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Response of bone turnover markers to raloxifene treatment in postmenopausal women with osteopenia.

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Abstract

Introduction: The change in bone turnover markers (BTM) in response to osteoporosis therapy can be assessed by a decrease beyond the least significant change (LSC) or below the mean of the reference interval (RI). We compared the performance of these two approaches in women treated with raloxifene.

Methods: Fifty postmenopausal osteopenic women, (age 51-72y) were randomised to raloxifene or no treatment for 2 years. Blood samples were collected for the measurement of BTM. The LSC for each marker was calculated from the untreated women and the RI obtained from healthy premenopausal women (age 35-40y). Bone mineral density (BMD) was measured at the spine and hip.

Results: There was a decrease in BTM in response to raloxifene treatment; percentage change at 12 weeks, CTX -39% (95% CI -48 to -28) and PINP -32% (95% CI -40 to -23) P<0.001. The proportion of women classified as responding to treatment using LSC at 12 weeks was: CTX 38%, PINP 52%, at 48 weeks CTX 60%, PINP 65%. For the RI approach; at 12 weeks CTX and PINP 38%, at 48 weeks CTX 40%, PINP 45%. There was a significant difference in the change in spine BMD in the raloxifene treated group compared to the no-treatment group at week 48; difference 0.031 g/cm2, (95% CI 0.016 to 0.046, P<0.001).

Conclusions: The two approaches identified women that reached the target for treatment using BTM. Both LSC and RI criteria appear useful in identifying treatment response but the two approaches do not fully overlap and may be complementary.

Key words: bone markers, raloxifene, bone resorption, osteopenia,

Conflicts of Interest

Dr Naylor, Miss Gossiel and Dr Jacques have no disclosures. Dr N Peel has received speaker's honoraria and funding to attend educational events from Warner Chilcott, Lilly, Servier, Merck, Roche, GSK and ProStrakan and consultancy fees from Internis Pharma, ProStrakan and Lilly. Professor Eastell has received funding from the National Institute for Health research (NIHR) and grant/consultancy funding from Warner Chilcott, Eli Lilly, Roche, Immunodiagnostic Systems and Merck.

Mini abstract:

We used two methods of identifying women who reached the target for raloxifene treatment with bone turnover markers. Both approaches identified women that responded to treatment, but did not fully agree and may be complementary.

Background

Raloxifene is a selective estrogen receptor modulator (SERM) that has beneficial effects on bone and lipids with no detrimental effects on the breast or endometrium [1, 2] and is licensed for the treatment and prevention of osteoporosis [3] . Raloxifene treatment results in an increase in bone mineral density (BMD), reduction in bone turnover markers (BTM) and reduced risk of vertebral fractures [4-6] . A systematic analysis of clinical trials demonstrated that raloxifene reduces the risk of vertebral fracture by 40 to 49% in postmenopausal women with osteoporosis [7] . The magnitude of change in BTM and BMD with raloxifene treatment is of a smaller magnitude compared to other, more potent treatments for osteoporosis such as bisphosphonates.

Bone turnover markers are established as useful tools in research studies of metabolic bone disease as they show a biological response to the treatment of osteoporosis [8, 9] . In clinical trials, the changes in bone turnover markers have been shown to be related to change in fracture risk [10] . There is evidence that there is an association between the change in bone formation markers but not bone resorption markers on raloxifene and the reduction in spine fracture risk [5, 11-13] . Changes in bone markers in response to treatment occur earlier and are of a greater magnitude than changes in bone density. A significant change in BMD can rarely be detected in an individual in response to oral anti-resorptive therapy in less than 18-24 months. Compliance with treatment is a concern in clinical practice and monitoring patients on treatment using biochemical markers can provide useful additional information for the management of patient care [14] .

There are limited data on the use of bone markers in clinical practice. In a retrospective study of the utility of urinary N telopeptide of type I collagen (NTX) to monitor bisphosphonate treatment, a poor response defined by change in NTX, was associated with early identification of non-compliance or the presence of secondary osteoporosis [15] . This suggests that bone turnover markers are useful tools in clinical practice for the management of patients with osteoporosis. However, the use of bone markers for patient management is not common practice. Considerations for their use in clinical practice include cost effectiveness and also the variability of bone markers [16, 17] . Studies have reported poor reproducibility

of bone markers between laboratories [18, 19] . The standardization of bone marker measurements is currently being addressed with the introduction of international reference standards [10] . It has been proposed that the C terminal telopeptide of type I collagen (CTX) and N terminal propeptide of type I procollagen (PINP) are adopted as reference bone markers.

There are two methods that have been proposed when using bone markers to assess response to osteoporosis treatment [10] . Firstly the least significant change (LSC) approach which is the minimum change in bone markers that can be attributed to the treatment effect rather than random variation in the marker, or secondly a reduction in bone markers to below the mean of the reference interval (RI) for premenopausal women [10, 12] . Both of these approaches can be useful to monitor the response to bisphosphonate treatment using bone markers [20] , however the use of BTM for monitoring less potent treatment may not be as informative. There are limitations to both described approaches; there is no consensus for the calculation of least significant change and the reference intervals should be appropriate and robust.

The aim of this work was to identify the proportion of women that reach the goal for response to raloxifene treatment using the LSC and RI criteria and thereby assess their potential utility to monitor treatment response to this agent in clinical practice.

Materials and methods

Subjects

Postmenopausal women with osteopenia, ages 50 to 80 years were recruited from general practice surgeries or new patient referrals for BMD measurements. Women were eligible if they had osteopenia at either the lumbar spine or proximal femur (BMD T-score between -1and -2.5 SD) measured by dual energy x-ray absorptiometry (DXA). The participants completed an osteoporosis risk factor questionnaire at the screening visit with laboratory investigations to identify co-morbidities which could affect bone metabolism (screening investigations: FBC, ESR, TSH, bone profile, electrolytes, liver function tests, serum and urine electrophoresis, and 24-hour urine calcium excretion). Exclusion criteria included the presence of metabolic bone disease or other medical condition that would affect bone metabolism; use of hormone replacement therapy or other antiresorptive treatments within the previous 6 months; history of hepatic or renal impairment, venous thromboembolic disease; unexplained uterine bleeding or malignancy. Women with degenerative disease of the spine or evidence of two or more vertebral fractures between L1 and L4 on the DXA lumbar spine scan were also excluded. Healthy premenopausal women ages 35 to 40 years were recruited to establish a premenopausal reference interval. This study population has been described elsewhere [20, 21] . The women who were included had regular menstrual cycles and were vitamin D replete (25OHD >50nmol/L). Exclusion criteria included the use of oral prednisolone (or equivalent) and bone active drugs, any disease that affects bone metabolism, presence of recent fracture (within 12 months), surgical operation (within 3 months), pregnancy or current use of contraceptive pill. The raloxifene study was approved by the Sheffield Research Ethics Committee and signed informed consent was obtained from each participant prior to inclusion into the study. The use of samples was approved by the Sheffield Musculoskeletal Biobank, which received ethics approval from the NRES REC South Central Oxford C, (REC ref 10/H0606/20) and is housed in the University of Sheffield Biorepository (HTA Licence no. 12182).

Study intervention

In this controlled open label study, 50 postmenopausal osteopenic women were randomised to receive raloxifene (60 mg/day Evista, Eli Lilly and Co., Indianapolis, IN) plus elemental calcium 500mg/day as

calcium carbonate (Calcichew: Shire Pharmaceuticals Ltd., Andover, Hants, UK) or to receive no treatment. Adherence was assessed using medical events monitoring system (MEMS) bottle caps (AARDEX, Zurich, Switzerland), which record the date and number of times a medication bottle is opened [22] . Complete MEMS data were available for 17 of the 21 women, 12 women had compliance >80% and 5 women >60%. The study was conducted in accordance with ethical recommendations for monitoring adherence [23] . The 87 healthy premenopausal women were not prescribed any medication during the study [20] .

Study assessments

Anthropometric measurements included height (to nearest 0.1cm), weight (to nearest 0.1kg) (Seca Birmingham UK). Bone mineral density (BMD, g/cm2) of the lumbar spine (LS), femoral neck (FN) and total hip (TH) were measured by dual energy x-ray absorptiometry (DXA) using Hologic QDR1000W, (Hologic Inc, Bedford MA, USA). The mean BMD was calculated from two DXA scans performed a week apart at baseline and again at 48 and 96 weeks. The coefficients of variation were calculated from baseline duplicate measurements the CVs were 1.5% (LS), 2.7% (FN), 1.9% (TH) [24]

Biochemistry

Samples for biochemistry were collected from the postmenopausal women who attended for regular visits over 2 years. Blood was collected after an overnight fast and serum stored at -80°C until analysis in one analytical batch. Fasting blood samples were collected at baseline from the healthy premenopausal women. The C-terminal telopeptide of type I collagen (CTX), intact pro-collagen I N-propeptide (PINP), 25 hydroxyvitamin D (25OHD) and parathyroid hormone (PTH) were measured in serum by IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, UK) inter-assay CV 6.5%, 7.2%, 6.7% and 6.5% respectively.

Statistical analysis

For analysis of change in bone turnover markers from baseline, a treatment group comparison was made using a mixed effects repeated measures model with baseline measurement fitted as a covariate. If an

overall statistically significant difference was found between treatment and no treatment groups then the effect of treatment was assessed at each time point. A within-treatment group analysis was performed to determine if the change in bone turnover marker was significant over time. If an overall statistically significant difference was found over time then the change from baseline was assessed at each time point. All bone turnover marker measurements were $\log_{10}$ transformed prior to analysis and changes from baseline were back transformed and expressed as a percentage change. Missing values were imputed using last observation carried forward.

The differences in the change from baseline in BMD between the treatment and no treatment group were assessed at 48 and 96 weeks using analysis of covariance (ANCOVA) with baseline measurement fitted as a covariate.

Least significant change (LSC) for the bone turnover markers was calculated using measurements from the no treatment group over 12 weeks. This is the minimum difference between two measurements that can be considered a true change due to treatment rather than random variability in the measurement. The distribution of the measurements was positive 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:

$$LSC_{log} = Z' \times \sqrt{2} \times SD_{RMS}$$

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:

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

The goal for LSC response was defined as a percentage decrease from baseline in bone marker greater than the lower limit of the LSC. This was calculated at 12 and 48 weeks of treatment.

The reference interval was calculated from premenopausal women who were vitamin D replete (25OHD >50 nmol/L) using log transformed data [20] . The goal for RI response was defined as a bone marker result below the mean for the premenopausal reference interval, calculated at 12 and 48 weeks of treatment.

The longitudinal mixed effects repeated measures models were fitted using IBM SPSS Statistics for windows Version 21.0 (Armonk, NY) and plots were created in R (http://cran.r-project.org/).

Bone mineral density

Change from baseline in DXA BMD was calculated for LS, FN and TH. The treatment group was compared to the no-treatment group at 48 and 96 weeks using analysis of covariance adjusting for baseline measurement.

Results

The baseline characteristics of the study participants are shown in Table 1. Spine BMD was lower and BTM higher in the postmenopausal osteopenic women compared to the healthy premenopausal controls. There were 6 participants that withdrew from the study before 48 weeks and so were not included in the analysis, (5 in the treatment group and 1 in the no treatment group). One participant in the treatment group had no sample for the week 48 visit. Data for the BTMs are shown up to 48 weeks as after this time-point there was no further decrease in response to treatment.

Percentage change from baseline

The time course and magnitude of response of BTM to raloxifene treatment are shown in Figure 1. There was an overall statistically significant difference between the no treatment and treatment groups for BTM $P< 0.001$. The within week analysis shows that the between group differences are statistically significant at every time point for the BTM; CTX and PINP ($P<0.001$). There is an overall difference from baseline over time in the treatment group CTX, PINP ($P<0.001$). The mean percentage change in BTM from baseline are shown in Table 2. The decrease in CTX was early and below baseline by week 1 (-21%, 95% CI -27 to -14, $P<0.001$), the decrease from baseline was significant at week 4 for PINP (-17% 95% CI -28 to -4, $P=0.014$).

Response to treatment

The target for response to treatment was defined as a percentage change in BTM greater than the LSC or as a result that was in the lower half of the premenopausal reference interval. The percentage change from baseline at 12 weeks for individual participants with the LSC for PINP and CTX are shown in Figure 2. Four women had an increase in BTM at this time point. The two women had an increase in PINP from baseline at 12 weeks, this had decreased by 48 weeks. Both had a high CTX at baseline and had good compliance as assessed by MEMS data (89% and 97%). One of the women had a high baseline PTH and low 25OHD. Two women had an increase in CTX at 12 weeks, one had low baseline CTX which may have influenced the large percentage change result and her PINP was decreased at 12 weeks (-37%). The

second woman had poor compliance throughout the study (65%) and only a small percentage change in PINP at 12 weeks (-5%).

The number of women classified as responders by the two criteria at 12 weeks and at 48 weeks are shown in Table 3. Using the LSC criteria more women reached the target for response at 12 weeks for PINP (52%), than for CTX (38%). By 48 weeks the number of women reaching the goal for treatment was 65% for PINP and 60% for CTX.

The absolute values for CTX and PINP are shown in Figure 3. At baseline 14% of women were below the mean of the reference interval for CTX and 10% for PINP (Table 3). At 12 weeks 8/21 (38%) were below the RI mean for CTX and PINP (Table 3). Of the 8 women below the RI mean for CTX at 12 weeks, 3 did not remain below at 48 weeks. However another 3 women were below the mean at 48 weeks only, so the total remained at 8 women. The 8 women below the RI for PINP at 12 weeks, remained the same at 48 weeks with one additional person reaching the premenopausal mean. The model used to calculate the mean change from baseline accounted for the baseline BTM values. The baseline BTM value did not influence the magnitude of change in BTM.

At the 12 week visit for PINP 6 of the 21 women were classified as responding to treatment by both LSC and RI methods, 7 by either LSC or RI and 8 non-responders. For CTX 4 of the 21 women were classified by both LSC and RI 6 by one of the criteria and 11 non-responders. Five women did not reach the target response for LSC or RI in either PINP or CTX. These included 3 of the 4 women who had an increase in BTM at 12 weeks compared to baseline. A total of 15 women were classified as reaching the target for response by LSC method and 9 for the RI criteria, by either CTX or PINP or both.

Bone Density

There was a significant difference in the absolute change in lumbar spine BMD in the raloxifene treated group mean 0.017 g/cm^2 (SD 0.019) compared to the no-treatment group -0.010 g/cm^2 (SD 0.027) at week 48; difference (adjusted for baseline) 0.031 g/cm^2, 95% CI 0.016 to 0.046, $P<0.001$. At 96 weeks the

difference was mean 0.029 g/cm^2 (95% CI 0.004 to 0.053) P=0.024. There was no difference between the treatment and no treatment groups in the change in BMD at total hip or femoral neck. Total hip week 96 difference 0.013 g/cm^2 (95% CI -0.008 to 0.035, P=0.219), FN difference 0.014 g/cm^2 (95% CI -0.014 to 0.042, P=0.306). At 96 weeks 3 women had a change in LS BMD greater than the LSC (-3.6%) and 1 for hip BMD (LSC TH -4.2%, FN -6.1%).

Discussion

There was a decrease in BTM in response to raloxifene treatment, at 12 weeks there was a 39% decrease in CTX, and 32% decrease in PINP. Other studies have reported a similar decrease in BTM in response to raloxifene for CTX measured using other methods [1, 2, 25] and for total PINP measured by a different autoanalyser method [26] . The changes in BTM occur earlier and are of a greater magnitude than the change in bone density.

The two approaches that have been proposed as ways to assess response to treatment using BTM are a change greater than the LSC or a reduction to below the mean of the premenopausal RI.
We found that both approaches identified women that responded to raloxifene treatment. Bone density measurements after 2 years were only greater than the LSC for spine BMD for 3 women. There were 5 women who did not reach the target for treatment by either method for bone markers. Two of the women had poor compliance and others had possible confounding factors including high PTH and low 25OHD, thyroidectomy and steroid use.

One of the limitations of the RI approach is the overlap of results for pre and postmenopausal women [8] . For women who are below the mean of the reference interval at baseline, the clinician would look for a change in response to raloxifene from the pre-treatment value within the reference interval.

The proportion of women classified as responders by the LSC method was fewer than reported for other treatments. This is not unexpected as the percentage change in BTM in response to raloxifene is less than that for other osteoporosis treatments such as bisphosphonates [4, 20, 27, 28] . For example we have reported previously in a study of postmenopausal women treated with bisphosphonates (TRIO study) that 12 weeks of alendronate therapy resulted in 98% responders by CTX and 82% by PINP for LSC; that contrasts with the response to raloxifene therapy at 12 weeks which was 38% for CTX and 52% for PINP (Table 3) [20] . The LSC was less for the raloxifene study -45% CTX and -27% PINP in comparison to the TRIO study (-56% CTX, -38% PINP). There are several ways that LSC can be calculated [10, 29] . Factors to consider include one-tailed or two tailed, level of significance, the population that it is calculated

from and over what time course. For monitoring treatment in clinical practice it has been suggested that a one-sided probability of 0.05 is appropriate as a decrease in BTM is expected. It has also been suggested that a probability of 80% is adequate for monitoring treatment. It would be useful to have a consensus on the LSC calculation.

The LSC threshold and the RI mean should be considered as a guideline to help with clinical decisions. The LSC represents a statistical approach and not a true biological change in response to treatment. Women who have had a decrease in BTM that has not exceeded the LSC have not reached the goal for response may have still had a change in bone markers in response to the treatment. For those women in the lower half of the reference interval at baseline it is clinically relevant to monitor their change within the RI during treatment. Many factors are considered when making clinical judgements and the change in BTM offers additional information that could help with decisions for patient management.

A response to treatment is dependent on patient compliance. However, treatment response may be suboptimal even in patients who take their treatment and may reflect intermittent compliance or poor absorption. Measuring the BTM response is useful in addition to talking to the patient to identify that although they are taking the treatment the desired response is not achieved, this would be identified earlier than measuring the BMD response. Earlier identification of poor response enables treatment to be reviewed, investigation for cause of poor response to be undertaken and changes in management to be instituted in a timely way.

When monitoring change in bone markers the regression to the mean should be considered. [30, 31] . Regression to the mean is a statistical phenomenon that can make natural variation in repeated data look like real change. It happens when unusually large or small measurements tend to be followed by measurements that are closer to the mean [32] . Regression to the mean should not be an issue in the change from baseline analysis because this is a randomised study (if subjects are randomly allocated to comparison groups then all groups should be equally affected by regression to the mean) and the baseline measurement was adjusted for in the mixed effects model that we used.

The limitations of this study are the small number of participants. We did not have sufficient numbers to assess whether the change in BMD was different for those that reached the target response compared to those that did not, or whether the change in bone markers was greater for those with the highest baseline results or better compliance. The long term storage of the samples may influence the BTM measurements, there are no published data on the effect of long term storage on the assays used.

In conclusion bone markers offer a way to monitor response to osteoporosis treatment so that the management of the patient can be adjusted if necessary at an early stage in treatment.
Both LSC and RI criteria appear useful in identifying treatment response but the two approaches do not fully overlap. The response of BTM provides additional information that can complement other clinical information for patient management. For markers to be useful in clinical practice, using either of these approaches for determining responders, there should be a consensus on LSC calculation and robust reference intervals established using data from large populations.

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Table 1. Baseline characteristics for postmenopausal women randomised to raloxifene or no treatment and the premenopausal reference group. Results are shown as mean (SD) for demographics and median (inter quartile range) for biochemistry results.

	Raloxifene	No treatment	Premenopausal
n	21	23	87
Age, years	63 (7)	61 (6)	38 (2)
Height, cm	161 (6)	161 (6)	165 (7)
Weight, kg	70 (12)	72 (16)	67 (11)
LS BMD g/cm^2	0.857 (0.060)	0.903 (0.098)	1.092 (0.117)
25OHD µg/L	17 (13 to 32)	19 (12 to 32)	28 (23 to 31)
PTH pg/mL	36 (25 to 45)	30 (25 to 40)	29 (23 to 37)
CTX µg/L	0.68 (0.47 to 0.93)	0.55 (0.34 to 0.73)	0.32 (0.23 to 0.41)
PINP µg/L	47 (37 to 59)	50 (27 to 60)	29 (22 to 35)

Table 2: Percentage change from baseline for BTM in the treatment group over 48 weeks.

	CTX			PINP		
	% Change	95%CI	P-value	% Change	(95% CI)	P-value
Week 1	-21	(-27, -14)	<0.001	-10	(-18, -1)	0.036
Week 2	-28	(-38, -16)	<0.001	-6	(-15, 4)	0.230
Week 4	-37	(-49, -23)	<0.001	-17	(-28, -4)	0.014
Week 8	-37	(-47, -25)	<0.001	-22	(-31, -11)	<0.001
Week 12	-39	(-48, -28)	<0.001	-32	(-40, -23)	<0.001
Week 24	-44	(-55, -31)	<0.001	-40	(-47, -32)	<0.001
Week 36	-48	(-59, -34)	<0.001	-42	(-49, -34)	<0.001
Week 48	-48	(-60, -33)	<0.001	-39	(-49, -28)	<0.001

Table 3. Responder analysis for least significant change (LSC) and reference interval (RI)

BTM	visit	n	LSC	LSC responders	Geometric mean (RI)	RI responders
CTX	Baseline	21	-45%	-	0.32 µg/L (0.13 to 0.81)	3 (14%)
	12 Weeks	21		8 (38%)		8 (38%)
	48 Weeks	20		12 (60%)		8 (40%)
PINP	Baseline	21	-27%	-	28 µg/L (15 to 54)	2 (10%)
	12 Weeks	21		11 (52%)		8 (38%)
	48 Weeks	20		13 (65%)		9 (45%)

Figure 1. The percentage change (mean and SEM) in CTX and PINP over 48 weeks. The women treated with raloxifene are shown as the grey line with circles, the women receiving no-treatment as the black line with squares.

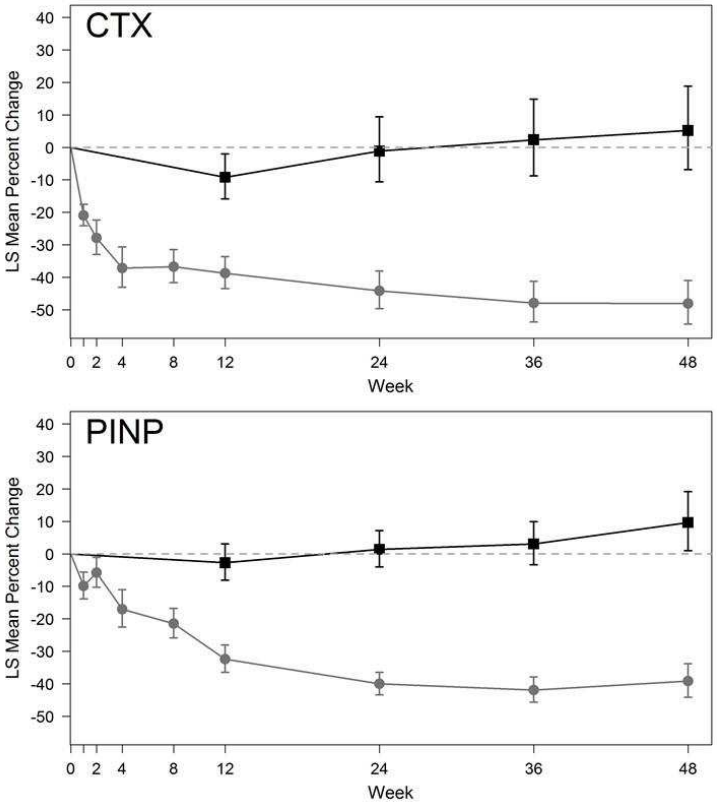

Figure 2. Percentage change from baseline for CTX and PINP at 12 weeks for the women treated with raloxifene; shaded area shows the LSC. The target for treatment is a decrease beyond the LSC.

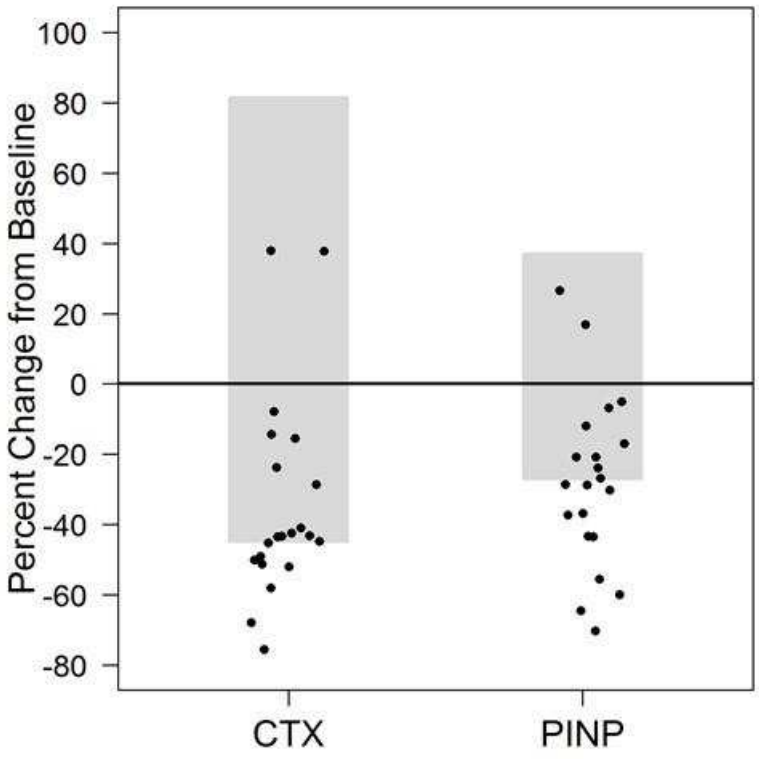

Figure 3. Box and whisker plot of absolute values for CTX and PINP over 48 weeks for the women treated with raloxifene; the box represents the interquartile range, the middle solid line is the median and the whiskers show the range of the data. The premenopausal reference interval is shown by the shaded area, the geometric mean shown by the black dashed line. The target for treatment is below the mean for the premenopausal reference interval.