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**Overcoming resistance to bisphosphonates through the administration of alfacalcidol:
results of a one-year, open follow-up study**

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Introduction

Osteoporosis is a disorder that leads to enhanced bone fragility through a decrease in the mass as well as deterioration of the microarchitecture of bones. Owing to its high prevalence and negative impact on quality of life, osteoporosis is the third most important public health disorder (after malignancies and cardiovascular disease), afflicting 200 million people worldwide [1]. Oral bisphosphonates are routinely prescribed for treatment for patients with osteoporosis. Adequate calcium and vitamin D sufficiency of the body is essential for antiresorptive treatment to be effective. According to HORVÁTH *et al.*, serum 25-hydroxyvitamin D₃ level is lower than normal in 40% of elderly nursing home residents, whereas data from BHATTOA *et al.* reflect the same in 56.7% of postmenopausal women [2, 3]. Some studies demonstrated statistically significant reduction of fractures during five years of high dose (100 000 E every four months) treatment with vitamin D₃ [4], but others not [5-7]. According to a recent study, in 8 to 25 per cent of osteoporotic patients, bone loss continues despite adequate treatment with bisphosphonates, administered in combination calcium and vitamin D₃ supplementation [8]. A proportion of these patients may have functional hypovitaminosis D potentially resulting from insufficient intake (despite supplementation), impaired activation, genuine resistance to vitamin D₃ or secondary hyperparathyroidism [9]. In the remainder, the cause underlying the lack of bisphosphonate effects is unknown.

In osteoporosis, genuine resistance to bisphosphonates is probably non-existent or may be considered extremely uncommon, at the least. In Paget's disease, by contrast, where therapy is aimed at achieving complete biochemical remission, resistance to bisphosphonates has been demonstrated by several researchers. The lack of a complete biochemical remission is regarded by some as the criterion for bisphosphonate resistance [10], whereas according to others [11], the latter is established by a lower than 50-per-cent decrease in serum alkaline phosphatase (AP) level. Notwithstanding the criteria, the prevalence of resistance varies with different bisphosphonates in the range between 83% (etidronate [10]) and 11% (zolendronate [12]).

According to additional, relevant evidence, the efficacy of activated analogues of vitamin D (alfacalcidol, calcitriol) in reducing bone fractures and increasing bone density is superior to that of native vitamin D. This has been demonstrated by several studies in various disease forms, i.e. in postmenopausal osteoporosis, as well as in osteoporosis related to inflammatory joint disease, or glucocorticoid therapy [13-16].

This study was intended to ascertain:

1. whether replacing vitamin D₃ with alfacalcidol is followed by an increase in BMD (i.e. bisphosphonate resistance can be overcome) in patients not responding to treatment with a bisphosphonate administered in combination with supplemental calcium and conventional vitamin D₃;
2. the incidence of hypovitaminosis D₃ in the study population;
3. the changes occurring in biochemical markers of bone turnover during one year of treatment with alfacalcidol,
4. the frequency of adverse events associated with the use of alfacalcidol.

Patients and methods

Patients

Seventy-six patients were enrolled into the study conducted between January 2006 and March 2007. The duration of follow-up was 12 months on average. Inclusion criteria were: diagnosed postmenopausal or senile osteoporosis (in females) or idiopathic osteoporosis in males, and greater than 3% decrease in BMD – as demonstrated by axial DEXA – despite adequate treatment (vitamin D₃ 400-1000 U/day, calcium 1000 mg/day and alendronate 70 mg/week) for a year or longer. Exclusion criteria comprised established secondary osteoporosis, other forms of calcipenic osteopathy, hypercalcaemia, and history of renal calculosis. The male-to-female ratio was 4:72; mean age of the study population was 70.5 (±8.2) years. Ten patients had a history of at least one prevalent vertebral or low-trauma peripheral fracture. The average serum 25-hydroxyvitamin D₃ level was 72,2± 36,2 nmol/l at the starting and 74,1 ± 34,0 nmol/l at the end of the study (the difference was not significant statistically). Below normal serum 25-hydroxyvitamin D₃ level (<75 nmol/l) was found in 38 patients, along with elevated (>72 pg/ml) serum PTH level in 23 of these subjects. The mean glomerular filtration rate (GFR) was 85,3 ml/min/1,73m² at baseline and 4 patients had GFR lower than 60 ml/min/1,73 m² at starting of the study, 2 of them had elevated serum PTH level. In the included patients, the average administered dose of D₃ vitamin was 530 U/day along with 1000 mg of calcium supplementation before the starting of the study.

Study design

During the follow-up period of one-year on average, control visits were scheduled at baseline and at 3-month intervals thereafter. At baseline, a detailed history (concomitant diseases, fractures, osteoporosis and risk factors) was recorded along with appraisal of clinical status by obtaining a lateral x-ray of the lumbosacral spine and performing a laboratory screen (ESR, CRP, CBC, serum calcium, phosphorus, BUN, creatinine, GOT, GPT, AP, albumin, osteocalcin, 25-hydroxyvitamin-D₃, parathormon [PTH] levels, urinary calcium/creatinine [UCa/Cr] and deoxypyridinoline crosslinks/creatinine [D-Pyr/creatinine] ratios). Bone density was measured in both peripheral bones (DTX200) and in the axial skeleton (DEXA, Lunar DPX Pro) and BMD values obtained from the radius distal segment, as well as the means of the values measured in the 1st through 4th lumbar vertebrae were taken into account. Serum calcium level and urinary calcium/creatinine ratio were determined at control visits repeated at 3-month intervals. After one year, information was gathered on fractures and renal

calculosis that had occurred during the follow-up period, as well as osteodensitometry, lumbosacral x-ray, and laboratory screen were repeated.

Treatment protocol

The pre-existing regimen of alendronate 70 mg once per week was left unchanged, but conventional vitamin D₃ and calcium treatment was replaced with 0.5 µg/day alfacalcidol (1α-hydroxvitamin D₃). The calcium supplementation was stopped also.

Endpoints

The primary endpoint of the study was defined as the change measurable (by DEXA) in bone mineral density (BMD) of the forearm and of axial bones one year after the introduction of alfacalcidol treatment. Secondary endpoints included changes in clinical chemistry parameters (serum calcium, phosphorus, and alkaline phosphatase levels), biochemical markers of bone turnover (serum osteocalcin, PTH, urinary D-Pyr/creatinine), UCa/Cr ratio determined in first-voided morning urine, treatment-emergent adverse reactions, and osteoporotic fractures that occur during follow-up.

Statistical analysis

The differences between the values of study parameters (BMD; serum Ca, P, AP, osteocalcin, PTH levels; urinary D-Pyr/creatinine and UCa/Cr ratios) determined at two time points were analysed using the SPSS 15.0 software package. First, the normality of differences was checked with the Kolmogorov-Smirnov test (normal distribution would allow performing a parametric [t or d] test). The differences between the paired values of all nine variables were significantly different from the normal distribution ($p < 0.01$ for UCa/Cr and $p < 0.001$ for the rest). As the data were unsuitable for analysis with the *t*-test, non-parametric Wilcoxon signed-rank test was performed to check the uniformity of repeated measurements. (This method is a non-parametric alternative to performing a paired *t*-test.)

Results

a) Changes in BMD during follow-up

The median of changes as well as the minimal and maximal changes are summarised in *Table 1*.

	<i>Unit</i>	<i>Median</i>	<i>Minimum</i>	<i>Maximum</i>
Se Calcium	mmol/l	0.06	-0.09	1.13
Se Phosphate	mmol/l	-0.05	-0.49	0.3
Alkaline phosphatase	U/l	-13	-46	287
Parathormone	pg/ml	-10.7	-150	22.5
Urinary Ca/creatinine		0.1	-0.32	0.49
Osteocalcin	ng/ml	-0.4	-5.8	4.2
BMD of forearm	g/cm ²	0.007	-0.011	0.039
BMD of lumbar 1-4 vertebrae	g/cm ²	0.012	-0.998	0.116
Relative change of forearm BMD	%	2.18	-3.5	16.3
Relative change of lumbar BMD	%	1.38	-11.3	15.8
Deoxypyridinoline crosslinks/creatinine	nmol/mmol	-0.2	-15.69	0.12

Table 1: Median, minimal and maximal changes in various parameters as a result of treatment

After treatment for $396,5 \pm 21,3$ days with alendronate – with no change in the original dosage regimen of 70 mg/week, but – in combination with 0.5 µg/day alfacalcidol instead of the previously prescribed conventional vitamin D₃ (administered with calcium), we observed the following changes (median values): forearm BMD increased by 0.007 g/cm², and lumbar (L₁₋₄) BMD increased by 0.012 g/cm². The changes in T-scores showed rather high scatter. The relative changes in BMD values were 2.18% and 1.38% for the forearm and lumbar vertebrae, respectively. BMD changes that have occurred during one-year follow-up are shown in *Figure 1*. Box and whiskers plots are summarizing the median, quartiles, and extreme values. The *box* represents the interquartile range which contains the 50% of values.

The *whiskers* are lines that extend from the box to the highest and lowest values, excluding outliers. A *line* across the box indicates the median.

Compared to baseline values, densitometry results (both BMD values and T-scores) obtained after one-year treatment revealed statistically significant improvement in both regions (Wilcoxon's signed rank test, $p < 0.001$).

b) Changes in clinical chemistry parameters

At baseline, mean serum calcium level was 2.34 (± 0.106) mmol/l, phosphorus level was 1.18 (± 0.169) mmol/l, and UCa/Cr ratio measured in first-voided morning urine was 0.25 (± 0.161). After one year of treatment, serum calcium level increased by 0.06 mmol/l (median), while serum phosphorus level decreased by 0.05 mmol/l. Urinary Ca to creatinine ratio measured in first-voided morning urine increased by 0.1 (median). All these changes were significant (Wilcoxon's signed rank test, $p < 0.001$) (see *Figure 2*). The decline of serum alkaline phosphatase level over the follow-up period by a median of 13 U/l was also significant, but clinically not relevant (see *Figure 3*).

c) Changes in biochemical markers of bone turnover

Changes in serum parathormone (PTH) level were as expected. At baseline, elevated PTH was ascertained in 32 patients; this number decreased to 16 during the one-year follow-up. The median change in serum PTH level was significantly lower one year later (median change: -10.7 pg/ml) (*Figure 3*). Serum osteocalcin level decreased by 0.4 ng/ml (median) after treatment; this was accompanied by a decrease in the D-Pyr/creatinine ratio by 0.2 nmol/mmol ($p < 0.001$) (*Figure 3*). Although the changes showed high scatter, they proved to be highly significant (Wilcoxon's signed rank test, $p < 0.001$). The ranges of relative changes expressed as percentages of the base values for all parameters (*Figure 4*).

d) Adverse events

Mild hypercalcaemia (serum calcium level < 3 mmol/l) was observed in 3 patients; no other clinically relevant adverse reactions were seen. There was no significant increase in urinary calcium based on alendronate therapy.

e) New fractures

As stated above, ten patients had a history of at least one prevalent vertebral or low-trauma peripheral fracture at starting of the study. Four new vertebral and two peripheral (wrist)

fractures were recorded during follow up. Two of the vertebral fractures were sustained by patients with a prevalent vertebral fracture.

Discussion

Some data from the literature suggest that activated vitamin D derivatives (alfacalcidol, calcitriol), also called D-hormone analogs, are superior to conventional vitamin D, as regards the mitigation of fracture risk. In their meta-analysis of 33 clinical studies, RICHY *et al.* found a 13.4-per-cent (delta RD, 95% CI 7.7-19.8) reduction of fracture risk during the use of activated vitamin D₃ derivatives, compared to a mere 6% (delta RD_i, 95% CI 1-12) accomplished by treatment with conventional vitamin D₃ [13]. The rate difference (RD) was statistically significant (ANOVA-1; $P < 0.001$) RINGE *et al.* studied the changes of BMD and bone fractures in patients with glucocorticoid-induced osteoporosis, over 3 years of treatment with either alfacalcidol or vitamin D₃ – both administered with calcium supplementation of 500 mg daily. Changes in the BMD of the lumbar spine were 2.4% vs. -0.8% ($p < 0.0001$), and of the femoral neck 1.2% vs. 0.8% ($p < 0.006$), respectively. The proportion of patients who have sustained at least one new vertebral fracture was 9.7% in the alfacalcidol and 24.8% in the control group (risk reduction: 0.61, $p = 0.005$), whereas the proportions of patients with at least one new non-vertebral fracture were 15% vs. 25% (risk reduction: 0.41, $p = 0.08$). Taking the occurrence of any type of incident fractures into account, the above proportions were 19.4% vs. 40.6%, respectively (risk reduction: 0.52, $p < 0.001$) [14].

During the AAC study published in 2007, 30-30 patients were randomised into either of the following 3 treatment arms: *a*) alfacalcidol 1 µg + calcium 500 mg/day; *b*) alendronate 70 mg/week + calcium 1000 mg/day + vitamin D₃ 1000 IU/day; *c*) alendronate 70 mg/week + calcium 500 mg/day + alfacalcidol 1 µg/day. During the 2 years of follow up, bone density of the lumbar spine and of the total hip increased by 3%/1.5%, 5.4%/2.4%, and 9.6%/3.8%, respectively. The magnitude of changes observed in patients treated with alendronate + alfacalcidol + calcium was significantly greater than of those seen in the other two groups ($p < 0.0001$ for lumbar BMD and $p < 0.0002$ for femur BMD). The number of new osteoporotic (vertebral + non-vertebral) fractures occurring over 2 years of treatment in the three treatment groups was 9, 10, and 2, respectively – this finding is also in support of the favourable effect of alfacalcidol and especially of the combination of alendronate + alfacalcidol on fracture prevention [16].

As suggested by several studies, administering antiresorptive agents (e.g. bisphosphonates) with activated vitamin D₃ derivatives may increase the success of therapy. The beneficial

effects of alfacalcidol on intestinal calcium absorption, osteoid mineralization, muscle function and motor coordination, serum PTH level, and the risk of falls may substantially enhance the efficacy of treatment with bisphosphonates [17-18].

The anabolic effect of alfacalcidol on bone is supported also by *in vivo* and *in vitro* data. Under the effect of alfacalcidol, osteoblasts have been shown to release various growth factors (TGF- β , IGF-1 and -2, bone morphogenetic proteins [BMPs], bone matrix proteins [collagen I, osteocalcin, osteopontin]) and thereby counterbalance the reduction of bone turnover during treatment with bisphosphonates [18-21]. This osteoanabolic effect of alfacalcidol administered in combination with alendronate has been demonstrated by several studies [21-24].

Our study was conducted on patients whose bone density was declining despite adequate treatment with a bisphosphonate and supplementation of calcium plus conventional vitamin D₃ since a year at least. Patients with renal impairment or secondary osteoporosis were not allowed to participate in the study. Notwithstanding this, the study population included a substantial number of patients with decreased serum 25-hydroxyvitamin D₃ levels (n=38), accompanied by higher than normal serum PTH level (n=23). This might suggest a potential relationship between resistance to bisphosphonates and the level of vitamin D₃ insufficiency and the increase of serum PTH. Persistence of secondary hyperparathyroidism reduces BMD response to alendronate in older women with osteoporosis [9]. The improvement of the biochemical markers of bone turnover and increasing BMD indicate that patients apparently resistant to treatment with a bisphosphonate plus supplementation with calcium and conventional vitamin D₃ are more likely to benefit from combination therapy with a bisphosphonate and alfacalcidol [9]. The enhanced efficacy of alendronate and alfacalcidol in combination is probably related to the synergism between the considerably different modes of action of the two components. In particular, the antiresorptive effect of alendronate is favourably supplemented by the mitigation of osteoclastogenesis [23, 25] and reduction of serum PTH level, along with the stimulation of osteoblast activity [21], improvement of bone quality [15,23] and the microarchitecture of trabecular bone by alfacalcidol [23]– which also enhances muscle strength and thereby activates the ‘mechanostat’ function of the skeleton [18]. The whole array of these effects might explain the outstanding effect on bones [17-18, 24]. Further elucidation of these mechanisms would require large-scale studies with fracture endpoints, defined in addition to monitoring changes in bone density.

Although the lack of a control group may be considered a potential flaw of this study, the inclusion of untreated patients losing more than 3 per-cent of their bone density each year would have been ethically questionable at the least.

Conclusion

As shown by the results of this study, patients whose bone density decreases inexorably despite adequate treatment with alendronate and supplemental calcium and vitamin D₃ might benefit from a combined treatment of alendronate with alfacalcidol. The latter combination accomplished significant increase of BMD along with improvement of the biochemical markers of bone turnover, but without any substantial increase in the incidence of adverse effects.

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Figure legends

Figure 1. The changes of BMD during follow-up. Box and whiskers plots are summarizing the median, quartiles, and extreme values.

Figure 2. Changes of serum calcium and phosphorus levels, as well as of UCa/creatinine ratio during the study.

Figure 3. The change of serum alkaline phosphatase activity (U/l), parathormone level (pg/ml), osteocalcin level (ng/ml) and of urinary deoxypyridinoline crosslinks/creatinine (D-Pyr/creatinine, nmol/mmol) by the end of the follow-up.

Figure 4. The ranges of relative changes expressed as percentages of the base values for all parameters.

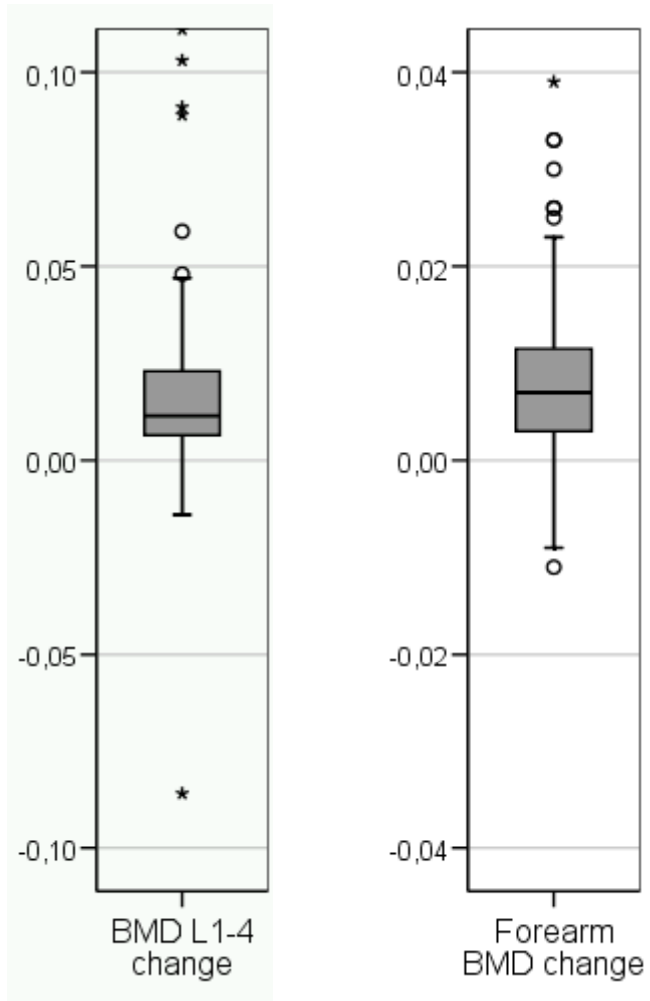


Figure 1. The changes of BMD during follow-up (g/cm²).

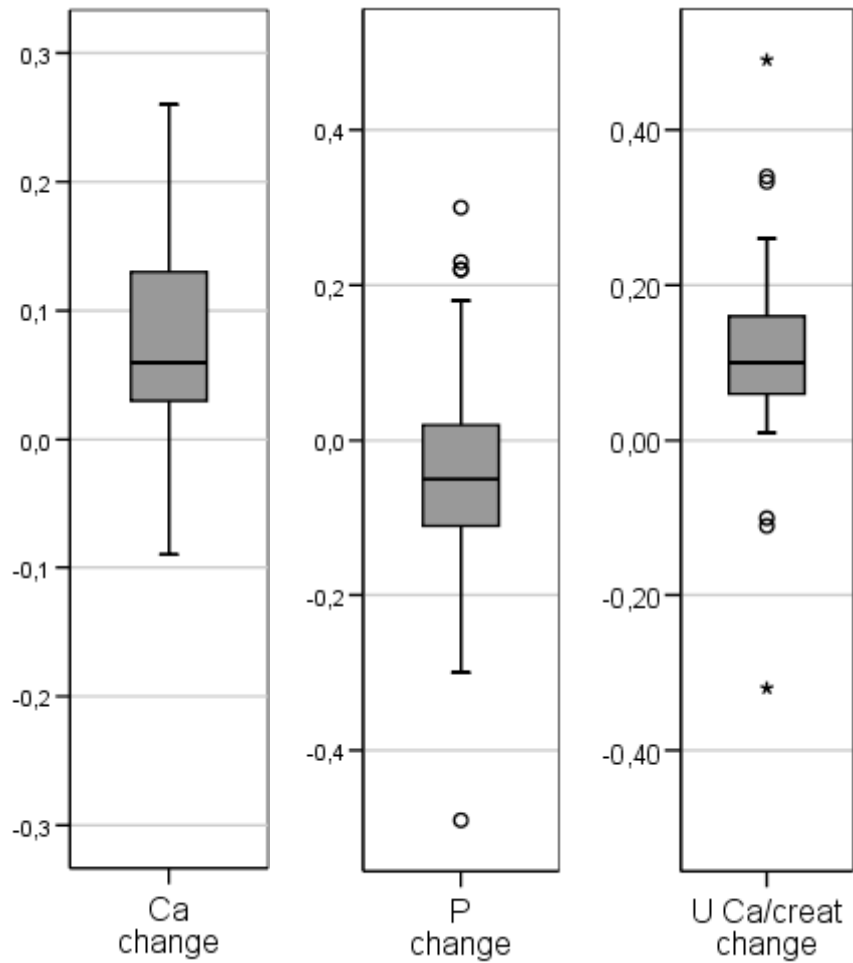


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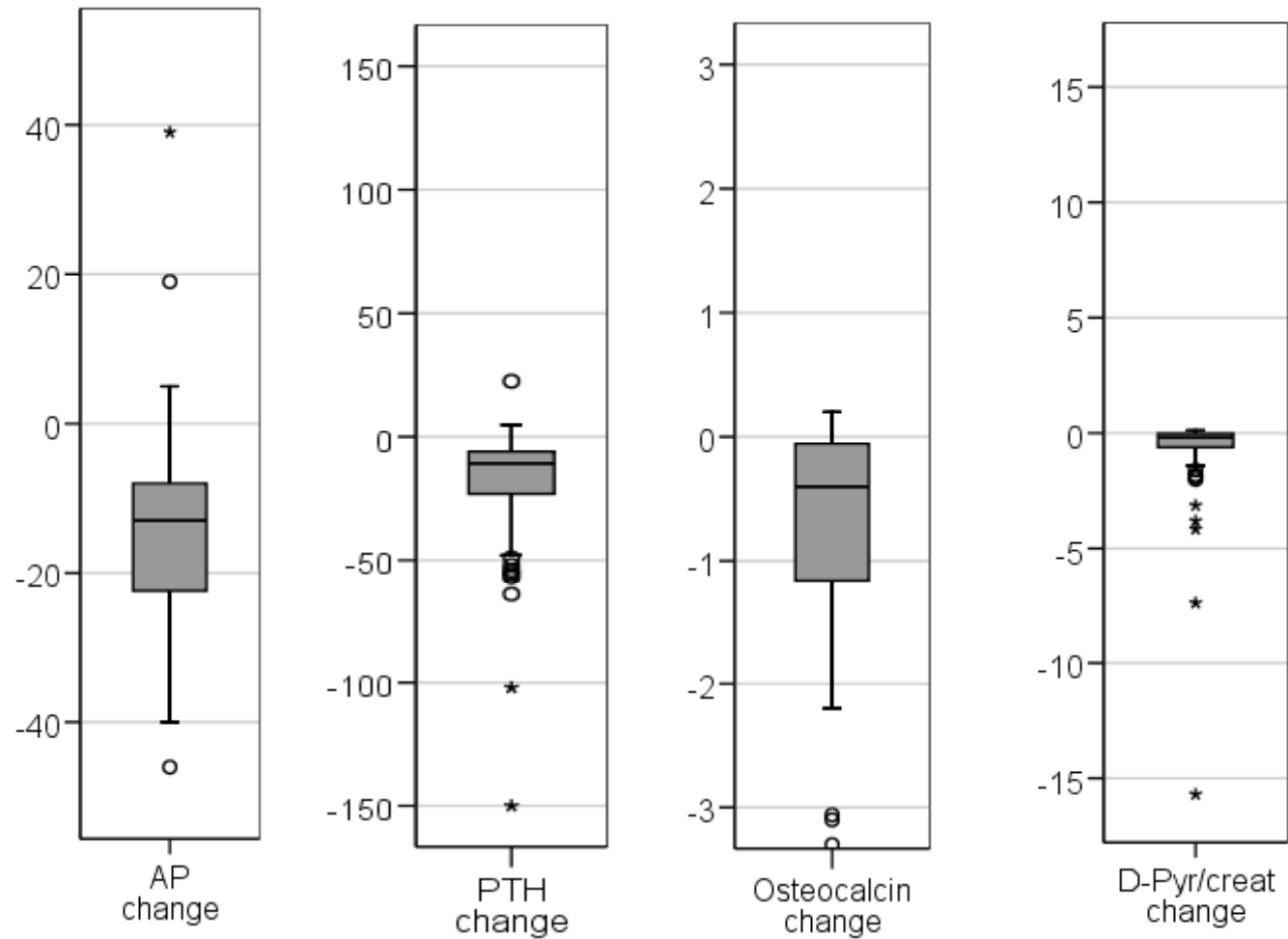


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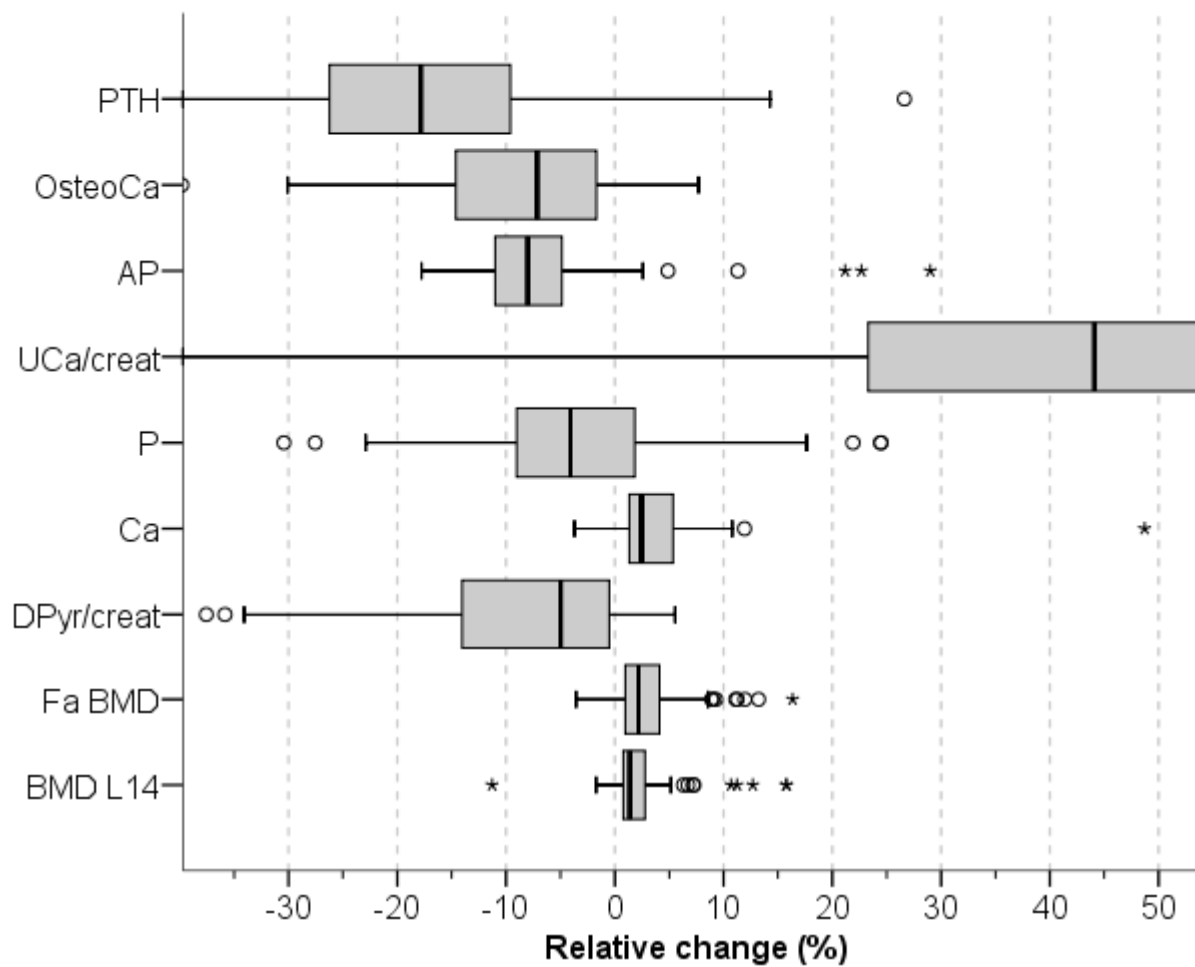


Figure 6. The ranges of relative changes expressed as percentages of the base values for all parameters.