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**Effect of age and gender on serum periostin: relationship to cortical measures, bone turnover and hormones**

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## **Abstract**

Periostin is an extracellular matrix protein, and in bone is expressed most highly in the periosteum. It increases bone formation through osteoblast differentiation, cell adhesion, Wnt signalling and collagen cross-linking.

We hypothesised that serum periostin would be high at times of life when cortical modeling is active, in early adulthood and in older age, and that it would correlate with cortical bone measures, bone turnover and hormones that regulate cortical modeling.

We conducted a cross-sectional observational study of 166 healthy men and women at three skeletal stages; the end of longitudinal growth (16-18 years), peak bone mass (30-32 years) and older age (over 70 years). We measured serum periostin with a new ELISA optimised for human serum and plasma which recognises all known splice variants (Biomedica). We measured the distal radius and distal tibia with HR-pQCT, and measured serum PINP, CTX, sclerostin, PTH, IGF-1, estradiol and testosterone.

Periostin was higher at age 16-18 than age 30-32 (1253 vs 842 pmol/l,  $p < 0.001$ ), but not different between age 30-32 and over age 70. Periostin was inversely correlated with tibia cortical thickness and density ( $R -0.229$ ,  $-0.233$ , both  $p = 0.003$ ). It was positively correlated with PINP ( $R 0.529$ ,  $p < 0.001$ ), CTX ( $R 0.427$ ,  $p < 0.001$ ) and IGF-1 ( $R 0.440$ ,  $p < 0.001$ ).

When assessed within each age group the correlations were only significant at age 16-18, except for PINP which was also significant over age 70.

We conclude that periostin may have a role in IGF-1 driven cortical modeling and consolidation in young adults, but it may not be an important mediator in older adults.

**Key words:** HR-pQCT, cortex, peak bone mass, aging, bone turnover, periostin

## 1 Introduction

1.1 Bone size, geometry, density and microarchitecture are important determinants of bone strength. Understanding the regulation of these properties during skeletal maturation and aging is important in developing therapeutic strategies to prevent osteoporosis and fragility fractures.

Periostin is an extracellular matrix protein expressed in bone, cartilage, ligaments, lung and other sites. It was first described in 1993 and initially named osteoblastic-specific factor 2 [1]. There are seven alternative splice variants in the C-terminal domain, leading to seven different isoforms [2, 3]. It is involved in heart valve development, wound healing, fibrotic processes and cancer invasion [4-6]. In bone, it is produced mainly by osteoblasts and osteocytes. It is expressed most highly in the periosteum [7] and in vitro and animal studies suggest that it enables bone formation through several mechanisms. It increases osteoblast differentiation and proliferation [7], and inhibits degradation of  $\beta$ catenin, partly through inhibition of sclerostin Wnt signalling [8]. It enables cell adhesion and mobility by acting as a ligand for integrins [7]. It increases collagen cross-linking through activation of lysyl oxidase [9]. Periostin knock-out mice have low bone mass, reduced cortical bone volume and increased intracortical modeling with aging [8, 10]. Periostin may be particularly important in the bone formation response to mechanical loading and intermittent PTH [11].

Genetic variability in periostin contributes to the heritability of bone microstructure at the radius and tibia; it is positively correlated with BV/TV and cortical thickness, and inversely correlated with cortical porosity [12]. However, periostin (using different assays) is not strongly correlated with BMD by DXA [12-15] and periostin alleles do not associate with DXA BMD in genome-wide analysis [16]. Serum periostin was positively correlated with

femoral neck cortical thickness by QCT in older men and women, but not after adjustment for age and gender [12].

Higher serum periostin has been associated with higher fracture risk in postmenopausal women, and the association with non-vertebral fracture seems to be stronger than with vertebral fracture (13, 14).

1.2 We have previously shown that there is cortical consolidation beyond the end of longitudinal growth to peak bone mass [17]. With aging there is an increase in cortical perimeter which is thought to be an adaptive response to maintain bone strength when bone mass decreases [18].

Therefore, circulating periostin might be high in young adults when there is active cortical modeling, and in older adults when periosteal circumference increases. Testosterone and IGF-1 are thought to increase periosteal apposition, particularly during growth and consolidation, and it is possible that their action is mediated through periostin.

Some of the indeterminate results of previous human clinical studies of periostin may be due to the different assays in use. Some of the previously reported assays were not optimised for use in human serum, and not all were well characterised for which splice variants they detected. A recently released ELISA (June 2016, Biomedica Gruppe, Vienna, Austria) for periostin may be an improvement on previous assays for clinical human studies. This new assay recognises all seven known splice variants of human periostin and the calibrators and controls are produced in a human serum matrix [19].

1.3 The aims of this study were 1) To describe how serum periostin measured by the Biomedica assay varies by gender and age, 2) To determine if serum periostin is associated

with radius and tibia HR-pQCT cortical bone measures at end of growth, peak bone mass and older age, 3) To determine if serum periostin is associated with biochemical markers of bone turnover and hormones at end of growth, peak bone mass and older age.

## **2 Methods**

We conducted a single-centre, cross-sectional, observational study.

### **2.1 Subjects**

We recruited 180 healthy volunteers through poster advertisements, emails to hospital and university staff and mailing from Sheffield general practices. We studied 30 men and 30 women in each of three age groups; 16-18 years to represent the end of longitudinal growth 30-32 years to represent peak bone mass and over 70 years to represent aging. Volunteers were excluded if they had any disease or were taking any medication known to affect bone metabolism (such as inflammatory disease or cancer; hormonal contraceptives other than Depo-Provera were allowed), if they did not have at least two evaluable vertebrae on dual-energy x-ray absorptiometry (DXA), had previously fractured both radii or tibiae, if there were any other conditions that would affect the reliability of the study measurements, if they were pregnant or trying to conceive or if they were unable to give informed consent. We screened 268 volunteers to recruit 180 participants who. Fourteen participants were excluded from this analysis due to significantly abnormal biochemical results (vitamin D deficiency, high PTH or high PINP) or insufficient volume of serum sample to make all the measurements. There was one missing result for serum testosterone, otherwise data were complete for all 166 participants included in this analysis. The study was approved by the Sheffield Research Ethics Committee, conducted according the Declaration of Helsinki, and all participants gave written informed consent.

## 2.2 Imaging

Bone density at the lumbar spine and hip in  $\text{g/cm}^2$  was measured by DXA (Hologic Discovery, Bedford, MA, USA). HR-pQCT images of the distal radius and distal tibia were obtained with the XtremeCT device (Scanco Medical AG, Zurich, Switzerland) using standard protocols. In subjects aged 16-18 who had visible growth plates on the scout image, the image stack was obtained from 1mm proximal to the proximal limit of the growth plate [20]. HR-pQCT images were analysed with standard software provided by Scanco Medical AG (version 6).

## 2.3 Biochemistry

Blood samples were all taken in the morning, after an overnight fast.

Serum PINP (CV 2.1%), CTX (CV 6.8%), PTH (CV 5.0%), estradiol (CV 3.9%) and testosterone (CV 2.2%) were measured with the Cobas e411 analyser (Roche Diagnostics, Painswick, Germany). In participants with serum estradiol below the lower limit of detection ( $<5$  pg/ml), a value of 4 pg/ml was assigned for analysis (this was done for 12 women over age 70). IGF-1 was measured using a manual ELISA (ImmunoDiagnostic Systems, Boldon, UK) (CV 5.7%). Serum sclerostin and periostin were measured with quantitative sandwich ELISA kits from Biomedica (Vienna, Austria). The intra-assay and inter-assay CVs are  $\leq 10\%$  and  $\leq 6\%$ , respectively. The periostin ELISA assay uses a mouse monoclonal antibody directed against the mid-region and a goat polyclonal antibody directed against 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.

## 2.4 Statistics

The original study included duplicate measurements to study the short term reproducibility of the HR-pQCT and method comparison with standard pQCT, so sample size was determined according to the ISCD in accordance with recommendations for estimating the reproducibility of repeat measurements[21].

Statistical analysis was done with SPSS version 22.0.

General linear model (GLM) univariate analysis with Tukey post-hoc comparison was used to compare groups by age and gender and assess gender-age interactions. Periostin was not normally distributed so was log-transformed for the GLM analysis, and Spearman's coefficient was used to assess correlations between periostin and other variables. P values less than 0.05 were accepted as statistically significant. We did conduct multiple comparisons, but as this was an exploratory analysis we did not use post-hoc significance corrections. The reference interval for serum periostin was calculated with Medcalc, using a normal distribution method (when the age 16-18 group were excluded, normality tests were acceptable).

### **3 Results**

3.1 Subject characteristics and spine and hip DXA BMD are shown in Table 1. Men and women were shorter at age 70+ than at younger ages. In women, body mass index was higher with increasing age. In men and women, lumbar spine BMD was lower at age 16-18 than at age 30–32. In women, lumbar spine and total hip BMD was lower at age 70+ than at age 30-32.

#### 3.2 Periostin

Serum periostin did not differ between men and women. It was higher at age 16-18 than 30-32 ( $p<0.001$ ), but not different between age 30-32 and age 70+ ( $p=0.08$ ) (Figure 1). There



was no significant gender\*age interaction. Because there was no gender difference, and no difference between age 30-32 and age 70+, used all the participants in these age groups (n=108) to estimate a mature skeleton reference interval. The reference interval is 454-1323 pmol/l.

### 3.3 Periostin and DXA BMD

There were no significant correlations between serum periostin and DXA BMD at the lumbar spine or total hip, when analysed as group and within each age group. However the correlation coefficients were greater in the age 16-18 group (R -0.222, p=0.093 and R -0.0238, p=0.072) than in the other age groups (age 30-32: R 0.096, p=0.487, R -0.035, p=0.801 and age 70+: R 0.176 p=0.212, R 0.079 p=0.572).

### 3.4 Radius and tibia HR-pQCT measures

#### 3.4.1 Cortical measures

See Figure 2 and Figure 3.

Radius cortical perimeter was greater in men than in women, and greater at age 70+ than at age 30-32. Radius cortical thickness did not differ by gender. It did not differ between age 16-18 and age 30-32, but was lower at age 70+ than at age 30-32. Radius cortical BMD was higher in women than in men. It was lower at age 16-18 and age 70+ than at age 30-32.

Tibia cortical perimeter was greater in men than women. It was higher at age 16-18 than age 30-32 but not different at age 70+ than age 30-32. Tibia cortical thickness was higher in men than in women. It was lower at age 16-18 and age 70+ than age 30-32. Tibia cortical BMD did not differ by gender. It was lower at age 16-18 and age 70+ than at age 30-32.

#### 3.4.2 Trabecular density

Radius and tibia trabecular BMD was higher in men than in women ( $p < 0.001$ ). It was lower at age 70+ than at age 30-32 (radius  $p = 0.012$ , tibia  $p = 0.011$ ), but not different at age 16-18 than at age 30-32.

### 3.5 Correlation of periostin with HR-pQCT measures

Periostin was not correlated with radius or tibia trabecular BMD, any radius cortical measures or tibia perimeter. It was negatively correlated with tibia cortical thickness and tibia cortical BMD (Table 2). When analysed within each age group, these correlations were only significant at age 16-18.

### 3.6 Correlation of periostin with biochemical markers of bone turnover and hormones

Periostin was positively correlated with PINP and CTX (Table 2). When analysed within each age group, these correlations were both significant at age 16-18 and the correlation with PINP was significant at age 70+. Neither correlation was significant at age 30-32.

Periostin was positively correlated with IGF-1 (Table 2). When analysed within each age group, this correlation was only significant at age 16-18.

There were no significant correlations between periostin and PTH, estradiol, testosterone or sclerostin in the whole study group or any of the three age groups. When correlations with estradiol and testosterone were tested separately in men and women they were still non-significant.

## 4 Discussion

4.1 This is the first study to measure circulating periostin during skeletal modeling, and assess the relationship with cortical measures, biochemical markers of bone turnover and

hormones in this young age group. It is also the first study to assess the relationship of the Biomedica periostin assay with age, gender, bone measures and hormones.

We found that serum periostin is higher at age 16-18 than at age 30-32. The range of periostin measurements was greater at age 16-18 than other ages, which is typical for biochemical markers due to the higher turnover and some heterogeneity of skeletal modeling and maturity at this age. At age 16-18 periostin is positively correlated with IGF-1, PINP and CTX and inversely correlated with cortical thickness and cortical density. This suggests that periostin does have a role in cortical consolidation after the end of growth, and could mediate IGF-1-driven cortical modeling. The inverse correlation with cortical density suggests that periostin is higher in young adults with ongoing cortical modeling, but lower in individuals who are nearer to peak bone mass where cortical consolidation is complete and modeling has slowed. There were no significant correlations between periostin and cortical measures, biochemical markers of bone turnover or hormones at age 30-32. This is consistent with our hypothesis that periostin would not have a significant biological role when there is little active cortical modeling.

In older adults, cortical perimeter was greater and cortical thickness and density were lower than at age 30-32, but periostin was not significantly different from age 30-32. There was a positive correlation of periostin with PINP in older adults, but no correlation with CTX, cortical measures or hormones. Therefore periostin may be a more active mediator during cortical modeling in younger life than during the different processes of cortical aging.

4.2 The only other study to report periostin by age and gender found that periostin was higher in men, and lower in older adults (mean age 65y) than their offspring (mean age 38y), but this study used a different assay [12]. Periostin was positively correlated with HR-pQCT bone density and architecture in older adults, but the correlations did not persist after adjustment

for gender, age, BMI, and years since menopause, and there were no significant correlations with biochemical markers of bone turnover, sclerostin, hormones or fracture. Serum periostin is not related to DXA BMD in older adults [12-15]. There may be a positive correlation between periostin and alkaline phosphatase in postmenopausal women, but no correlation with CTX [13], consistent with our findings of a correlation with PINP but not CTX. Serum periostin does not change after treatment with zoledronic acid in postmenopausal women with osteoporosis, again suggesting that it may not have such a significant role in age-related bone loss [13].

However, higher serum periostin has been associated with prevalent and incident fractures and non-vertebral fractures in postmenopausal women independently of BMD. The evidence for association with vertebral fracture is not as strong, which might be expected if periostin is more important in cortical than trabecular bone [14, 15]. It is counterintuitive to see a positive association between fracture risk and a factor which increases bone formation and tends to be positively associated with bone density. It may be that high levels of periostin in older adults are a response to increased strain in weaker bone.

4.3 We hypothesised that periostin would be associated with cortical perimeter, but the significant relationships were with cortical thickness and density. Although periostin is most highly expressed in the periosteum, serum periostin seems to reflect activity in the whole cortical compartment rather than just the periosteum.

Periostin was not correlated with estradiol or testosterone which are thought to regulate periosteal apposition, so sex steroids may exert their specific actions on the periosteum through other pathways.

Although periostin does not seem to be strongly associated with bone measures in older adults, there are some situations where it could be a useful marker. Experimental evidence

suggests it may have an important role in the action of teriparatide on bone, and in the response to mechanical loading through interaction with sclerostin and Wnt signalling [22]. Therefore it will be of interest in studies of anabolic bone agents, and especially in studies of combined anabolic agents with physical stimulation.

4.4 The main strength of this new periostin assay is that based on epitope mapping and the use of isoform measurements, all 7 known isoforms are recognised [23]. This is not the case for any other assay. The assays from USCN [24] and Roche Elecsys [25] did not evaluate any recognition of isoforms. The assay from Adipogen recognises isoforms 1 and 2, and possibly 3 and 4 [26] and the Abbott Architect assay recognises 6 of the 7 known isoforms [27].

4.5 There are some limitations to this study. It is cross-sectional and observational, so causal relationships cannot be inferred. The Biomedica assay recognises all seven isoforms of periostin, but the tissue-specificity of the splice variants is not yet known, so it may be that an assay which only recognises bone isoforms would give more informative results. The correlations of periostin with cortical measures were stronger at the tibia than at the radius. This may reflect the poorer short term precision of these measurements at the radius than at the tibia [28]. This is partly because the cortex is very thin at the standard XtremeCT distal radius site. Motion artefacts are more common at the radius than at the tibia, but we repeated scans where necessary to get good quality images. We used the standard Scanco software for HR-pQCT analysis but there are more advanced methods (particularly defining the endocortical perimeter in older adults with endocortical resorption [29]) which might have given different results for the cortical thickness and density.

4.6 In conclusion, serum periostin is higher at the end of growth (age 16-18) than at peak bone mass and older age. At this age it is positively correlated with IGF-1 and biochemical markers of bone turnover, and negatively correlated with cortical thickness and density, suggesting a role in IGF-1 driven bone modeling and cortical consolidation. The relationship with bone turnover and cortical measures is not seen at peak bone mass (age 30-32), and there is a weaker correlation with PINP only in older adults (age 70+).

Periostin may be an important mediator of cortical bone during skeletal development, but less important in older age.

## **5 Acknowledgments**

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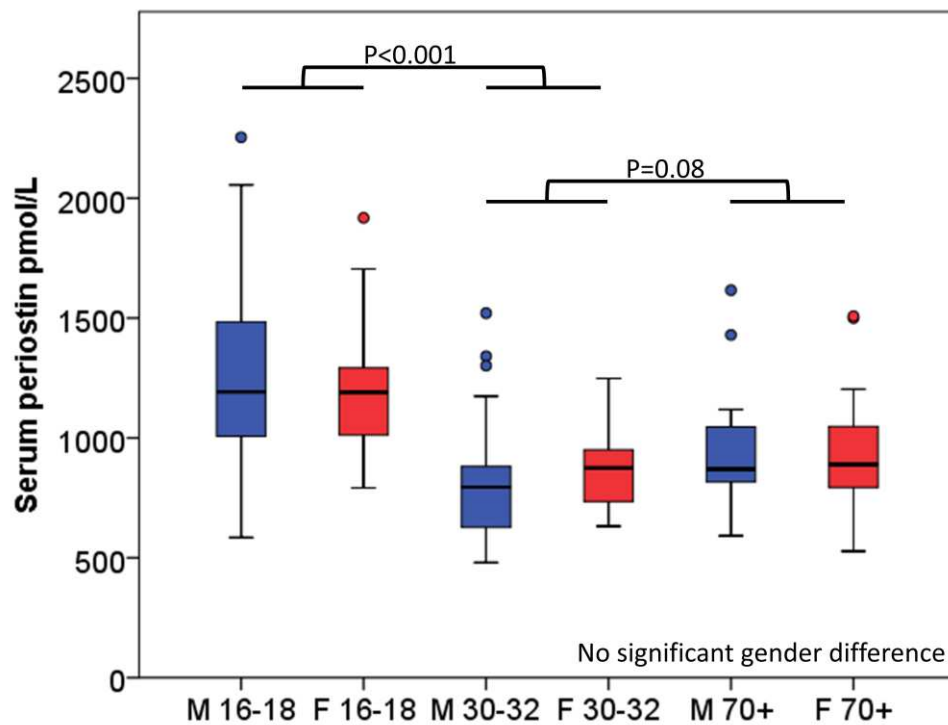
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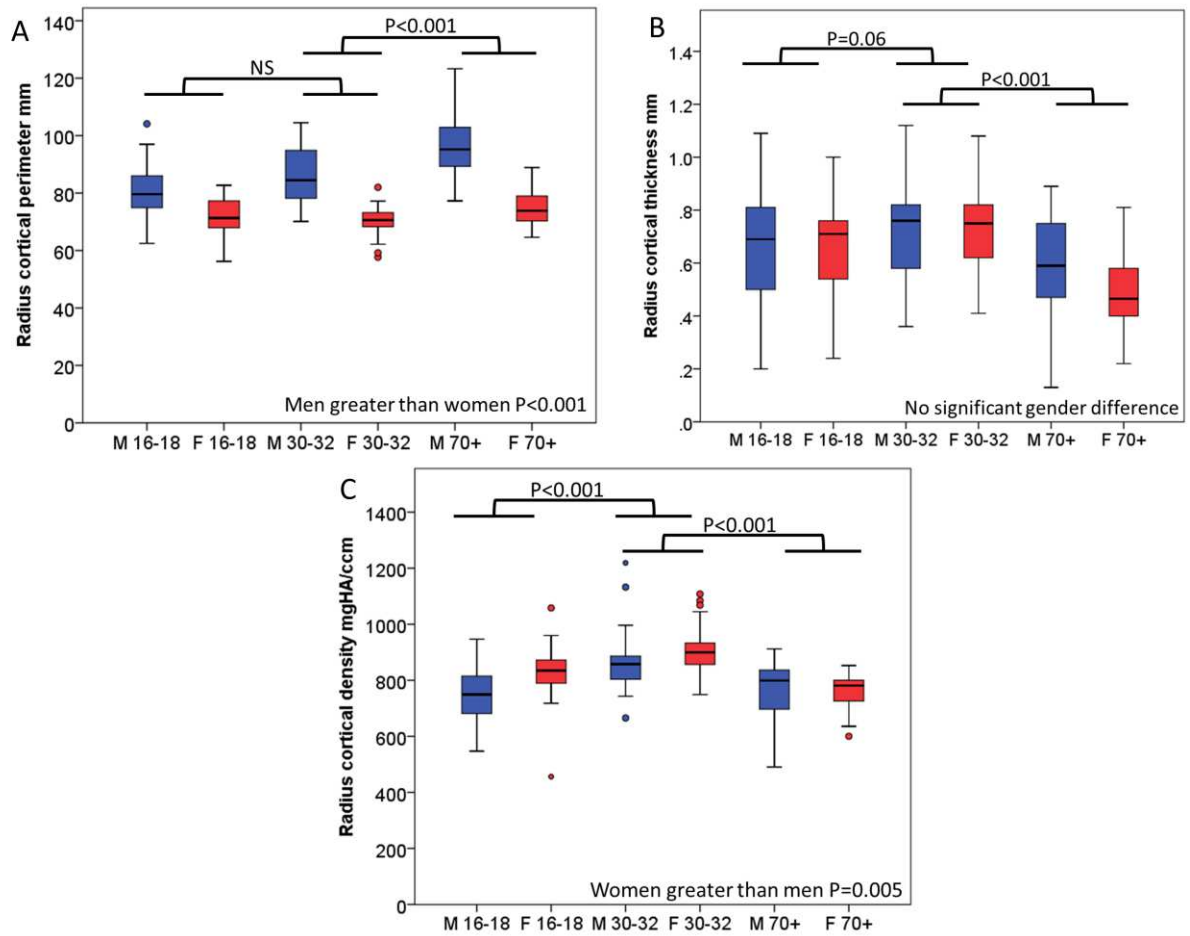


## Figure Legends

**Figure 1 Serum periostin by age and gender groups;** Boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> centiles, central lines indicate the median, whiskers indicate the minimum and maximum values that are not outliers, circles represent outliers.

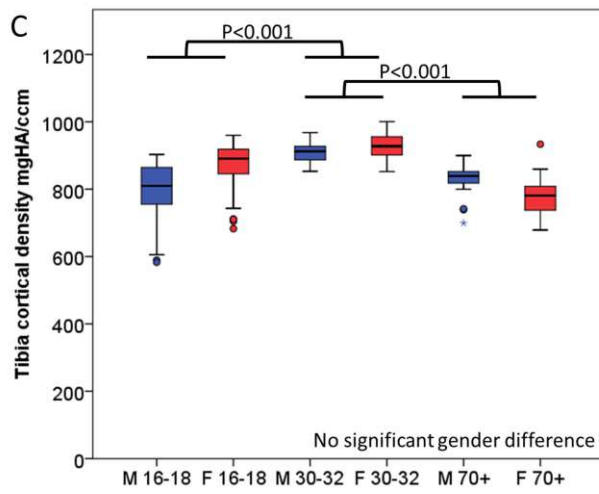
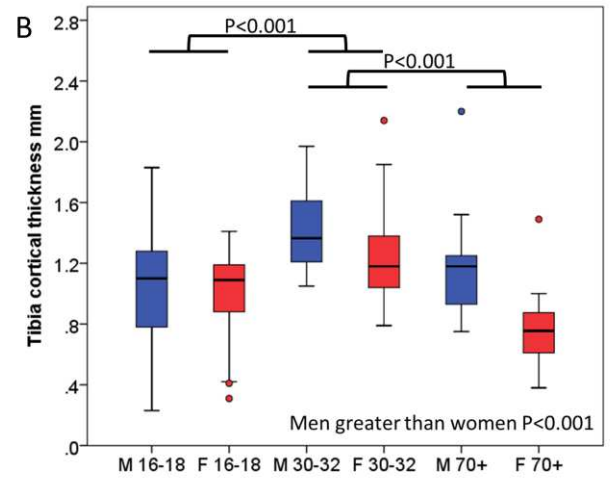
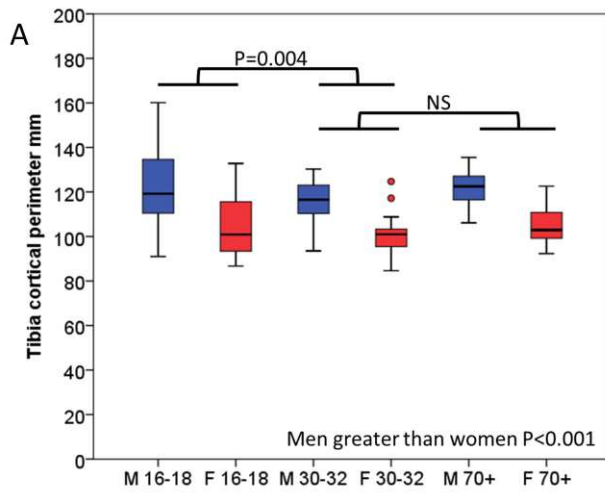


**Figure 2 Radius cortical measures;** Perimeter (A), thickness (B), bone mineral density (C). Boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> centiles, central lines indicate the median, whiskers indicate the minimum and maximum values that are not outliers, circles represent outliers. NS=not significant.



**Figure 3 Tibia cortical measures; Perimeter (A), thickness (B), bone mineral density (C).**

Boxes indicate the 25<sup>th</sup> and 75<sup>th</sup> centiles, central lines indicate the median, whiskers indicate the minimum and maximum values that are not outliers, circles represent outliers. NS=not significant.



**Table 1 Group characteristics; Results given as mean (SD).**

\*different to age 30-32 p<0.05

Age group	Women			Men		
	16-18 n=29	30-32 n=29	70+ n=28	16-18 n=29	30-32 n=26	70+ n=25
Age, years	17.5 (0.8)	31.2 (0.8)	75.0 (3.5)	17.4 (0.7)	31.4 (0.8)	75.3 (3.4)
Height, cm	165.1 (5.1)	165.1 (4.9)	157.6* (5.9)	178.9 (7.3)	178.7 (6.1)	174.8* (6.0)
BMI, kg/m <sup>2</sup>	22.2* (3.7)	24.3 (4.0)	27.4* (4.6)	24.5 (6.0)	25.8 (3.6)	26.8 (3.3)
Lumbar spine BMD g/cm <sup>2</sup>	0.965* (0.084)	1.040 (0.108)	0.949* (0.167)	0.993* (0.107)	1.073 (0.186)	1.115 (0.139)
Total hip BMD g/cm <sup>2</sup>	0.961 (0.089)	0.974 (0.123)	0.866* (0.138)	1.077 (0.153)	1.074 (0.125)	1.021 (0.128)

**Table 2 Correlations (by Spearman’s Rho) of serum periostin with cortical measures, biochemical markers of bone turnover and hormones, in all subjects and within each age group.**

	All subjects		Age 16-18		Age 30-32		Age 70+	
	R	p	R	p	R	p	R	p
Radius cortical perimeter	-0.089	0.255	-0.098	0.464	-0.117	0.394	-0.014	0.919
Radius cortical thickness	-0.010	0.894	-0.020	0.884	0.024	0.862	0.181	0.194
Radius cortical density	-0.141	0.069	-0.075	0.578	0.185	0.177	0.090	0.522
Tibia cortical perimeter	0.060	0.442	0.160	0.230	-0.196	0.151	-0.037	0.792
<b>Tibia cortical thickness</b>	<b>-0.229</b>	<b>0.003</b>	<b>-0.368</b>	<b>0.004</b>	0.043	0.757	0.051	0.715
<b>Tibia cortical density</b>	<b>-0.233</b>	<b>0.003</b>	<b>-0.423</b>	<b>0.001</b>	0.180	0.188	0.118	0.401
<b>PINP</b>	<b>0.529</b>	<b>&lt;0.001</b>	<b>0.493</b>	<b>&lt;0.001</b>	-0.085	0.536	<b>0.285</b>	<b>0.039</b>
<b>CTX</b>	<b>0.427</b>	<b>&lt;0.001</b>	<b>0.262</b>	<b>0.047</b>	-0.174	0.203	0.166	0.236
Sclerostin	-0.028	0.718	0.111	0.407	0.220	0.106	0.115	0.413
PTH	-0.067	0.394	-0.040	0.764	-0.117	0.394	0.228	0.101
<b>IGF-1</b>	<b>0.440</b>	<b>&lt;0.001</b>	<b>0.401</b>	<b>0.002</b>	0.096	0.484	-0.060	0.671
Estradiol	0.042	0.588	0.109	0.414	0.134	0.328	-0.089	0.528
Testosterone	0.002	0.980	-0.004	0.978	-0.230	0.094	-0.051	0.717

