Title

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Journal

Clinical Orthopaedics and Related Research®, 467(12)

ISSN

1528-1132

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Publication Date

2009-12-01

DOI

10.1007/s11999-009-0896-5

Peer reviewed

Clin Orthop Relat Res (2009) 467:3079–3086
DOI 10.1007/s11999-009-0896-5

SYMPOSIUM: TRIBUTE TO DR. MARSHALL URIST: MUSCULOSKELETAL GROWTH FACTORS

Cancellous Bone Properties and Matrix Content of TGF-β2 and IGF-I in Human Tibia

A Pilot Study

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Published online: 27 May 2009

Abstract Transforming and insulin-like growth factors are important in regulating bone mass. Thus, one would anticipate correlations between matrix concentrations of growth factors and functional properties of bone. We therefore investigated the relationships of (1) TGF-β2 and (2) IGF-I matrix concentrations with the trabecular microstructure, stress distribution, and mechanical properties of tibial cancellous bone from six male human cadavers. Trabecular stress amplification ($VMExp/\sigma_{app}$) and variability (VMCOV) were calculated using microcomputed tomography (μCT)-based finite element simulations. Bone volume fraction (BV/TV), surface/volume ratio (BS/BV), trabecular thickness (Tb.Th), number (Tb.N) and separation (Tb.Sp), connectivity (Eu.N), and anisotropy (DA) were measured using 3-D morphometry. Bone stiffness and strength were measured by mechanical testing. Matrix concentrations of TGF-β2 and IGF-I were measured by ELISA. We found higher matrix concentrations of TGF-β2 were associated with higher Tb.Sp and $VMExp/\sigma_{app}$ for pooled data and within subjects. Similarly, a higher matrix concentration of IGF-I was associated with lower stiffness, strength, BV/TV and Tb.Th and with higher BS/BV, Tb.Sp, $VMExp/\sigma_{app}$ and VMCOV for pooled data and within subjects. IGF-I and Tb.N were negatively associated within subjects. It appears variations of the stress distribution in cancellous bone correlate with the variation of the concentrations of TGF-β2 and IGF-I in bone matrix: increased local matrix concentrations of growth factors are associated with poor biomechanical and architectural properties of tibial cancellous bone.

One or more of the authors has received funding from grants from the National Institutes of Health (YNY, AR049343; GJG, AR 44712; DPF, AR40776).
This work was performed at Department of Orthopaedics and Rehabilitation, Henry Ford Hospital, Detroit, MI.

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Introduction

Transforming growth factor β1 (TGF-β1), TGF-β2 and insulin-like growth factors (IGF-I and IGF-II) are believed important local regulators of osteoblast and osteoclast activity [2, 12]. These growth factors can be synthesized and stored in bone matrix during bone formation, released during bone resorption and affect bone remodeling [21, 44]. There is substantive evidence that TGF-βs and IGFs, specifically TGF-β2 and IGF-I, affect osteoblastic cell proliferation, differentiation, and survival [5, 19, 37, 60, 62]. Insulin-like growth factor-I also regulates bone resorption by enhancing osteoclast activity [24, 31, 44]. TGF-β can affect osteoclast differentiation and survival and, depending on the dose, can enhance or reduce

resorption [11, 27, 33]. Increased or decreased mechanical loading also affect the IGF and TGF-β expression in osteocytes [39, 40, 51, 52, 70].

The importance of TGF-βs and IGF-I in the control of bone growth and remodeling is further established by rodent models. In genetically modified mice, enhanced bone mass and enhanced stiffness, strength and mineral concentration of the cortical bone matrix are generally associated with reduced TGF-β signaling [3, 20, 23]. Cancellous bone volume fraction is higher in the IGF-I deficient mice than in the wild type [4]. On the other hand, over-expression of IGF-I in the osteoblasts of transgenic mice also causes an increase in the cancellous bone volume fraction in transgenic mice [45]. While subcutaneous administration of IGF-I to adult rats can reduce trabecular bone formation [61], local administration of IGF-I to old rats can increase the trabecular bone volume [63].

Studies with human bone tissue indicate that bone matrix concentrations of TGF-β1, TGF-β2, and IGF-I are related to aging, metabolic bone disease, and fracture risk but the relationships may be gender and skeletal site dependent [1, 7, 29, 38, 46, 49, 50, 54, 57, 64]. A few studies have examined the relationships of local matrix TGF-β1, TGF-β2, and IGF-I with bone mass and mechanical properties in human bone, however, the results have been conflicting [1, 46, 56, 57]. For example, despite strong demonstrations in animal experiments, an association between bone volume fraction and the matrix concentrations of TGF- β1 or TGF-β2 was not found in the human iliac crest, femoral shaft, or lumbar spine [6, 50]. The matrix concentration of IGF-I is correlated with bone density in the iliac crest and lumbar spine [49, 57], but not in the proximal femoral shaft, femoral neck, or Ward's triangle [49, 56]. Apparent density and stiffness differences between the superior and inferior regions of the human femoral head are also not accompanied by differences in the matrix concentrations of IGF-I or IGF-II [58].

Our previous work demonstrated the matrix concentration of IGF-I in cancellous bone is negatively correlated with the bone volume fraction, strength, and stiffness of cancellous bone from proximal tibiae of human male cadavers within subjects [18]. The current objectives were (1) to examine the relationship of TGF-β2 with the mechanical, microarchitectural, and stress distribution properties and (2) to examine the relationship of IGF-I matrix levels with the microarchitectural and stress distribution properties of tibial cancellous bone.

Materials and Methods

Right tibiae from six male human cadavers that were free of bone and joint disease (average age, 48 ± 14 years; range, 26–63 years) were utilized. A total of 45 cylindrical specimens of cancellous bone were prepared. These are the same specimens used in our previous study [18]; the details of specimen processing have been previously described. Briefly, bone slabs were sectioned from the proximal tibiae such that the subchondral bone plate was completely removed at the center of the condyles during the first cut. The second cut was made 35 mm distal to the first cut. From the 35-mm-thick slab of cancellous bone, cancellous bone cores (diameter, 8 mm) were cut out using a diamond-tipped coring tool (Starlite, Rosemont, PA). Starting 6 mm distal to the proximal end of the core, each core was trimmed to a 10-mm-long cylinder. Because we were interested in within-individual variations rather than an anatomic site effect, as many cores as possible were cut out from each slab to represent the entire section but their exact location in the transverse plane was not recorded. All specimens were then scanned by a 3-D microcomputed tomography (μCT) system with a resolution of 21 μm using previously developed techniques [26, 53].

μCT images were used to construct finite element (FE) models by directly converting image voxels representing bone tissue to eight-node hexahedral finite elements using a special purpose program [26, 32]. The trabecular bone tissue in the model was assumed isotropic and uniform with a Young's modulus of 5 GPa and a Poisson's ratio of 0.3. Prescribed displacements equivalent to 0.5% strain were applied in the longitudinal direction through sliding interfaces on the top and bottom surfaces of the cylinder with all other surfaces unconstrained. The apparent uniaxial stress (σ_{app}) was calculated by summing nodal reaction forces and dividing by the apparent cross-sectional area of specimens. The mean (VMExp) and standard deviation (VMSD) of trabecular von Mises stress distribution were calculated from a three-parameter Weibull cumulative probability function fitted to the stress distribution for each specimen [25, 65–67]. The variability of trabecular shear stress was expressed as the coefficient of variation: VMCOV = VMSD/VMExp. The magnitude of trabecular shear stress was expressed as the average trabecular shear stress per apparent superoinferior uniaxial stress (VMExp/σ_{app}). Both VMExp/σ_{app} and VMCOV are considered structural indices of shear stress concentration in the hard tissue [25, 66, 68, 69].

A custom-written program was used to compute architectural parameters from μCT images [22, 28, 36]. Bone volume fraction (BV/TV), bone surface-to-volume ratio (BS/BV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were calculated using 3-D stereology principles. Connectivity (Eu.N) of the trabecular network was calculated using the topological approach based on the Euler-Poincare number [47]. The degree of anisotropy (DA) was calculated as the maximum to minimum mean intercept length ratio.

Measurement of apparent modulus and ultimate strength of cylindrical cancellous bone samples under uniaxial compression was reported in the previous study [18]. These modulus and strength data were used to correlate mechanical properties with matrix concentrations of growth factors in our study.

Growth factor extraction methods were as described in our previous study [18]. Cancellous bone cylinders were ground into small particles using a biopulverizer (BioSpec Products, Inc., Bartlesville, OK). The particles were placed within dialysis units (Slide-A-Lyzer®, MW cutoff = 3500, Pierce, Rockford, IL) and growth factors extracted by dialysis against 4 M guanidine hydrochloride, 30 mM Tris (pH 7.4), 0.05 M EDTA, and a mixture of protease inhibitors at 4°C for 48 hours. Bone residue and extract were separated by centrifugation at 10,000 rpm for 5 minutes. The extracts were redialyzed against phosphate buffer saline solution for 72 hours and then stored at −20°C until subsequent assay.

TGF-β2 generally exists as a latent form in bone matrix, requiring activation before it can exert biological activities [2]. In order to activate latent TGF-β2, the supernatant extracted from bone samples was acidified by addition of 25 μl of 1 M HCl to 125 μl extract sample and neutralized with 25 μl 1.2 M NaOH/0.5 M HEPES. Meanwhile, in order to avoid the IGF-binding protein artifacts, the supernatant extracted from bone samples was pretreated (10 min) to release IGF-I from binding proteins by the use of an acidic buffer (pretreatment reagent, composition proprietary, IGF1 ELISA assay R&D Systems, Minneapolis, MN).

The concentrations of TGF-β2 and IGF-I in bone matrix were determined by sandwich enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions. Duplicate assays were performed for each extract, and the results were averaged. The sensitivity of TGF-β and IGF-I assays were determined to be 0.75 ng/g and 2.79 ng/g, respectively. The matrix concentrations of TGF-β2 and IGF-I were expressed as growth factor concentration per dry weight of the bone powder. Initial studies demonstrated that TGF-β1, TGF-β2, and IGF-I could be efficiently isolated from powdered human bone using two 24 hours extractions in guanidine (4 M) EDTA (0.05 M), 0.03 M Tris buffer. Growth factor concentrations were within the range of those previously reported. (TGF-β1: From 250 ± 0.34 ng/g for lumbar spine to 710 ± 400 ng/g for femoral head; TGF-β2: 9.29 ± 4.72 ng/g for lumbar spine to 14.48 ± 4.63 for femoral shaft; IGF-I: 80-870 ng/g for iliac crest to ~300–1000 ng/g for femur) [50, 56, 57]. The concentrations of TGF-β2 and IGF-I in the series of bone samples in the present study was within the range of initial analyses, however; TGF-β1 concentrations were considerably lower than those obtained in our initial analyses and lower than the previously published range. As we have no reasonable explanation for this discrepancy, the TGF-β1 data have not been included in the present analysis.

Correlation analysis was used to test the presence of relationships between matrix growth factor densities and other cancellous bone properties. Regression analysis was performed to examine the relationships. The relationships between growth factors and parameters within a subject were examined using mixed models in which each subject was treated as a random effect. JMP (SAS Institute, Cary, NC) was used for the analyses. Because there was no more than one subject at a given age, the mixed models that used donor as a variable would automatically address age variations in the data. Therefore, results were not adjusted for age.

An adjustment for p values was calculated for multiple tests, taking into account the correlations between multiple factors [55]. This calculation suggested a p value of 0.012 corresponding to $\alpha = 0.05$.

Results

Matrix concentration of TGF-β2 was positively associated with Tb.Sp and VMExp/σ_{app} for pooled data (Table 1; Fig. 1) and within subjects (Table 2; Figs. 2, 3).

Matrix concentration of IGF-I was associated with lower stiffness, strength, BV/TV, and Tb.Th and with higher BS/BV, Tb.Sp, VMExp/$\sigma_{app,}$ and VMCOV for pooled data (Table 1; Figs. 1, 4, 5) and within subjects (Table 2; Figs. 6, 7). IGF-I and Tb.N were negatively associated within subjects (Table 2).

Discussion

Biomechanical properties of bone tissue are influenced by bone remodeling, in which growth factors play an important role. Our objectives were to investigate the relationships of matrix concentrations of (1) TGF-β2 and (2) IGF-I with biomechanical, microarchitectural, and trabecular stress distribution properties of cancellous bone in the proximal tibiae of men.

We note several limitations. First, the proximal tibia is not a site of common osteoporotic fractures. However, it does experience cancellous bone density and architectural changes with age [16, 17], ligament injury [35, 41] knee replacement [42] and osteoarthritis [34, 71]. Therefore, we believe studies of the proximal tibial bone are relevant to the understanding of degenerative diseases and the design, fixation and durability of total joint prostheses of the knee. Second, our study is correlational and although the results support a relationship between growth factor signaling and mechanical property regulation, causation cannot be

Table 1. Degree of freedom-adjusted coefficients of determination (r^2_{adj}) and p values associated with the effect of TGF-β2 and IGF-I on mechanical and architectural properties of cancellous bone in simple linear regression

Response ↓ Effect →	TGF-β2	IGF-1
E_{app}	$r^2_{adj} = -0.010$; p = 0.457 (−)	$r^2_{adj} = 0.197$; p = 0.001 (−)
σ_u	$r^2_{adj} = 0.005$; p = 0.273 (−)	$r^2_{adj} = 0.222$; p = 0.000 (−)
VMCOV	$r^2_{adj} = 0.050$; p = 0.074 (+)	$r^2_{adj} = 0.278$; p = 0.000 (+)
$VMExp/\sigma_{app}$	$r^2_{adj} = 0.088$; p = 0.027 (+)	$r^2_{adj} = 0.222$; p = 0.000 (+)
BV/TV	$r^2_{adj} = 0.035$; p = 0.115 (−)	$r^2_{adj} = 0.226$; p = 0.000 (−)
BS/BV	$r^2_{adj} = 0.040$; p = 0.100 (+)	$r^2_{adj} = 0.239$; p = 0.000 (+)
Tb.Th	$r^2_{adj} = 0.032$; p = 0.125 (−)	$r^2_{adj} = 0.205$; p = 0.001 (−)
Tb.N	$r^2_{adj} = 0.045$; p = 0.086 (−)	$r^2_{adj} = 0.185$; p = 0.001 (−)
Tb.Sp	$r^2_{adj} = 0.105$; p = 0.016 (+)	$r^2_{adj} = 0.213$; p = 0.000 (+)
Eu.N	$r^2_{adj} = -0.023$*; p = 0.871 (−)	$r^2_{adj} = -0.023$*; p = 0.889 (−)
DA	$r^2_{adj} = 0.020$; p = 0.176 (−)	$r^2_{adj} = 0.051$; p = 0.073 (−)

* A negative r^2_{adj} means the assumption of a linear relationship is worse than the assumption of a constant. The sign in parentheses show whether the relationship is negative or positive.

Table 2. Degree of freedom-adjusted coefficients of determination (r^2_{adj}) and p values associated with the effect of TGF-β2 and IGF-I on mechanical and architectural properties of cancellous bone in mixed models

Response ↓ Effect →	TGF-β2	IGF-1
E_{app}	$r^2_{adj} = 0.300$; p = 0.496 (−)	$r^2_{adj} = 0.376$; p = 0.015 (−)
σ_u	$r^2_{adj} = 0.380$; p = 0.184 (−)	$r^2_{adj} = 0.460$; p = 0.006 (−)
VMCOV	$r^2_{adj} = 0.290$; p = 0.156 (+)	$r^2_{adj} = 0.425$; p = 0.003 (+)
$VMExp/\sigma_{app}$	$r^2_{adj} = 0.484$; p = 0.011 (+)	$r^2_{adj} = 0.561$; p = 0.000 (+)
BV/TV	$r^2_{adj} = 0.578$; p = 0.110 (−)	$r^2_{adj} = 0.599$; p = 0.021 (−)
BS/BV	$r^2_{adj} = 0.290$; p = 0.098 (+)	$r^2_{adj} = 0.469$; p = 0.000 (+)
Tb.Th	$r^2_{adj} = 0.288$; p = 0.112 (−)	$r^2_{adj} = 0.442$; p = 0.002 (−)
Tb.N	$r^2_{adj} = 0.636$; p = 0.088 (−)	$r^2_{adj} = 0.618$; p = 0.204 (−)
Tb.Sp	$r^2_{adj} = 0.609$; p = 0.011 (+)	$r^2_{adj} = 0.580$; p = 0.039 (+)
Eu.N	$r^2_{adj} = 0.421$; p = 0.419 (−)	$r^2_{adj} = 0.427$; p = 0.416 (+)
DA	$r^2_{adj} = -0.367$*; p = 0.050 (−)	$r^2_{adj} = -0.194$*; p = 0.278 (−)

* A negative r^2_{adj} means the assumption of a linear relationship is worse than the assumption of a constant. The sign in parentheses show whether the relationship is negative or positive.

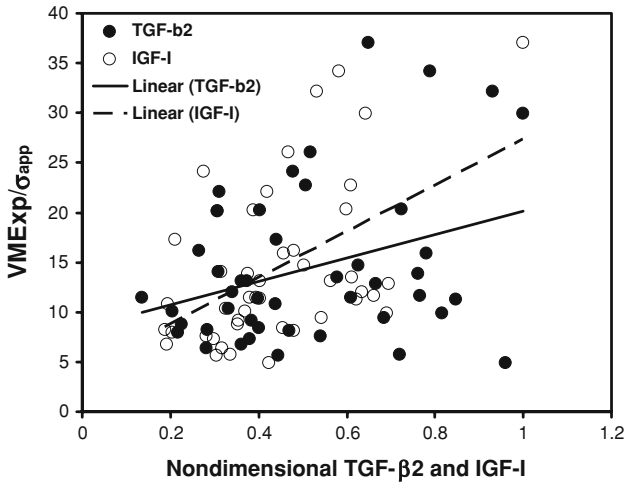

Fig. 1 Magnitude of trabecular shear stresses ($VMExp/\sigma_{app}$) increases with increasing matrix concentrations of TGF-β2 and IGF-I in the same bone. The growth factor values are normalized using the maximum of each type of measurement for ease of comparison.

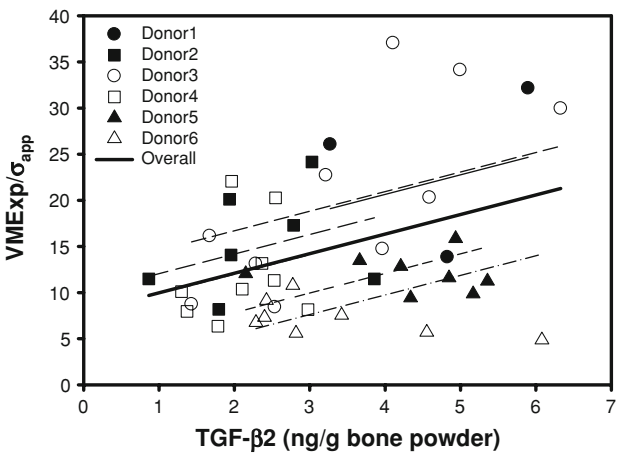

Fig. 2 Mixed model fit to $VMExp/\sigma_{app}$ indicated a positive linear trend with TGF-β2 within an individual tibia.

established from the current data. Third is the use of a constant tissue modulus in finite element analyses of cancellous bone. The stress distribution properties calculated from FE models are expected to be different between homogenous and variable-modulus models [8] but not to the extent that our conclusions would be affected. Finally, the architectural parameters examined in this study are based on stereological principles. Stereology-based

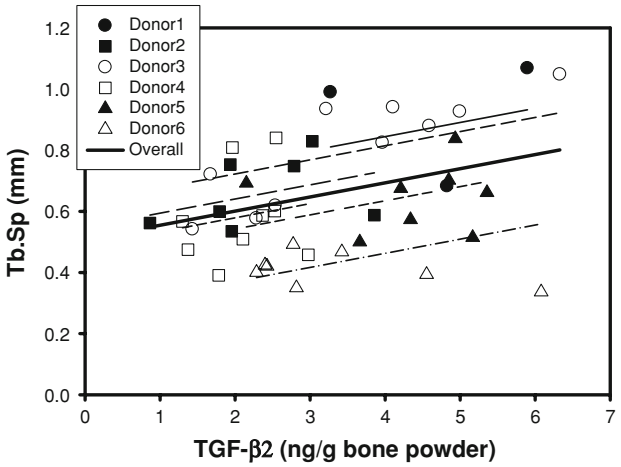

Fig. 3 Mixed model fit to trabecular separation (Tb.Sp) indicated a positive linear trend with TGF-β2 within an individual tibia.

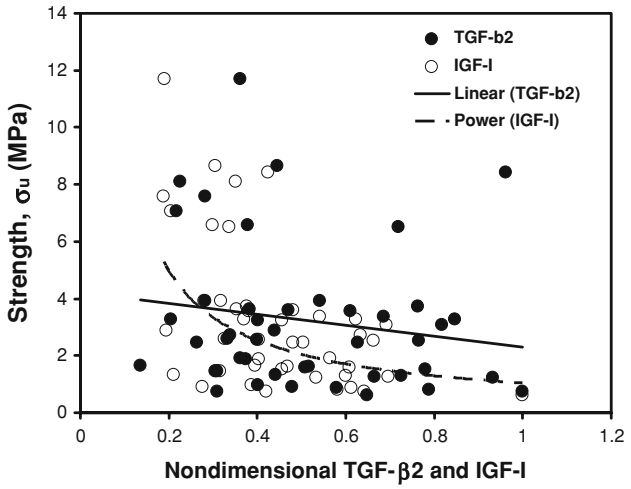

Fig. 4 Apparent strength of cancellous bone decreases with increasing matrix concentrations of TGF-β2 (NS) and IGF-I in the same bone. The growth factor values are normalized using the maximum of each type of measurement for ease of comparison. Because the linear fit to the IGF-I data passes through $\sigma_u = 0$ within the range of measured IGF-I values, it was deemed inadequate. A power-fit, although equally explanatory ($r^2_{adj} = 0.238$, $p < 0.001$) as the linear model ($r^2_{adj} = 0.222$, $p < 0.001$), is presented as a simple function to illustrate the nonlinear nature of the relationship.

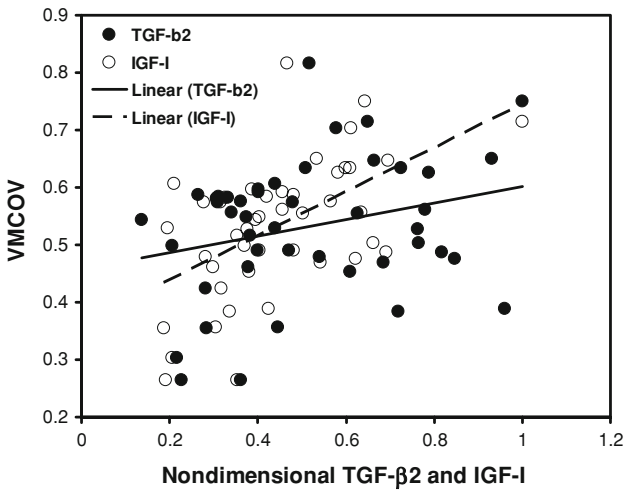

Fig. 5 Coefficient of variation of trabecular shear stresses (VMCOV) increases with increasing matrix concentrations of TGF-β2 (NS) and IGF-I in the same bone. The growth factor values are normalized using the maximum of each type of measurement for ease of comparison.

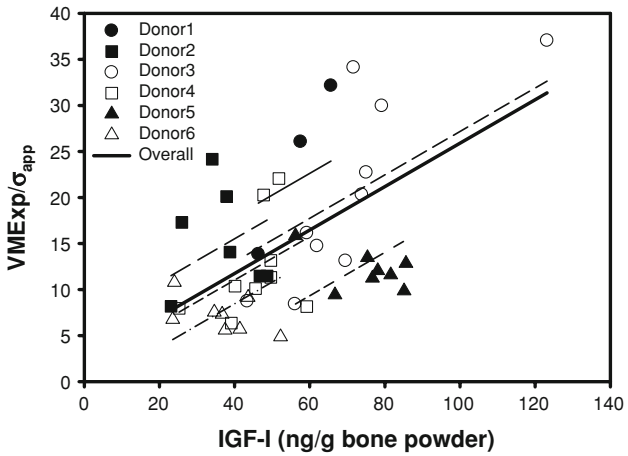

Fig. 6 Mixed model fit to $VMExp/\sigma_{app}$ indicated a positive linear trend with IGF-I within an individual tibia.

calculation of microstructural parameters (other than BV/TV, Eu.N and DA) results in values that are different from those obtained by direct calculation [15, 30] but microstructural parameters calculated using one method are highly correlated to those calculated using the other method [15].

We did not find a relationship between TGF-β2 and cancellous bone strength or stiffness. This is similar to results from other human bone studies that failed to find an association between bone volume and matrix TGF-β1 or TGF-β2 [6, 50]. However, it is in contrast with results from a genetically modified mouse study where reduced TGF-β signaling was associated with enhanced cortical bone strength and stiffness [3].

We did find an increase in Tb.Sp and $VMExp/\sigma_{app}$ with increasing matrix levels of TGF-β2. These results are consistent with TGF-β overexpression and inhibition studies in animals where increased TGF-β expression is associated with lower bone mass [20, 23]. The effect of the microstructure is separated from that of matrix properties in our stereology and FE analyses. Based on the presence of a relationship of TGF-β with microstructural properties but not with stiffness and strength, further work focusing on the relationship between growth factor concentrations

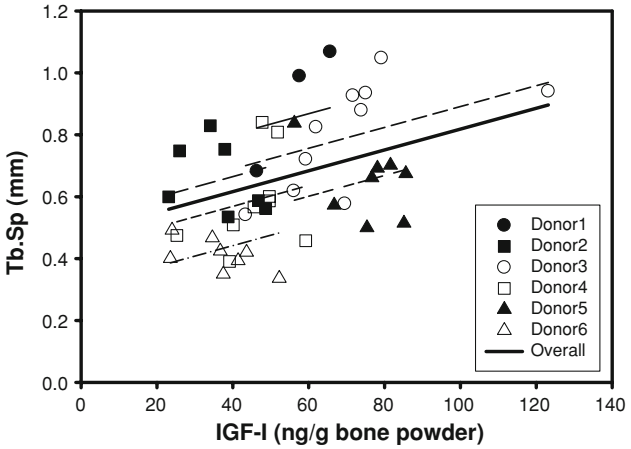

Fig. 7 Mixed model fit to trabecular separation (Tb.Sp) indicated a positive linear trend with IGF-I within an individual tibia.

and matrix mechanical properties in human bone may be warranted [3].

Decreasing cancellous bone strength and stiffness with increasing matrix concentration of IGF-I has been reported previously [18]. The increasing VMExp/σ_{app}, VMCOV, BS/BV and Tb.Sp as well as decreasing BV/TV, Tb.Th and Tb.N with increasing IGF-I is consistent with the strength and stiffness results from the same bones and bone volume results from IGF-I-deficient mice [4]. Negative relationship between architectural parameters and IGF-I matrix concentration within individual subjects is in contrast with a previous study in which a positive relationship between skeletal IGF-I concentration and bone volume fraction of cancellous bone from iliac biopsies was observed [57]. This inconsistency may be due to donor differences as the tissue in the Seck study was obtained from female donors with breast cancer. The difference between the results may also indicate that the effect of local IGF-I on bone is site-specific; site specificity can be between different bones and between different regions of the same bone. The IGF regulatory system is relatively complex and other components of this system such as insulin-like growth factor binding proteins (IGFBPs) may be involved in modulating the action of IGF-I on remodeling. The production of IGFBPs in human bone cells under similar conditions is different between cells from different skeletal sites [43] that may explain, in part, the site-specific relationship of IGF-I with bone mass.

Increasing levels of BV/TV, modulus and strength with decreasing levels of growth factors in our study may be associated with an adaptive response of bone to increasing mechanical demands in these bone regions. This idea is consistent with the finding that matrix deposition of both IGF-I and TGF-β1 decreases as the result of increased mechanical loading in rats exercised on a treadmill [9]. Experiments with rat osteoblast cultures indicate that proliferation associated with estrogen and testosterone is mediated by IGF-I whereas proliferation associated with mechanical strain is not [10, 13, 14]. On the other hand, increased mechanical loading causes a strong expression of IGF-I mRNA in the osteocyte as reported in rat caudal vertebrae and cortical bone in vivo mechanical loading experiments [39, 40, 51, 52]. TGF-β increases IGF-I expression in human osteoblast cells [48]. The response of TGF-β to mechanical stimulation depends on the TGF-β isoform, nature of the mechanical perturbation, cell type considered, and the anatomic site [59, 70] but methodologic differences such as mRNA expression versus matrix concentration of the protein and in vitro versus in vivo model systems may amplify these dependencies. Overall, these data suggest that mechanical strain-related proliferation of osteoblasts may not be directly mediated by IGF-I but could be affected by IGF-I in a strain-dependent manner through its interactions with TGF-β and estrogen. The greater effect of IGF-I on bone mass, architecture, and mechanical properties than TGF-β2 may be explained by IGF-I having both systemic and local roles in bone metabolism and TGF-β being a more local regulator of bone remodeling.

Our data suggest the variation of biomechanical, microarchitectural, and trabecular stress distribution properties of cancellous bone in human tibiae is correlated with the variation of the concentrations of TGF-β2 and IGF-I in bone matrix. Increased local matrix concentrations of growth factors are associated with poor biomechanical and architectural properties of tibial cancellous bone.

References

1. Aerssens J, Boonen S, Joly J, Dequeker J. Variations in trabecular bone composition with anatomical site and age: potential implications for bone quality assessment. *J Endocrinol.* 1997;155:411–421.
2. Alliston T, Derynck R. Transforming growth factor beta in skeletal development and maintenance. In: Canalis E, ed. *Skeletal Growth Factors*. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:233–249.
3. Balooch G, Balooch M, Nalla RK, Schilling S, Filvaroff EH, Marshall GW, Marshall SJ, Ritchie RO, Derynck R, Alliston T. TGF-beta regulates the mechanical properties and composition of bone matrix. *Proc Natl Acad Sci USA.* 2005;102:18813–18818.
4. Bikle D, Majumdar S, Laib A, Powell-Braxton L, Rosen C, Beamer W, Nauman E, Leary C, Halloran B. The skeletal structure of insulin-like growth factor I-deficient mice. *J Bone Miner Res.* 2001;16:2320–2329.

5. Bikle DD. Integrins, insulin like growth factors, and the skeletal response to load. *Osteoporos Int.* 2008;19:1237–1246.
6. Bismar H, Kloppinger T, Schuster EM, Balbach S, Diel I, Ziegler R, Pfeilschifter J. Transforming growth factor beta (TGF-beta) levels in the conditioned media of human bone cells: relationship to donor age, bone volume, and concentration of TGF-beta in human bone matrix in vivo. *Bone.* 1999;24:565–569.
7. Boonen S, Aerssens J, Dequeker J, Nicholson P, Cheng X, Lowet G, Verbeke G, Bouillon R. Age-associated decline in human femoral neck cortical and trabecular content of insulin-like growth factor I: Potential implications for age-related (type II) osteoporotic fracture occurrence. *Calcif Tissue Int.* 1997;61:173–178.
8. Bourne BC, van der Meulen MC. Finite element models predict cancellous apparent modulus when tissue modulus is scaled from specimen CT-attenuation. *J Biomech.* 2004;37:613–621.
9. Bravenboer N, Engelbregt MJ, Visser NA, Popp-Snijders C, Lips P. The effect of exercise on systemic and bone concentrations of growth factors in rats. *J Orthop Res.* 2001;19:945–949.
10. Cheng M, Zaman G, Rawlinson SC, Mohan S, Baylink DJ, Lanyon LE. Mechanical strain stimulates ROS cell proliferation through IGF-II and estrogen through IGF-I. *J Bone Miner Res.* 1999;14:1742–1750.
11. Chenu C, Pfeilschifter J, Mundy GR, Roodman GD. Transforming growth factor beta inhibits formation of osteoclast-like cells in long-term human marrow cultures. *Proc Natl Acad Sci USA.* 1988;85:5683–5687.
12. Conover CA. Insulin-like growth factors and the skeleton. In: Canalis E, ed. *Skeletal Growth Factors*. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:101–116.
13. Damien E, Price JS, Lanyon LE. The estrogen receptor's involvement in osteoblasts' adaptive response to mechanical strain. *J Bone Miner Res.* 1998;13:1275–1282.
14. Damien E, Price JS, Lanyon LE. Mechanical strain stimulates osteoblast proliferation through the estrogen receptor in males as well as females. *J Bone Miner Res.* 2000;15:2169–2177.
15. Day JS, Ding M, Odgaard A, Sumner DR, Hvid I, Weinans H. Parallel plate model for trabecular bone exhibits volume fraction-dependent bias. *Bone.* 2000;27:715–720.
16. Ding M. Age variations in the properties of human tibial trabecular bone and cartilage. *Acta Orthop Scand Suppl.* 2000;292:1–45.
17. Ding M, Hvid I. Quantification of age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone. *Bone.* 2000;26:291–295.
18. Dong XN, Yeni YN, Zhang B, Les CM, Gibson GJ, Fyhrie DP. Matrix concentration of insulin-like growth factor I (IGF-I) is negatively associated with biomechanical properties of human tibial cancellous bone within individual subjects. *Calcif Tissue Int.* 2005;77:37–44.
19. Dufour C, Holy X, Marie PJ. Transforming growth factor-beta prevents osteoblast apoptosis induced by skeletal unloading via PI3 K/Akt, Bcl-2, and phospho-Bad signaling. *Am J Physiol Endocrinol Metab.* 2008;294:E794–E801.
20. Erlebacher A, Derynck R. Increased expression of TGF-beta 2 in osteoblasts results in an osteoporosis-like phenotype. *J Cell Biol.* 1996;132:195–210.
21. Farley JR, Tarbaux N, Murphy LA, Masuda T, Baylink DJ. In vitro evidence that bone formation may be coupled to resorption by release of mitogen(s) from resorbing bone. *Metabolism.* 1987;36:314–321.
22. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M. The direct examination of three-dimensional bone architecture in vitro by computed tomography. *J Bone Miner Res.* 1989;4:3–11.
23. Filvaroff E, Erlebacher A, Ye J, Gitelman SE, Lotz J, Heillman M, Derynck R. Inhibition of TGF-beta receptor signaling in osteoblasts leads to decreased bone remodeling and increased trabecular bone mass. *Development.* 1999;126:4267–4279.
24. Fiorelli G, Formigli L, Zecchi Orlandini S, Gori F, Falchetti A, Morelli A, Tanini A, Benvenuti S, Brandi ML. Characterization and function of the receptor for IGF-I in human preosteoclastic cells. *Bone.* 1996;18:269–276.
25. Fyhrie DP, Hoshaw SJ, Hamid MS, Hou FJ. Shear stress distribution in the trabeculae of human vertebral bone. *Ann Biomed Eng.* 2000;28:1194–1199.
26. Fyhrie DP, Lang SM, Hoshaw SJ, Schaffler MB, Kuo RF. Human vertebral cancellous bone surface distribution. *Bone.* 1995;17:287–291.
27. Gingery A, Bradley EW, Pederson L, Ruan M, Horwood NJ, Oursler MJ. TGF-beta coordinately activates TAK1/MEK/AKT/NFkB and SMAD pathways to promote osteoclast survival. *Exp Cell Res.* 2008;314:2725–2738.
28. Goulet RW, Goldstein SA, Ciarelli MJ, Kuhn JL, Brown MB, Feldkamp LA. The relationship between the structural and orthogonal compressive properties of trabecular bone. *Journal of Biomechanics.* 1994;27:375–389.
29. Hering S, Isken E, Knabbe C, Janott J, Jost C, Pommer A, Muhr G, Schatz H, Pfeiffer AF. TGFbeta1 and TGFbeta2 mRNA and protein expression in human bone samples. *Exp Clin Endocrinol Diabetes.* 2001;109:217–226.
30. Hildebrand T, Laib A, Muller R, Dequeker J, Ruegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res.* 1999;14:1167–1174.
31. Hill PA, Reynolds JJ, Meikle MC. Osteoblasts mediate insulin-like growth factor-I and -II stimulation of osteoclast formation and function. *Endocrinology.* 1995;136:124–131.
32. Hou FJ, Lang SM, Hoshaw SJ, Reimann DA, Fyhrie DP. Human vertebral body apparent and hard tissue stiffness. *J Biomech.* 1998;31:1009–1015.
33. Janssens K, ten Dijke P, Janssens S, Van Hul W. Transforming growth factor-beta1 to the bone. *Endocr Rev.* 2005;26:743–774.
34. Kamibayashi L, Wyss UP, Cooke TD, Zee B. Changes in mean trabecular orientation in the medial condyle of the proximal tibia in osteoarthritis. *Calcif Tissue Int.* 1995;57:69–73.
35. Kannus P, Sievanen H, Jarvinen M, Heinonen A, Oja P, Vuori I. A cruciate ligament injury produces considerable, permanent osteoporosis in the affected knee. *J Bone Miner Res.* 1992;7:1429–1434.
36. Kim DG, Christopherson GT, Dong XN, Fyhrie DP, Yeni YN. The effect of microcomputed tomography scanning and reconstruction voxel size on the accuracy of stereological measurements in human cancellous bone. *Bone.* 2004;35:1375–1382.
37. Langdahl BL, Kassem M, Moller MK, Eriksen EF. The effects of IGF-I and IGF-II on proliferation and differentiation of human osteoblasts and interactions with growth hormone. *Eur J Clin Invest.* 1998;28:176–183.
38. Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF. A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. *Bone.* 1997;20:289–294.
39. Lean JM, Jagger CJ, Chambers TJ, Chow JW. Increased insulin-like growth-factor-I messenger-RNA expression in rat osteocytes in response to mechanical stimulation. *Am J Physiol.* 1995;268(2 Pt 1):E318–E327.
40. Lean JM, Mackay AG, Chow JW, Chambers TJ. Osteocytic expression of mRNA for c-fos and IGF-I: an immediate early gene response to an osteogenic stimulus. *Am J Physiol.* 1996;270(6 Pt 1):E937–E945.

41. Leppala J, Kannus P, Natri A, Pasanen M, Sievanen H, Vuori I, Jarvinen M. Effect of anterior cruciate ligament injury of the knee on bone mineral density of the spine and affected lower extremity: a prospective one-year follow-Up study. *Calcif Tissue Int.* 1999;64:357–363.
42. Levitz CL, Lotke PA, Karp JS. Long-term changes in bone mineral density following total knee replacement. *Clin Orthop Relat Res.* 1995;321:68–72.
43. Malpe R, Baylink DJ, Linkhart TA, Wergedal JE, Mohan S. Insulin-like growth factor (IGF)-I, -II, IGF binding proteins (IGFBP)-3, -4, and -5 levels in the conditioned media of normal human bone cells are skeletal site-dependent. *J Bone Miner Res.* 1997;12:423–430.
44. Mochizuki H, Hakeda Y, Wakatsuki N, Usui N, Akashi S, Sato T, Tanaka K, Kumegawa M. Insulin-like growth factor-I supports formation and activation of osteoclasts. *Endocrinology.* 1992;131:1075–1080.
45. Nakasaki M, Yoshioka K, Miyamoto Y, Sasaki T, Yoshikawa H, Itoh K. IGF-I secreted by osteoblasts acts as a potent chemotactic factor for osteoblasts. *Bone.* 2008;43:869–879.
46. Nicolas V, Prewett A, Bettica P, Mohan S, Finkelman RD, Baylink DJ, Farley JR. Age-related decreases in insulin-like growth-factor-I and transforming growth-factor-beta in femoral cortical bone from both men and women-Implications for bone loss with aging. *J Clin Endocrinol Metab.* 1994;78:1011–1016.
47. Odgaard A, Gundersen HJ. Quantification of connectivity in cancellous bone, with special emphasis on 3-D reconstructions. *Bone.* 1993;14:173–182.
48. Okazaki R, Durham SK, Riggs BL, Conover CA. Transforming growth-factor-beta and forskolin increase all classes of insulin-like growth-factor-I transcripts in normal human osteoblast-like cells. *Biochem Biophys Res Commun.* 1995;207:963–970.
49. Pepene CE, Seck T, Diel I, Minne HW, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I in iliac crest bone matrix in premenopausal women with idiopathic osteoporosis. *Exp Clin Endocrinol Diabetes.* 2004;112:38–43.
50. Pfeilschifter J, Diel I, Scheppach B, Bretz A, Krempien R, Erdmann J, Schmid G, Reske N, Bismar H, Seck T, Krempien B, Ziegler R. Concentration of transforming growth factor beta in human bone tissue: relationship to age, menopause, bone turnover, and bone volume. *J Bone Miner Res.* 1998;13:716–730.
51. Reijnders CM, Bravenboer N, Holzmann PJ, Bhoelan F, Blankenstein MA, Lips P. In vivo mechanical loading modulates insulin-like growth factor binding protein-2 gene expression in rat osteocytes. *Calcif Tissue Int.* 2007;80:137–143.
52. Reijnders CM, Bravenboer N, Tromp AM, Blankenstein MA, Lips P. Effect of mechanical loading on insulin-like growth factor-I gene expression in rat tibia. *J Endocrinol.* 2007;192:131–140.
53. Reimann DA, Hames SM, Flynn MJ, Fyhrie DP. A cone beam computed tomography system for true 3D imaging of specimens. *Appl Radiat Isot.* 1997;48:1433–1436.
54. Rivadeneira F, Houwing-Duistermaat JJ, Beck TJ, Janssen JA, Hofman A, Pols HA, Van Duijn CM, Uitterlinden AG. The influence of an insulin-like growth factor I gene promoter polymorphism on hip bone geometry and the risk of nonvertebral fracture in the elderly: the Rotterdam Study. *J Bone Miner Res.* 2004;19:1280–1290.
55. Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustment methods in clinical trials. *Stat Med.* 1997;16:2529–2542.
56. Seck T, Bretz A, Krempien R, Krempien B, Ziegler R, Pfeilschifter J. Age-related changes in insulin-like growth factor I and II in human femoral cortical bone: Lack of correlation with bone mass. *Bone.* 1999;24:387–393.
57. Seck T, Scheppach B, Scharla S, Diel I, Blum WF, Bismar H, Schmid G, Krempien B, Ziegler R, Pfeilschifter J. Concentration of insulin-like growth factor (IGF)-I and -II in iliac crest bone matrix from pre- and postmenopausal women: Relationship to age, menopause, bone turnover, bone volume, and circulating IGFs. *J Clin Endocrinol Metab.* 1998;83:2331–2337.
58. Sharp CA, Brown SJ, Davie MW, Magnusson P, Mohan S. Increased matrix concentrations of IGFBP-5 in cancellous bone in osteoarthritis. *Ann Rheum Dis.* 2004;63:1162–1165.
59. Sterck JG, Klein-Nulend J, Lips P, Burger EH. Response of normal and osteoporotic human bone cells to mechanical stress in vitro. *Am J Physiol.* 1998;274(6 Pt 1):E1113–E1120.
60. Thomas T, Gori F, Spelsberg TC, Khosla S, Riggs BL, Conover CA. Response of bipotential human marrow stromal cells to insulin-like growth factors: effect on binding protein production, proliferation, and commitment to osteoblasts and adipocytes. *Endocrinology.* 1999;140:5036–5044.
61. Tobias JH, Chow JW, Chambers TJ. Opposite effects of insulin-like growth factor-I on the formation of trabecular and cortical bone in adult female rats. *Endocrinology.* 1992;131:2387–2392.
62. Triplett JW, O'Riley R, Tekulve K, Norvell SM, Pavalko FM. Mechanical loading by fluid shear stress enhances IGF-1 receptor signaling in osteoblasts in a PKCzeta-dependent manner. *Mol Cell Biomech.* 2007;4:13–25.
63. Wakisaka A, Tanaka H, Barnes J, Liang CT. Effect of locally infused IGF-I on femoral gene expression and bone turnover activity in old rats. *J Bone Miner Res.* 1998;13:13–19.
64. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, Hase M, Takai H, Harada A, Ikeda K. Association of a polymorphism of the transforming growth factor-beta 1 gene with generic susceptibility to osteoporosis in postmenopausal Japanese women. *J Bone Miner Res.* 1998;13:1569–1576.
65. Yeni YN, Christopherson GT, Dong XN, Kim DG, Fyhrie DP. Effect of microcomputed tomography voxel size on the finite element model accuracy for human cancellous bone. *J Biomech Eng.* 2005;127:1–8.
66. Yeni YN, Hou FJ, Ciarelli T, Vashishth D, Fyhrie DP. Trabecular shear stresses predict in vivo linear microcrack density but not diffuse damage in human vertebral cancellous bone. *Ann Biomed Eng.* 2003;31:726–732.
67. Yeni YN, Hou FJ, Vashishth D, Fyhrie DP. Trabecular shear stress in human vertebral cancellous bone: intra- and inter-individual variations. *J Biomech.* 2001;34:1341–1346.
68. Yeni YN, Kim DG, Divine GW, Johnson EM, Cody DD. Human cancellous bone from T12-L1 vertebrae has unique microstructural and trabecular shear stress properties. *Bone.* 2009;44:130–136.
69. Yeni YN, Zelman EA, Divine GW, Kim DG, Fyhrie DP. Trabecular shear stress amplification and variability in human vertebral cancellous bone: Relationship with age, gender, spine level and trabecular architecture. *Bone.* 2008;42:591–596.
70. Zhang R, Supowit SC, Hou X, Simmons DJ. Transforming growth factor-beta2 mRNA level in unloaded bone analyzed by quantitative in situ hybridization. *Calcif Tissue Int.* 1999;64:522–526.
71. Zysset PK, Sonny M, Hayes WC. Morphology-mechanical property relations in trabecular bone of the osteoarthritic proximal tibia. *J Arthroplasty.* 1994;9:203–216.