Increased serum concentrations of type I procollagen C-terminal propeptide and osteocalcin during a short course of calcitriol administration to adult male volunteers

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Abstract. To investigate bone collagen metabolism during vitamin D treatment, 15 healthy males (aged 28-45 years, median 34) were treated orally with calcitriol, 2 µg daily for 7 days and followed for a total of 2 weeks. The serum concentration of calcitriol rose markedly (median difference and 95% confidence limits: 49% (5-82), \( p<0.005 \)) during treatment, whereas serum levels of calcidiol, and calcium remained unchanged. The serum level of procollagen type I C-terminal propeptide rose 15\% (7-33, \( p<0.003 \)), whereas no alterations were observed concerning serum procollagen type III N-terminal propeptide, and serum hyaluronan. The serum concentration of osteocalcin rose concomitantly (26\% (12-45), \( p<0.003 \)). All values returned to baseline levels within seven days after the treatment week. The serum levels of osteocalcin and procollagen type I C-terminal propeptide were positively correlated (\( r_s=0.71, p<0.004 \)) during the study. Serum procollagen type I C-terminal propeptide and serum osteocalcin did not correlate with serum procollagen type III N-terminal propeptide or serum hyaluronan either at baseline or after treatment. It is concluded that a short course of calcitriol administration to healthy males stimulates the biosynthesis of bone-related matrix proteins. By contrast, connective tissue components of predominantly extrasosseous origin are not affected.

The active vitamin D metabolite, calcitriol has been proposed to play an important role in the regulation of bone collagen metabolism (1). However, conflicting results have emerged from in vitro studies, indicating that the hormone can both stimulate and suppress bone collagen synthesis (2,3).

Type I collagen secreted by osteoblasts (4,5) is the major organic constituent of bone (6). By contrast, type III collagen is virtually absent from normal bone, but present in varying proportions in e.g. skin, vessel wall and muscle (7). Both collagen species are secreted as high molecular weight precursor molecules termed procollagens. In the extracellular space, C- and N-terminal extension peptides are cleaved off by specific proteases on a molar-to-molar ratio. Two of the resulting propeptides, the N-terminal type III propeptide (PIIINP) and the C-terminal type I propeptide (PICP) have been demonstrated in various body fluids, where they are supposed to reflect the present biosynthetic activity of their parent collagen types (8). Hyaluronan is a high molecular weight polysaccharide which is particularly abundant in soft connective tissue where it is thought to be of importance in the regulation of interstitial fluid volume (9). Hyaluronan is transported from the tissues via the lymphatic system to the general circulation where increased levels have been observed in various fibrosing conditions (10,11).

The present study was undertaken in order to investigate the in vivo effect of short-term calcitriol administration to healthy male volunteers on bone and soft connective tissue metabolism as estimated by seromarkers of extracellular matrix components and osteoblast activity.
Subjects and Methods

Fifteen male volunteers aged 28-45 years (median 34) were recruited from the medical staff. The subjects were healthy and without any medication. After a run-in period, all received an oral dose of 2 μg calcitriol (Rocaltril®, Roche) daily for 7 days. Fasting blood samples were collected before, 1, and 2 weeks after initiation of treatment. The investigation was carried out in late autumn and winter where vitamin D levels are at lowest in the Danish population (12). The volunteers were on normal diet and without restriction of physical activity. Data on calcium and non-collagen bone metabolism for 7 of the participants have been published previously (13).

PICP in serum was quantified by a newly developed equilibrium type RIA using human propeptide as tracer (Farmos Diagnostica, Oulunsalo, Finland) (14). Normal serum only contains propeptide antigen of similar size as standard PICP. Intra- and inter-assay coefficients of variation (cv) were 3.2 and 7.0%, respectively. Measurement of serum PIIINP was carried out as previously described (15) by means of a similarly designed RIA (Farmos Diagnostica, Oulunsalo, Finland) (16). The assay system is insensitive to low molecular weight degradation products of the propeptide. Intra- and inter-assay cv were 5.2 and 10.0%, respectively. Serum hyaluronan was determined by means of a radiometric assay (Pharmacia, Uppsala, Sweden) based on the use of specific hyaluronan binding proteins isolated from bovine cartilage (11,17). Inter-assay cv was 8.3%. Serum osteocalcin (bone gla protein) was measured by RIA (18). Intra- and inter-assay cv were 5 and 10%, respectively. Alkaline phosphatase activity in serum was measured spectrophotometrically as recommended by the Scandinavian Committee on Enzymes (19). Intra- and inter-assay cv were 2.5 and 5%, respectively. Serum calcidiol and calcitriol were determined by competitive protein binding assays. The intra- and inter-assay variations were 9.1 and 13.5% for calcidiol, and 11 and 11% for calcitriol (12,20). Serum level of calcium was measured by standard laboratory method and corrected for variation of serum albumin.

The study was approved by the local ethical committee and by the National Board of Health and conducted according to the declaration of Helsinki II.

**Fig. 1.**
The effect of calcitriol administration to 15 healthy male volunteers on biochemical variables of osteoblast and connective tissue metabolism. Calcitriol, 2 μg/day, was given the first week. Mean ± SEM are shown. Friedman test for analysis of variance (**=p<0.01) and Wilcoxon's paired rank sum test (*=p<0.005; significantly different from week 0). S-HYA: serum hyaluronan; S-PIIINP: serum procollagen type III N-terminal propeptide; S-PICP: serum procollagen type I C-terminal propeptide; S-BGP: serum osteocalcin.
Statistical methods
One-way analysis of variance (Friedman test) was performed for each parameter, and a posteriori one sample rank sum test (Wilcoxon’s test) was done. Changes in serum levels are given as median differences with 95% confidence limits. Relationships between variables were tested using Spearman rank correlation test. p<0.05 was chosen as level of significance.

Results
All subjects completed the study, and no adverse reactions were recorded. The serum level of calcitriol rose markedly during the treatment week (49% (5-82), p<0.005 (median difference and 95% confidence limits)), but in all subjects the serum levels remained within the normal range (Fig. 1). By contrast the serum level of calcidiol and albumin-corrected serum calcium did not change significantly (not shown). During the week of treatment the serum level of PICP rose by 15% (7-33, p<0.003), whereas no alterations were observed concerning serum PIIINP and hyaluronan (Fig. 1). The serum concentration of osteocalcin rose comitantly (26% (12-45), p<0.003) (Fig. 1), whereas the rise in serum alkaline phosphatase was not significant (not shown). All values returned to baseline level within seven days after the treatment week.

A positive correlation was found between serum osteocalcin and PICP at week 1 (r<sub>i</sub>=0.71, p<0.004) (Fig. 2), and at week 2 (r<sub>i</sub>=0.57, p<0.04). The correlation at baseline was insignificant (r<sub>i</sub>=0.45, p<0.10). The increase in serum osteocalcin was not significantly correlated to the increase in PIIINP (r<sub>i</sub>=0.40, p<0.15). No associations were evident between serum levels of PICP, PIIINP, and hyaluronan either at baseline or after the treatment week (not shown). No correlations could be shown between serum levels of osteocalcin and PIIINP or hyaluronan (not shown).

Discussion
Oral administration of 2 μg calcitriol for 7 days to healthy male volunteers increased in parallel the serum levels of the bone-related matrix constituents, PICP and osteocalcin, which returned to baseline one week after its withdrawal. The serum concentrations of the mainly extrasosseous matrix components, PIIINP and hyaluronan showed no significant deviations from baseline during and after calcitriol administration. There was an increase in the serum concentration of calcitriol, but in contrast to our previous findings (13) serum calcium did not change.

Osteocalcin is the most abundant non-collagen organic constituent of bone, and is solely produced by osteoblasts (21). Serum osteocalcin mainly reflects newly synthesized protein (21,22) and correlates with bone formation at organ and tissue level (23). However, osteocalcin release to serum may be rapidly regulated by several factors (24,25) and such changes may occur independently of bone formation. Calcitriol stimulates the production of osteocalcin in vitro (4). The augmentation of serum osteocalcin under the present experimental conditions (Fig. 1) accords with previous observations in healthy control subjects (13,26) and osteoporotic postmenopausal women (26).

Recently, Parfitt et al. demonstrated a positive correlation between serum PICP and iliac bone formation rate as well as total alkaline phosphatase activity in normal subjects and patients with various metabolic bone diseases (27). However, since type 1 collagen is also prevalent in extraskeletal connective tissues, the use of serum PICP as a biochemical marker of bone metabolism should be adopted.
with caution. Yet, the covariation of serum osteocalcin and serum PICP in the present experimental setting supports the concept of circulating PICP as a marker of osteoblastic activity and bone collagen formation. The lack of significant correlation between increases in osteocalcin and PICP may be due to differences in rate of release to the bloodstream or in the kinetics of elimination. Osteocalcin is cleared from serum by the kidneys, whereas it has been demonstrated by Smedsrød et al. that the two species of procollagen peptides applied in the present study and hyaluronan are taken up by liver endothelial cells via receptor mediated endocytosis (28,29).

Although an effect of calcitriol on the PICP receptor expression or its binding could be considered as a cause of the serum PICP increment, this would appear an unlikely explanation in view of the concordant change in serum concentration of the structurally distinct osteocalcin molecule. In addition, the lack of changes in serum hyaluronan and PIINP point to a specific effect of calcitriol on the biosynthetic pathway of type I collagen.

In conclusion, we have shown that a short course of calcitriol administration to healthy adult male volunteers leads to selective rises in the serum levels of bone matrix-related constituents, whereas seromarkers of mainly extraskelletal connective tissue elements remain unchanged. The results imply that osteoblast activation by short-term calcitriol treatment is accompanied by increased bone collagen formation.

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References


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