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The effect of teriparatide treatment on circulating periostin and its relationship to regulators of bone formation and BMD in postmenopausal women with osteoporosis

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Context: Treatment of postmenopausal osteoporosis with teriparatide (PTH 1-34) increases bone formation and improves bone microarchitecture. A possible modulator of this mechanism of action is periostin. *In vitro* experiments have shown that periostin may regulate osteoblast differentiation and bone formation through Wnt signaling. Periostin secretion is increased by PTH in preclinical models, but the effect of teriparatide treatment of postmenopausal osteoporosis on periostin is not currently known.

Objectives, to: i) determine the effect of teriparatide treatment on circulating levels of periostin and other regulators of bone formation and ii) investigate how changes in periostin relate to changes in bone turnover markers, regulators of bone formation and bone mineral density

Participants and design: 20 women with postmenopausal osteoporosis, a two-year open-label single-arm study. **Intervention:** Teriparatide 20 mcg was administered by subcutaneous injection daily over 104 weeks. Periostin, sclerostin and DKK-1, PINP and CTX were measured in fasting serum collected at baseline (two visits) then at weeks 1,2,4,12,26,52,78 and 104. BMD was measured at the lumbar spine, total hip and femoral neck by DXA.

Results: Periostin levels increased by 6.6% (95% CI -0.4, 13.5) after 26 weeks teriparatide treatment and significantly by 12.5% (95% CI 3.3,21.0, P<0.01) after 52 weeks. Change in periostin was positively correlated with change in lumbar spine BMD at week 52 (r=0.567(95% CI 0.137,0.817), P<0.05) and femoral neck BMD at week 104(r=0.682(95% CI 0.261,0.885), P<0.01).

Conclusion: Teriparatide therapy increases periostin secretion; it is unclear whether this increase mediates the effect of the drug on bone.

Teriparatide therapy increases periostin secretion in postmenopausal women with osteoporosis. It is unclear whether this increase mediates the effect of the drug on bone.

Introduction

Teriparatide, parathyroid hormone amino terminal (PTH 1-34) is an anabolic agent that stimulates bone remodeling. It increases bone formation, and subsequently bone resorption (1-3). It improves bone microarchitecture, increasing cortical thickness at the radius and tibia and trabecular connectivity (4-6). It may also induce bone formation on the periosteal surface and therefore have an effect on bone size, geometry and strength (7-11).

The molecular mechanism(s) underlying the anabolic action of teriparatide remains unclear. A possible modulator of the increased bone formation observed with teriparatide treatment is periostin. (12). In bone, it is primarily expressed in the periosteum of long bones and by the osteocytes. Its expression is regulated by factors involved in bone homeostasis such as mechanical strain, PTH, growth factors and cytokines (12, 13).

Periostin enhances the activation of lysyl oxidase and regulates collagen cross-link formation (14). Through interaction with receptor tyrosine kinases, several signaling pathways are initiated to activate transcription factors Notch1 and β -catenin (15, 16). One pathway regulated in this manner is the Wnt signaling pathway. *In vitro* experiments suggest that periostin may increase bone formation through Wnt signaling by direct action on β -catenin (16, 17), inhibition of osteocyte sclerostin expression (a Wnt inhibitor), (18) or direct interaction with sclerostin(19). Periostin null mice have altered bone microarchitecture, lower BMD, reduced bone strength and bone turnover (12, 14, 20).

The expression of periostin is influenced by PTH treatment (21). *In vitro* and *in vivo* experiments have demonstrated that periostin is upregulated in response to PTH in human osteoblast cultures, in mice and in patients with hyperparathyroidism (18, 22-24). The increases in some cortical bone parameters may be partly mediated by the inhibition of sclerostin (25) or by directly activating β -catenin Wnt signaling, increasing osteoblast differentiation and hence bone formation (18).

Clinical studies have demonstrated that postmenopausal women with osteoporosis treated with teriparatide have increased levels of DKK-1 (a Wnt signaling inhibitor) but the data for sclerostin are more variable (26-30). The observed increase in DKK-1 suggests that Wnt signaling shows some response to teriparatide, and could possibly explain the reduction in the anabolic effect seen after 12 months treatment with teriparatide (29). There is no current data describing the effects of teriparatide on circulating levels of periostin in postmenopausal women with osteoporosis. We hypothesised that teriparatide may stimulate periostin expression, and that the increase in periostin contributes to the anabolic action of teriparatide through interaction with DKK-1, sclerostin and Wnt signalling.

Circulating levels of periostin have previously been measured in serum and plasma in several clinical studies using two commercially available enzyme-linked immunosorbent assays (ELISA) (31-34).. More recently in 2016 a third assay has been developed (Gadermaier E, 2017)(Biomedica Gruppe, Vienna, Austria). This is the first study to report effects of osteoporosis treatment on periostin with this assay.

Our aims were to; i) determine the effect of teriparatide treatment on circulating levels of periostin and the regulators of bone formation in postmenopausal women with osteoporosis and ii) explore the changes in periostin in relation to the changes in bone turnover markers, regulators of bone formation, BMD and cortical thickness.

Study design and methods

We conducted a two-year open-label single-arm study to investigate the Mechanisms Of Action of Teriparatide in postmenopausal women with osteoporosis (MOAT Study). The study was registered with clinicalTrials.gov (<http://clinicaltrials.gov/>, number - NCT01293292) and with the European Union Drug Regulating Authorities Clinical Trials (EudraCT, number - 2010-021009-19).

The treatment was teriparatide (Forsteo, Eli Lilly and Company), 20 mcg by subcutaneous injection daily. To assess compliance patients were asked to return the used teriparatide syringes.

A 100,000 IU cholecalciferol (vitamin D3) load was given orally at the before starting teriparatide and six-monthly throughout the study. Patients also received a daily supplement of 600 mg calcium and 400 IU vitamin D (marketed as AdCal D3 (Prostrakan)) or an equivalent preparation. The Summary of Product Characteristics (SmPC) instructions for treatment, including information about dosing and interactions was followed. Response to teriparatide therapy was assessed by measurements of bone density (by QCT, HRpQCT and DXA), and bone turnover markers. The study was carried out at the Centre for Biomedical Research (CBR), Northern General Hospital, Sheffield. Patients with osteoporosis were identified from Sheffield Metabolic Bone Clinic referrals and General Practice registers.

Twenty postmenopausal women with osteoporosis were recruited. The inclusion criteria were: BMD T-score (at the lumbar spine or total hip) less than or equal to -2.5, at least 5 years since last menstrual period, ambulatory, able and willing to participate in the study and provide written informed consent, and serum 25OH vitamin D3 >50 nmol/L (after 100,000 unit cholecalciferol bolus). All participants were bisphosphonate naive. Other exclusion criteria were diseases, treatments or lifestyle factors known to affect bone metabolism. This study was approved by the North West 2 Research Ethics Committee – Liverpool Central and the Medicines and Healthcare Products Regulatory Agency (MHRA), UK, and all participants gave full informed written consent prior to their participation. All investigations were carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, and in accordance with the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines.

Laboratory methods

Venous blood samples were collected from each patient, between 08:00 and 10:00 following an overnight fast at baseline (two visits) then at weeks 1, 2, 4, 12, 26, 52, 78 and 104. Serum and plasma was separated and stored at -70°C until the time of biochemical measurements

Periostin was measured in plasma using a recently released (June 2016) ELISA from Biomedica (Vienna, Austria). The ELISA recognizes all seven known splice variants of human periostin and the calibrators and controls are produced in a human serum matrix. It uses a mouse monoclonal antibody directed against the mid-region and a goat polyclonal antibody directed against the epitopes that spread across the whole periostin molecule and that are mostly conserved between the isoforms. The range for the assay is 125 to 4000 pmol/L, the intra-assay precision was 5%, and the assay was linear with spike samples (99 to 115%). A precision profile to assess the assay was performed. 50 samples were each measured in duplicates. The standard deviation and coefficient of variation were calculated for each duplicate, Figure 1. The samples used had not been through a previous freeze thaw cycle. Sclerostin and DKK-1 were measured in serum using a manual sandwich ELISA from Biomedica (Vienna, Austria) with interassay precision between 5 and 6%.

PINP, CTX, bone ALP, TRAP 5b, 1,25 (OH)₂D and 25OHD were measured in serum using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, United Kingdom). The inter assay CVs were between 3 and 7%.

Bone mineral density (BMD) and high resolution peripheral quantitative computed tomography (HR-pQCT) methods

Areal BMD (in g/cm²) of the lumbar spine, femoral neck and total body was measured at baseline and 26, 52 and 104 weeks by DXA using a Discovery A densitometer (Hologic Inc, Bedford MA).

Images of the distal radius and tibia (non-dominant, non-fractured limb) were obtained using high-resolution peripheral quantitative computed tomography. Cortical thickness (Ct.Th; mm) was measured at the radius and tibia using the standard in-built software (version 6.0, Scanco Medical AG, Zurich, Switzerland). Detailed methods and quality control assessment have been previously published (35).

Statistical analysis

On the basis of a 1 standard deviation change in lumbar spine BMD from baseline and a 10% drop-out per year, we calculated that 20 participants would provide greater than 90% power to detect a change at the 5% significance level. A similar power was calculated for the bone formation markers. Early bone formation marker changes (14 and 28 days) in excess of 1 standard deviation have previously been reported in 15 patients treated with teriparatide (36).

Baseline characteristics were described as a mean and standard deviation for each variable, Table 1. Periostin, sclerostin, DKK-1, PINP and CTX were expressed as mean percentage changes from baseline (and 95 % confidence interval) at each visit. One-way ANOVA was used to test for between-group differences. Bonferroni post hoc analysis was used to test for differences between the different time points. Spearman rank coefficients were used to test for correlations between changes in periostin and change in other variables. A P-value of less than 0.05 was considered to be statistically significant in all tests. Statistical analysis was performed with SPSS for Windows version 21 (SPSS Inc., Chicago, IL, USA).

Effect of teriparatide on periostin

Periostin levels were increased at 26 weeks after teriparatide treatment by 6.6%, Figure 2. It remained increased for the remainder of the study period with a significant increase at 52 weeks, of 12.5% (3.3, 21) ($P < 0.01$). 15/20 patients had periostin data by the end of the study. To assess the effect of the 5 patients with the missing data at week 104, we used the week 26 time point because 20/20 patients had periostin data. The mean percentage change in levels of periostin from baseline at week 26 for these 5 was 1.96% The mean percentage change of the remaining 15 was 7.4%.

Effect of teriparatide on the regulators of bone formation

Sclerostin was increased 12 weeks after baseline by 20.8% (95% CI 3.2 to 38.4) ($P < 0.05$). It was increased through the remainder of the study period with a peak increase at 52 weeks, of 28.9% (-1.5, 59.2). DKK-1 was increased 26 weeks after baseline by 15.3% (-0.5, 31.1). It was increased for the remainder of the study period with a significant increase at 52 weeks, of 28.8% (3.9, 53.6) ($P < 0.05$), Figure 3.

Effect of teriparatide on bone turnover markers

PINP and CTX were increased significantly during teriparatide treatment, the peaks were at week 52 by 204% (95% CI 119 to, 289) and 227% (95% CI 116 to 338) respectively, ($P < 0.001$).

Periostin correlations

Change in periostin levels was positively correlated with changes in sclerostin at week 104 ($r = 0.518$ (0.008, 0.814), $P < 0.05$) and DKK-1 at week 52 ($r = 0.494$ (0.036, 0.780), $P < 0.05$), (Table 2 and Figure 4). The correlation coefficients have been recalculated with the outliers removed and changes in periostin levels were not correlated with sclerostin and DKK-1, ($r = 0.386$ (-0.81, 0.760) and (0.205 (-0.305, 0.624), ($P > 0.05$)). Changes in periostin did not correlate with change in bone turnover markers or cortical thickness at the radius or tibia. Changes in periostin levels at week 52 were positively correlated with changes in total hip BMD and femoral neck BMD at

week 104 ($r = 0.547$ (0.070, 0.820), $P < 0.05$) and ($r = 0.682$ (0.261, 0.885), ($P < 0.01$), respectively (Table 2 and Figure 4).

Discussion

Teriparatide and periostin

In this study we found that teriparatide treatment of postmenopausal women with osteoporosis significantly increased circulating levels of periostin. This offers some support to our hypothesis that the anabolic actions of teriparatide may be partially related to periostin. A number of *in vitro* and animal experiments support our finding. Periostin mRNA was upregulated in osteoblasts by PTH (21). Periostin expression was also increased when PTH was added to IDG-W3 cell line (12), osteocytes *in vivo* (18) and human osteoblasts (22). However, our study is the first to show that levels of periostin are also increased by teriparatide treatment in postmenopausal women.

Teriparatide and regulators of bone formation

Significant increases in circulating levels of the Wnt inhibitors sclerostin and DKK-1 were also observed. Therefore, the anabolic action of teriparatide is unlikely to be mediated by these regulators of bone formation. In rat bone and osteoblast experiments, sclerostin and DKK-1 decrease in response to intermittent PTH (37-39). However, our data is consistent with some previous clinical studies of teriparatide. (26, 29). Idolazzi L et al, 2016 reported significant increases in DKK-1 and sclerostin by about 15% and 5% respectively at 12 months from baseline ($p < 0.01$) using ELISAs (Biomedica Medizinprodukte, Vienna, Austria) (26). Similarly, Anastasilakis AD et al, 2010 reported a significant increase in DKK-1 by around 30% at 12 months from baseline ($p < 0.05$) (28). Others have reported an acute decrease in sclerostin from baseline four hours after injection, by 12.7% ($\pm 1.9\%$) ($P < 0.0001$) in 27 non-osteoporotic, healthy postmenopausal women treated with teriparatide, measured using Luminex kits (Millipore/Linco, St. Charles MO) (30) or remained unchanged (27, 29). The differences in the data could be attributed to the different assays, study populations used and timing of injection.

We observed that DKK-1 levels remained increased after 52 weeks of treatment. This finding supports the concept that the anabolic effects of teriparatide may be down-regulated through continuous increased secretion of DKK-1 in order to achieve homeostasis through the mechanostat (29). This observation is supported by and may explain the partial reduction in bone turnover markers that we and others (29, 40-42) have observed in the second year of the treatment period.

The increase in DKK-1 may be partly due to the increase in 1,25 dihydroxy-vitamin D ($1,25(\text{OH})_2\text{D}_3$) observed with teriparatide treatment; it has been reported that $1,25(\text{OH})_2\text{D}_3$ induces DKK-1 gene expression and protein production in cell culture (43). We observed a significant increase in $1,25(\text{OH})_2\text{D}_3$ but these changes were not correlated with changes in DKK-1 (data not shown).

It is important to recognise the limitation that sclerostin and DKK-1 in circulation may not accurately reflect their concentration at tissue level, and this could contribute to the conflicting observations.

Periostin correlations

Changes in periostin during teriparatide treatment were positively correlated with changes in sclerostin and DKK-1. This finding does not support our second hypothesis that periostin mediates the effects of teriparatide by inhibiting these regulators of bone formation. This is in contrast to results of mouse experiments (18) in which expression of sclerostin was inhibited by

PTH in the bones of periostin KO mice. However, it may be that periostin does mediate bone formation and Wnt signalling through direct stimulation of β -catenin (44) or by down-regulating the action of sclerostin (19). Both of these explanations would not necessarily be reflected by the circulating levels of sclerostin.

Changes in periostin were not correlated with changes in bone turnover markers. Similar findings were demonstrated by others using a different periostin ELISA. Bonnet et al 2015 reported that periostin was not correlated with PINP, CTX, PTH, sclerostin, or serum 25-hydroxyvitamin D using 432 healthy subjects from the Geneva Retired Workers Cohort (GERICO) (33). This lack of correlation does not necessarily imply that periostin is not a mediator of the bone anabolic effect of teriparatide on the periosteum. Periosteal bone formation only forms a small part of the bone formation in response to teriparatide treatment and the increase in PINP relates also to trabecular bone changes. The increase in bone formation markers observed may be due to the increase in the rate of bone remodelling. An increase in DXA BMD at the spine of 7% to 10% after 18 months of treatment has been reported (4, 45). The lack of correlation between periostin and bone formation markers may be due to the higher ratio of trabecular to cortical bone at this site (46).

Furthermore, changes in periostin were positively correlated with changes in BMD at the proximal femur. This is an important finding due to the lack of correlation with PINP. Changes in BMD in the spine, may be related to changes in BTMs (42, 47) whereas periostin is related to changes in BMD at the hip. This finding has not previously been explored and it could be that the action of teriparatide on BMD is mediated through periostin. This suggests a stronger relationship to the hip structure due to there being more cortical bone. There were no changes in cortical thickness and cortical density at the radius and tibia with teriparatide treatment and these were not correlated with changes in periostin. In contrast a study has reported significant increases in cortical thickness with teriparatide treatment using HR-pQCT (4) and histomorphometry methods (11), implying that bone formation is markedly increased in cortical bone. Our study was powered for DXA BMD and bone turnover markers. Sample size and measurement variability will have affected the ability to detect these changes.

This study was performed to explore the role of periostin during teriparatide treatment. There are some limitations such as a small number of patients receiving only one dose to teriparatide and a lack of a control group receiving no treatment. By the end of the study, there was missing data for 5 patients, due to drop-outs. If the 5 patients remained in the study until week 104 then we would have expected the mean change to be lower. However, despite these limitations some of the results obtained were consistent with previous studies.

Conclusion

This is the first study to describe the circulating periostin response to teriparatide treatment in osteoporosis, and explore correlations of periostin with bone turnover markers, Wnt inhibitors, BMD and cortical thickness. We conclude that periostin increased in response to teriparatide. This increase may partly mediate the anabolic mechanism of action on BMD, but it does not affect bone formation through Wnt signalling as it does not reduce levels of sclerostin and DKK-1.

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Figure 1: The precision profile of the assay showing CV% at different concentration of periostin.

Figure 2: Changes in serum circulating levels of periostin over a 104 weeks treatment period with teriparatide. Expressed as mean (95% confidence intervals) percentage change from baseline (0) (N = 20) at several time points. * P<0.05 and ** P<0.01.

Figure 3: Changes in serum circulating levels of sclerostin (left) and DKK-1 (right) over a 104 weeks' treatment period with teriparatide. Expressed as mean (95% confidence intervals) percentage change from baseline (0) (N = 20) at several time points. *P<0.05 compared with baseline

Figure 4: Graphs showing changes between periostin and DKK-1, sclerostin, total hip BMD and femoral neck BMD.

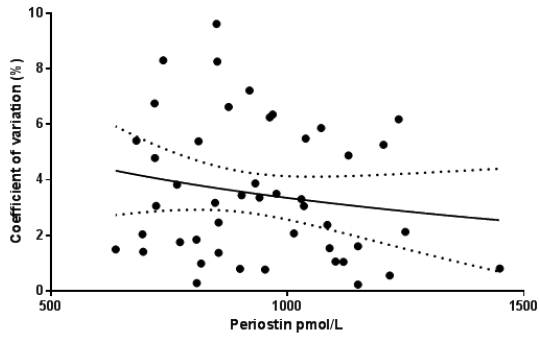
Table 1: Baseline characteristics of the participants of the MOAT study, (n=20 postmenopausal women with osteoporosis)

Variable	Mean (SD)
Age (years)	65.8 (5.1)
Height (cm)	161.2 (4.6)
Weight (kg)	64.3 (8.5)
BMI (kg/m ²)	24.8 (4.0)
Lumbar spine BMD T-score	-2.8 (0.3)
Total hip BMD T-score	-1.5 (0.6)
βCTX, (ng/ml)	0.55 (0.20)
Intact PINP, (ng/ml)	60.4 (17.2)
Bone TRAP 5b, (IU/L)	5.1 (0.8)
Bone ALP, (ng/ml)	20.8 (4.5)
Osteocalcin, (ng/ml)	24.1 (8.4)
PTH, (pg/ml)	34.3 (10.2)
25(OH)D ng/ml,	34.0 (8.7)
1,25(OH) ₂ D, (pg/ml)	62.0 (17.6)
Periostin, (pmol/L)	955.1 (224.3)
Sclerostin, (pmol/L)	20.3 (11.4)
DKK-1, (pmol/L)	34.0 (11.9)
Radius Ct.Th, (mm)	0.48 (0.12)
Tibia Ct.Th, (mm)	0.79 (0.22)

Table 2: Spearman rank correlations (95% confidence intervals) between changes in periostin and PINP, CTX, regulators of bone formation and BMD. Assessed from percentage changes from baseline at weeks 52 and 104

	WEEK 52 Periostin		WEEK 104 Periostin	
	Spearman Correlation Coefficient (95% CI)	P-Value	Spearman Correlation Coefficient (95% CI)	P-Value
PINP	0.217 (-0.332, 0.656)	0.438	-0.167 (-0.676, 0.450)	0.603
CTX	0.157 (-0.334, 0.581)	0.533	-0.201 (-0.677, 0.393)	0.509
Sclerostin	0.026 (-0.446, 0.486)	0.918	0.518 (0.008, 0.814)*	0.048
DKK-1	0.494 (0.036, 0.780)*	0.037	0.323 (-0.226, 0.716)	0.240
WEEK 104 BMD				
Femoral neck	0.424 (-0.091, 0.760)	0.102	0.682 (0.261, 0.885)**	0.005
Total hip	0.547 (0.070, 0.820)	0.028*	0.461 (-0.067, 0.787)	0.084
Lumbar spine	0.432 (-0.081, 0.764)	0.094	0.486 (-0.034, 0.799)	0.066

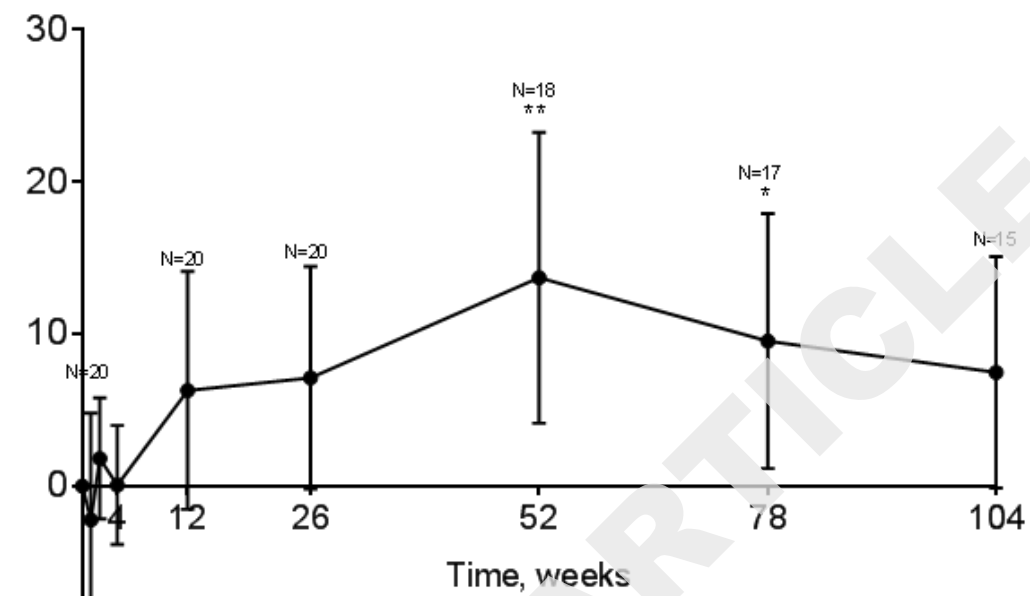
*P<0.05, **P<0.01, significant correlations



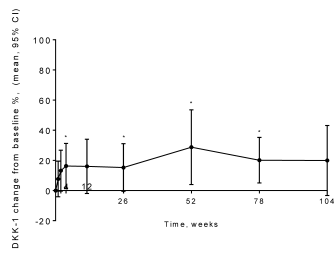
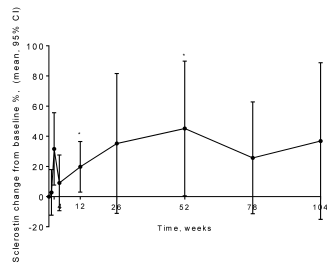
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Periostin change from baseline %, (mean, 95% CI)

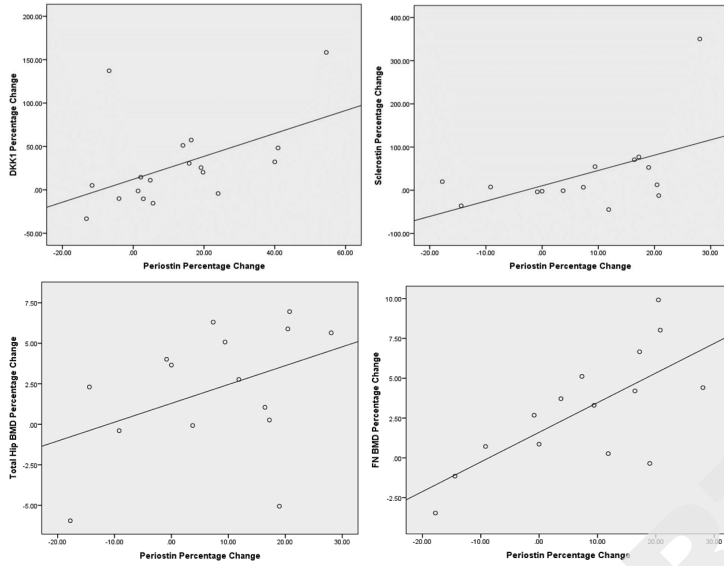


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