

# BONE MASS, STRENGTH AND STIFFNESS ARE REDUCED IN HUMAN TIBIAL CANCELLOUS BONE TISSUE WITH HIGH TGF- $\beta$ 2 CONTENT

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## INTRODUCTION

Transforming growth factor  $\beta$  (TGF- $\beta$ ), abundant in bone matrix, is believed to be an important local regulator of osteoblast and osteoclast activity during bone growth and remodeling. Understanding how TGF- $\beta$  controls bone remodeling is important to determine the underlying causes of the universal age-related loss of bone mass that can cause osteoporosis, debilitating fracture and death. It has been demonstrated that the concentrations of TGF- $\beta$ 1 and TGF- $\beta$ 2 in bone matrix from different sites of the human skeleton are significantly correlated with the rate of bone turnover [1]. Furthermore, an important pair of studies on transgenic mice also supports the importance of TGF- $\beta$  in the control of remodeling and development of bone. Overexpression of TGF- $\beta$ 2 behind the osteocalcin promoter results in progressive bone loss associated with an increased osteoclastic bone resorption, which is similar to bone abnormalities seen in human osteoporosis [2]. Conversely, inhibiting the TGF- $\beta$ 2 receptor in osteoblasts leads to decreased bone remodeling and increased trabecular bone mass and strength [3]. Each of these studies supports the hypothesis that TGF- $\beta$  can be identified as a key player in the control of bone remodeling. In addition, there are data supporting the significance of TGF- $\beta$  in bone osteoporosis. The TGF- $\beta$ 1 concentration from the bone samples after menopause is significantly higher as compared with age-matched premenopausal women [1]. Certain polymorphisms of the TGF- $\beta$  gene also have a higher prevalence in osteoporotic patients compared to normal women [4, 5].

To our knowledge, however, no study has ever addressed the relationship between TGF- $\beta$  and mechanical properties of bone tissue. In this study, we are examining the hypothesis that TGF- $\beta$  is correlated with apparent strength and stiffness of cancellous bone.

## MATERIALS AND METHODS

Fourteen 8 mm diameter, 10 mm high cylindrical specimens of cancellous bone from two human tibiae were prepared using a diamond-coring tool in the metaphyseal portion of proximal tibiae and subsequently trimming to length using a slow speed diamond saw. The

specimens were scanned by 3D micro computed tomography ( $\mu$ CT) with a voxel size of 21 $\mu$ m and bone volume fraction (BV/TV) was calculated using previously developed techniques [6]. Bone marrow was removed using a water jet. The specimens were uniaxially compressed with a strain rate of 0.1%  $s^{-1}$  using a servo-hydraulic testing system (Model 8501, Instron Corp, Canton, MA). The specimens were glued to brass end caps to eliminate the effect of end artifacts on measured stiffness. The apparent strength and stiffness were determined from the stress-strain curve as the maximum stress supported by the sample and maximum slope of the linear region of the stress-strain curve, respectively [7].

After mechanical testing, cancellous bone cylinders were trimmed using a low speed diamond saw. Bone samples were ground with a mortar and pestle in liquid nitrogen. The bone powder was washed in cold distilled water and growth factors not strongly bound to the organic matrix were extracted in buffered EDTA containing proteinase inhibitors (0.05 M EDTA, 0.03 M Tris pH7.4 with proteinase inhibitors 2mM phenylmethylsulfonyl fluoride, 2  $\mu$ m/ml leupeptin). The organic matrix was then extracted in guanidine buffer (4M guanidine HCl, 0.05 M EDTA, 0.03 M Tris pH 7.4 with proteinase inhibitors). After dialysis against phosphate buffered saline, TGF- $\beta$ 2 was quantified using commercially available ELISA assays (R&D systems, Minneapolis, MN). All extraction procedures were conducted at 4°C. Duplicate extractions were performed from each bone sample, and the results of the TGF- $\beta$ 2 measurements were averaged.

Statistical analysis was performed using GraphPad (GraphPad Software, Inc., San Diego, CA). The correlations among apparent strength, apparent stiffness, bone volume fraction, and TGF- $\beta$ 2 concentration of cancellous bone were assessed by simple linear regression and Pearson's correlation.

## RESULTS

The bone volume fraction of cancellous bone specimens had a significantly ( $p < 0.05$ ) negative correlation with the concentration of

TGF- $\beta$ 2 in bone matrix (Figure 1), indicating that bone mass decreased with the increasing concentration of TGF- $\beta$ 2.

The concentration of TGF- $\beta$ 2 in bone matrix was significantly ( $p < 0.05$ ) negatively correlated with the apparent strength (Figure 2) and the apparent stiffness (Figure 3) of cancellous bone.

## DISCUSSION

The significant correlations between the concentration of TGF- $\beta$ 2 and mechanical properties of cancellous bone demonstrated that TGF- $\beta$ 2 is indeed an important factor in determining bone mass and bone strength.

Particularly noteworthy is the finding that bone mass and TGF- $\beta$ 2 content are negatively correlated in human cancellous bone (Figure 1). This is consistent with the previous work on transgenic mice that demonstrated higher concentration of TGF- $\beta$ 2 in bone matrix accompanied by progressive, age-dependent loss of bone mass due to overexpression of TGF- $\beta$ 2 behind the osteocalcin promoter [2]. Increasing bone mass with decreasing hard tissue TGF- $\beta$ 2 content is also consistent with increased cancellous bone mass due to inhibition of the TGF- $\beta$ 2 receptor in osteoblasts shown in a previous study [3].

It naturally follows from the negative relationship between BV/TV and TGF- $\beta$ 2 concentration that both the apparent strength and stiffness of cancellous bone are reduced in the tissue with greater hard tissue TGF- $\beta$ 2 content (Figures 2 and 3). However, demonstration of this association is significant in that this is the first quantitative evidence that physiologically relevant variations in hard tissue TGF- $\beta$ 2 concentration have a direct mechanical consequence on tissue performance. The relationship between the hard tissue TGF- $\beta$ 2 content and cancellous bone apparent properties found in this study may provide significant insight into molecular mechanisms that bridge cellular activities to the mechanical function of bone tissue.

## ACKNOWLEDGMENTS

This research was supported by grants AR40776 and AR44712 from National Institutes of Health.

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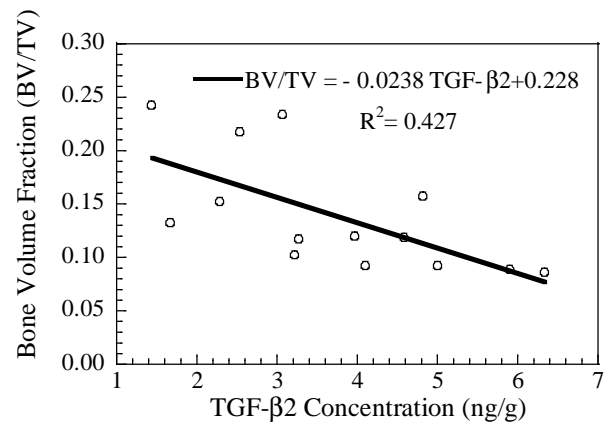


Figure 1. TGF- $\beta$ 2 concentration decreased significantly with bone volume fraction of cancellous bone ( $p=0.01$ ).

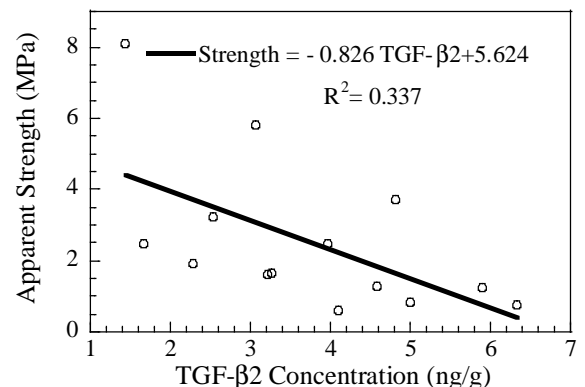


Figure 2. TGF- $\beta$ 2 concentration in bone matrix decreased significantly with apparent strength of cancellous bone ( $p=0.03$ ).

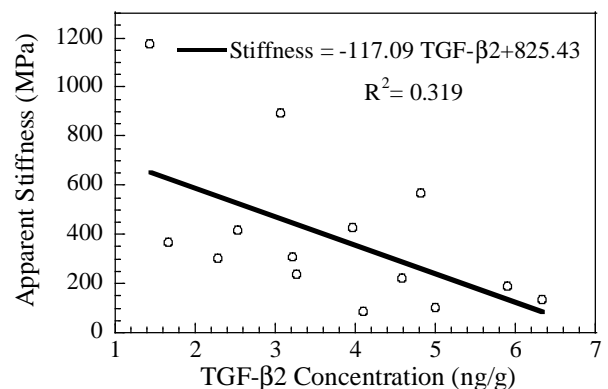


Figure 3. TGF- $\beta$ 2 concentration decreased significantly with apparent stiffness of cancellous bone ( $p=0.04$ ).