effect on BMD within one year of stopping treatment; however, the fracture risk reduction was maintained [24, 25]. There are limited comparative data available on the effect of stopping different BPs and to the best of our knowledge, none available from randomised studies [26].

We have previously carried out a randomised, open-label, parallel, single-centre study of three BPs for the treatment of postmenopausal osteoporosis (TRIO Study) [27, 28]. Participants were randomised to one of three BP (ibandronate, alendronate and risedronate) for two years of treatment. The end of the TRIO study provided the opportunity to examine the effect of stopping three randomised oral BP treatments for postmenopausal osteoporosis on BTMs and BMD in a two-year observational extension of the TRIO study.

Methods

Study Design

The original study comprised a two-year, open-label, parallel, randomised, controlled intervention trial of three oral BPs (TRIO study). The effects on BMD and BTMs have previously been published [27, 28]. At the end of the TRIO study, eligible participants were invited to participate in a further two-year observational extension study in which the BP treatment was stopped (TRIO offset study).

Study Population

For the original TRIO study, we recruited postmenopausal women, less than 85 years old, with osteoporosis defined by dual-energy x-ray absorptiometry (DXA) at the lumbar spine or proximal femur as i) a BMD T score $\leq$-2.5 (stratum 1) or ii) a BMD T score $\leq$-1.0 plus a prevalent non-traumatic fracture (stratum 2). The recruitment details have been described previously [27]. Exclusion criteria included recent fracture, the use of medications or diagnosis of a medical condition known to affect bone, or a BMI <18 or >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 participants.

We invited women who had completed the two-year TRIO BP treatment study to participate in a further two-year observational extension study with no BP treatment. The criteria for invitation and subsequent inclusion in the TRIO Offset study were based on: 1) BP treatment compliance of more than 80% assessed using medical

events monitoring system (MEMS) bottle caps (AARDEX, Zurich, Switzerland), 2) femoral neck BMD T-score of -2.5 or above, 3) no accelerated bone loss defined as more than 5% decrease in BMD at hip or spine, 4) no recent fracture. These criteria were consistent with the algorithm from the report of the ASBMR task force for managing patients on long-term BP therapy [17].

Study patients who stopped BPs continued to receive calcium and vitamin D supplements consistent with clinical practice. Participants were assessed at one year after treatment had stopped. Any participants with accelerated bone loss (>5% per year at hip or spine, after allowing for artefacts such as weight change) or new vertebral fracture were referred to the Metabolic Bone Clinic for further assessment and if any anti-resorptive or anabolic treatment was reintroduced, the participant was withdrawn from the study.

Healthy premenopausal women (ages 35 to 40 years) were recruited as an observational control group to monitor variability in measurements for BTMs and BMD over the study period [27]. The premenopausal participants had regular menstrual cycles and were not using hormonal contraceptives. Exclusion criteria included recent fracture, the use of medications or diagnosis of a medical condition known to affect bone, or a BMI <18 or >35 kg/m^2. At the end of the two-year TRIO study, the premenopausal women were asked to attend for another visit at the end of the two-year extension study.

Study Interventions

In the original (TRIO) two-year open-label study, participants were randomised to receive one of three oral BPs at the licensed dose: (i) ibandronate (Bonviva, Roche, 150mg), (ii) alendronate (Fosamax, Merck, 70 mg), or (iii) risedronate (Actonel, Warner-Chilcott, 35 mg) [27]. 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, ProStrakan), which was initiated one week before the BP treatments started.

Study Outcomes and Assessments

Participants had blood samples collected at baseline after calcium and vitamin D treatment had commenced but before BP treatment, then at 12, 48 and 96 weeks during treatment (TRIO study) and at 24, 48, 72 and 96 weeks off treatment for the extension study (TRIO offset) to total 192 weeks. In the premenopausal control group, samples were collected at baseline, 96 weeks and 192 weeks. Blood was collected after an overnight fast and left to clot for 30 minutes before centrifugation at 2500g for 10 minutes and morning, second void, fasting urine

samples were collected. All samples were stored at -80°C until analysis and those collected as part of the initial TRIO study (weeks 0 to 96) were re-measured with samples from the TRIO offset study in one analytical batch.

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 6.0%). 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 3.0%). The C-telopeptide of type I collagen (CTX), intact pro-collagen I N-propeptide (PINP), N-mid osteocalcin (OC), and bone alkaline phosphatase (BoneALP) were measured using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, UK). The inter-assay coefficients of variation (CVs) were 4.4%, 4.5%, 5.3% and 4.3% respectively.

BMD (g/cm^2) of the total hip (TH) and lumbar spine (LS) were measured by DXA using a Discovery A densitometer (Hologic Inc, Bedford, MA). Vertebral fracture assessments (VFA) were also performed using the Discovery A to identify prevalent and incident vertebral fractures. All VFA images were visually assessed using the algorithm-based qualitative approach [29]. All imaging measurements were performed at baseline and weeks 12 and 96 on treatment, then at week 48 and 96 off treatment, the visit window was $\pm$ 2 weeks.

The primary endpoint for the TRIO offset study was the change in urinary NTX at 96 weeks off treatment. The secondary endpoints were the change in BTMs (NTX, CTX, PINP, OC, Bone ALP) at 24, 48, 72 and 96 weeks off treatment, and the change in BMD at 48 and 96 weeks off treatment. The change in each measure was calculated in relation to i) the pre-treatment value and ii) the end of treatment value for BMD.

Statistical Considerations

The sample size for the TRIO offset study was constrained by the proportion of eligible patients from the original TRIO study, but anticipating an uptake of 33% (i.e. 20 patients per group), the study had a 90% power to detect a 10 nmol BCE/mmol Cr change in NTX assuming a standard deviation (SD) of 8 and a two-sided, 2% significance level, which equates to a 5% overall type I error incorporating a Tukey adjustment for the three-way multiple comparisons. Data were analysed using linear regression for each time point, with the covariates being pre-treatment age, BMI, stratum (osteoporotic/osteopenic), LS BMD and the treatment group. Changes in BMD are presented as least square (i.e. adjusted) mean percentage changes with two-sided 95% confidence intervals. Biochemistry outcomes were log-transformed before analysis with comparisons given as geometric means (defined as 100 x exp [adjusted log-mean – 1]) with 95% confidence intervals. The relationship between changes in BTMs and changes in BMD during the period of no treatment was investigated by correlation of the absolute change in BTMs with the percentage change in TH BMD compared to the end of treatment visit

(Pearson's correlation). Analyses were conducted using Stata statistical software (StataCorp. 2015 Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

Results

Baseline Characteristics

The study flow is described in Fig 1. Of the original TRIO participants, 94 were assessed for eligibility and 59 consented to continue into the TRIO offset study, two of whom were subsequently withdrawn with no further follow-up (one screen failure based on blood results, one changed her mind). The baseline characteristics of the remaining 57 participants at pre-treatment randomisation are presented in Table 1 for each of the three BP treatment groups and the premenopausal control group.

At the pre-treatment baseline, the characteristics of the participants of the TRIO offset study (n=57) were; mean age 66.6 years (SD 6.9), BMI 26.1 (SD 3.9), lumbar spine BMD T-score mean -2.0 (SD 0.7), total hip BMD T-score mean -1.14 (SD 0.7), 35% in stratum 1, 65% stratum 2. This compared with; mean age 65.9 years (SD 7.3), BMI 25.1 (SD 3.8), lumbar spine BMD T-score mean -2.6 (SD 1.0), total hip BMD T-score mean -1.7 (SD 0.9) and 57% stratum 1, 43% stratum 2 for those who did not participate in the TRIO offset study (n=35). After two years of BP treatment (end of the TRIO study) the lumbar spine BMD T-score was mean -1.6 (SD 0.8) for the women entering the offset study and mean -2.3 (SD 1.0) for those who did not participate (difference -0.7 95% CI -1.1 to -0.3, P<0.001). Total hip BMD T-score was mean -0.9 (SD 0.7) and -1.6 (SD 1.3) respectively (difference -0.7 95% CI -1.2 to -0.2, P=0.006). Vertebral fractures were identified by VFA in six participants at pre-treatment baseline, no new vertebral fractures were identified during the two year TRIO treatment period for the participants entering the offset study.

Bone Turnover Markers

The percentage changes from pre-treatment values for BTMs are shown in Fig 2. All BTMs exhibited similar patterns, with a rapid decrease in the first weeks of treatment followed by stabilisation up to the end of treatment (96 weeks) then a gradual increase towards, but not reaching, pre-treatment levels during the two years off treatment.

The magnitude of suppression of BTMs in the risedronate group during the treatment phase was consistently smaller than that observed for ibandronate or alendronate (Table 2). The mean decrease in NTX (the primary endpoint) at the end of treatment for risedronate was significantly different to that of ibandronate (41% vs 57%, ratio of means=1.35, 95% CI 1.07 to 1.71,p=0.013) or alendronate (41% v 60%, ratio of means 1.46, 95% CI 1.16 to 1.83, p=0.002). At 24 weeks off treatment, the change from pre-treatment for risedronate (17%) was

significantly less than for ibandronate (36%; ratio of means 1.29, 95% CI 1.09 to 1.54, p=0.005) and alendronate (40%, ratio of means 1.40, 95% CI 1.18, 1.66, p<0.001). All three BP groups exhibited a similar pattern of change from 48 weeks off treatment onwards, with no further pairwise differences. The three BP groups combined showed a statistically significant decrease from pre-treatment baseline at all time points during the off-treatment period.

The effect of BPs on CTX was more pronounced than observed for NTX. All three groups demonstrated a suppression of CTX throughout the two-year treatment period, with the effect of risedronate (79% change from pre-treatment) being significantly different to that of alendronate (91%, ratio of means 2.27, 95% CI 1.45 to 3.57, p=0.001) but not ibandronate (86%, ratio of means 1.46, 95% CI 0.92 to 2.31, p=0.11) (Table 2). The CTX values increased over the two years off treatment but remained significantly lower than the pre-treatment baseline. At 24 weeks off treatment, the percentage decrease from pre-treatment was 23% for risedronate, significantly less than alendronate (60%; ratio of means 1.93, 95% CI 1.34, 2.78, p=0.001) and with a borderline significant difference from ibandronate (50%; ratio of means 1.55, 95% CI 1.07, 2.24, p=0.023). The difference between alendronate and risedronate was maintained at 48 weeks off treatment (49% v 15%, the ratio of means 1.67, 95% CI 1.14 to 2.45, p=0.009). After that, CTX remained at significantly lower levels compared to pre-treatment, but no further differences were noted between the three treatment groups.

The bone formation markers had similar patterns to bone resorption (Fig 2). All of the bone formation markers increased during the two years off treatment (Fig 2) but did not reach the pre-treatment values; there was no difference between the treatment groups two years after stopping BPs.

The difference between the treatment groups for percentage change in bone formation markers is shown in Table 2. For PINP, the decrease from pre-treatment baseline at the end of the two-year treatment period in the risedronate group was 60%, which was significantly different to ibandronate (73%, ratio of means 1.47, 95% CI 1.15, 1.88, p=0.003) and alendronate (71%, ratio of means 1.39, 95% CI 1.10, 1.77, p=0.007). At 24 weeks off treatment, the percentage decrease from pre-treatment for risedronate was less than for the other two BPs (34% vs 51% with ibandronate, ratio of means 1.34, 95% CI 1.07 to 1.67, p=0.012; 34% vs 50% with alendronate, ratio of means 1.31, 95% CI 1.06 to 1.63, p=0.015) and also at 48 weeks off treatment (23% vs 42% with ibandronate, ratio of means 1.34, 95% CI 1.08 to 1.67, p=0.008; 23% vs 43% with alendronate, ratio of means 1.35, 95% CI 1.10 to 1.66, p=0.006). After two years of stopping treatment, there was no significant difference in the percentage change from baseline between the three treatment groups (Table 2). The difference between treatment groups for osteocalcin and Bone ALP are shown in table 2.

In the premenopausal control group, there was no change in CTX, NTX or Bone ALP during the study. However, there was a decrease in PINP and OC (mean -13%, 95% CI -20 to -4 and mean -20%, 95% CI -28 to -10 respectively).

Bone Mineral Density

The change in BMD for the original TRIO study has been previously published [27]: in summary, two years of treatment with BPs was associated with an increase in BMD in central skeletal sites, but with a smaller percentage change with risedronate treatment.

The percentage change in BMD for the 57 women in the TRIO offset study, compared to pre-treatment BMD measurements are shown in Fig 3. BMD increased during two years of treatment; total hip BMD by 3.6% (95% CI 2.2, 5.0), 4.2% (95% CI 2.9, 5.6) and 2.0% (95% CI 0.6, 3.3) for ibandronate, alendronate and risedronate respectively (Table 3). After two years off-treatment, the percentage changes from pre-treatment were 1.6% (95% CI 0.0, 3.3), 3.2% (95% CI 1.6, 4.8) and 0.7% (95%CI -0.9, 2.3) for ibandronate, alendronate and risedronate respectively, and 1.9% (95% CI 0.9, 3.0) for all groups combined (P<0.001). The overall change in BMD at the total hip two years after stopping treatment (4yr), compared to the end of treatment (2yr) was -1.6% (95% CI -1.9 to -1.2, P<0.001) for all BP groups combined, with no difference between the three treatment groups (P=0.85).

At the lumbar spine, BMD increased by 5.7% (95% CI 4.1, 7.4), 7.5% (95% CI 5.9, 9.0) and 4.1% (95% CI 2.6, 5.6) for ibandronate, alendronate and risedronate respectively during two years of treatment, with a significant difference between the BP groups (P=0.015) Table 3. Two years after treatment had been stopped, the change in lumbar spine BMD compared to pre-treatment baseline, was 4.7% (95% CI 2.6, 6.7), 8.0% (95% CI 6.0,10.0) and 2.5% (95% CI 0.5,4.5) for ibandronate, alendronate and risedronate respectively, with a significant difference between groups (P=0.002). The overall change in lumbar spine BMD during the off-treatment period (4yr visit), compared to the end of treatment (2yr visit) was -0.6% (95% CI -1.1 to -0.2, P=0.17) with no between-group difference (P=0.48). There was no significant change in BMD at either site for the control group over the study period (TH BMD mean -0.3%, 95%CI -1.6 to 1.0, LS BMD mean -0.7%, 95%CI -1.8 to 0.3).

Vertebral fractures were identified by VFA in four participants during the two year offset study (three women in the risedronate group and one in the ibandronate group).

The absolute difference in BTMs after stopping treatment (compared to the end of treatment visit) was related to the percentage change in TH BMD during two years off treatment. Greater bone loss was associated with the

largest increase in BTM. At week 24 after stopping treatment PINP r=-0.30 (P=0.035), CTX r=-0.50 (P=0.0002), at week 48 offset PINP r=-0.41 (P=0.004), CTX r=-0.58 (P<0.001), at week 96 offset PINP r=-0.41 (P=0.004) CTX r=-0.46 (P=0.0014). Similar results were obtained using absolute and relative changes.

Among the 51 participants completing the offset period, the height decreased by a mean of 0.40cm during BP treatment (95%CI 0.22cm to 0.59cmm P=0.0001) and by 0.32cm during the offset period (95% CI 0.16 to 0.49, P=0.0002). The average weight change in the same periods were +0.18kg (95% CI -0.54 to +0.89, P=0.62) and +0.56kg (-0.19kg to +1.31kg, P=0.14). There was no significant difference between the three BP groups for height or weight changes at either visit. The difference in height during the study is comparable to normal age related change over 3 years as reported by Siminoski *et al* that was not attributed to incident fracture [30].

Discussion

To our knowledge, this is the first randomised controlled trial of three BPs (ibandronate, alendronate and risedronate) licensed in the UK, that includes the measurement of several BTMs and BMD measurements.

We found that within 24 weeks of stopping oral BPs, bone turnover increases towards the pre-treatment baseline level. Stopping oral BPs might be expected to increase activation frequency, and this would increase bone resorption followed by an increase in bone formation, due to coupling; if there were full offset of effect, then these would return to the pre-treatment baseline. Even allowing for the small changes observed in the control group or possible changes with long term storage of samples [31], the levels of BTMs remained lower than pre-treatment baseline, suggesting that all three oral BPs continued to suppress bone turnover to a similar extent two years after stopping treatment.

The mechanism(s) underlying the continued suppression of bone turnover after oral BP treatment has stopped are not clear. It has been proposed that the half-life of BPs in bone may be up to 10 years and so the drug will continue to be released from bone and inhibit bone turnover [32]. However, this theory also proposes that the suppression will be greater for those BPs that have a greater affinity for hydroxyapatite (greater for alendronate). We found no difference in the effect of stopping treatment between the three oral BPs. The BPs also have a different binding affinity for the enzyme farnesyl pyrophosphate synthase (FPPS), a key enzyme of osteoclast function. Risedronate is a strong inhibitor of FPPS; whereas alendronate and ibandronate appear less effective inhibitors of the enzyme [8]. It may be that these two effects could balance each other out, and is in keeping with our observations. Bisphosphonates have potent effects on osteoclastic bone resorption and agents which

affect one arm of the remodelling process usually affect the other, primarily by a decrease in remodelling surface but also perhaps by osteoclast derived factors which regulate coupling [33]. An alternative, but speculative, hypothesis is that all oral BPs reduce the number of osteoclast precursors during the first year of treatment and this reduction remains after stopping treatment [34-36].

The practical implication of this study is that it provides information about the timing of the change in BTMs and BMD of the commonly used treatments for osteoporosis and it shows that 24 weeks after treatment has stopped there is a smaller change in BTM from pre-treatment baseline in risedronate group compared to the other treatment groups. Despite greater suppression of BTMs by alendronate and ibandronate, the rate of increase and the final values are similar between all three BPs. Two years after treatment stopped, all three oral BPs have similar residual effects on bone turnover and BMD. The observation of bone loss from the hip but not the spine, after cessation of treatment, is similar to that observed in the FLEX study [19]. The rate of bone loss from the total hip after treatment stopped was 1.6% over two years overall and didn't differ between the three BPs. This is a similar rate of bone loss to that found in the placebo group of other clinical trials [37, 38]. The lack of bone loss from the spine could be an artefact due to the development of degenerative changes in the spine but is in contrast to studies showing that when denosumab is stopped there is accelerated bone loss from the spine as well as from the hip due to an 'overshoot' of bone turnover markers [39, 40]. It is, therefore, more likely that the time course of the offset of effect of BPs differs by skeletal region with longer-term effects on bone turnover in cancellous bone containing red bone marrow, such as the spine. The delivery and uptake of BPs may be greater at the spine than non-vertebral sites.

The question remains as to whether the measurement of bone turnover markers at 24 weeks after stopping BPs can identify offset of treatment effect. We found that a greater increase in BTMs 24 weeks after treatment had stopped was associated with greater bone loss at the total hip during the offset period. Furthermore, we might expect continued suppression of bone turnover whether we use ibandronate, alendronate or risedronate. The changes in BMD are much too small to identify offset of effect reliably in the individual over a short timescale, as have been reported for alendronate [12, 19, 22, 41]. These data help plan the frequency of monitoring patients undergoing a pause in treatment and enable decisions about restarting treatment to be made in a timely and evidence-based manner. Clinically, the reintroduction of treatment would be considered when the patient is again at high fracture risk – this will also depend on factors other than BMD and BTMs such as recent fracture. However, reintroduction of treatment could be considered once a significant change was detectable in the individual, i.e. an increase in BTMs or decrease in BMD, which is greater than the least significant change. The

findings in our study support the recommendation of the ASBMR task force report [17]; for women not at high fracture risk after 3–5 years of BP treatment, a pause in the treatment of 2–3 years could be considered, with periodic reassessment.

The study has a number of limitations. We acknowledge that the two-year duration of treatment in the original TRIO study is not representative of usual clinical practice, where usually up to five years is administered before review, so that the observed effects may not represent those after longer exposure. The relatively small number of subjects, with consequently low power, limited our analyses to changes in BTMs and BMD rather than effects on fracture rates. Furthermore, these data only relate to postmenopausal women and may not apply to other patient groups treated with BPs.

We used a control group of premenopausal women. We didn't use a control group of untreated osteoporotic women as effective treatments are widely available and guidelines are in place in the UK that recommend treatment. An advantage of using a control group of premenopausal women is that no bone loss is expected to occur over the study period. This allowed us to monitor any issues with drift in calibration of equipment as identified in the TRIO study ultrasound measurements [27]. We also noted a difference from baseline for some of the BTMs, which provided an estimate of change due to sample storage.

Finally, though allocation to BP was originally randomised, the rules for discontinuation may have resulted in a non-random comparison amongst those entering into the TRIO offset study. There was a significant difference in the T-score at the spine and hip in those that entered into the TRIO offset study compared with those who did not participate, at both pre-treatment baseline and after two years of treatment. We recognise that the women in the TRIO offset study have relatively good BMD T-scores and we can't exclude a difference in the offset between the three bisphosphonates in women with more severe osteoporosis. However, Bauer *et al* examined fracture outcomes after stopping alendronate and found the age- and baseline BMD–adjusted risk of fracture after the first year of follow-up among those in the tertile with the greatest total hip bone loss did not differ from those in the other 2 tertiles (RHR, 1.06 [95% CI, 0.67–1.68]). Results were similar for 1-year change in femoral neck BMD [23].

In conclusion, two years after stopping BP treatment the increase in bone markers was similar for all three BPs with a decrease in the hip but not in spine BMD. Neither BTMs nor BMD returned to pre-treatment baseline after two years off treatment.

Figure Legends

Fig 1 Consort chart for the TRIO Offset study

Fig 2 The percentage change from baseline in biochemical markers of bone resorption (NTX, CTX) and bone formation (PINP, OC, BoneALP). Data are shown as least squares mean and 95% CI for the three bisphosphonate treatments. The period on treatment is shown as the shaded area, the period off treatment is not shaded (Difference amongst treatment groups; *P<0.02, **P<0.004).

Fig 3 The percentage change from baseline in BMD at the total hip (TH) and lumbar spine (LS). Data are shown as least squares mean and 95% CI for the three bisphosphonate treatments. The period on treatment is shown as the shaded area, the period off treatment is not shaded. (Difference amongst treatment groups; *P<0.02, **P<0.004).

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