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Mice lacking thrombospondin 2 show an atypical pattern of endocortical and periosteal bone formation in response to mechanical loading

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Abstract

Thrombospondin 2 (TSP2) is an extracellular matrix (ECM) protein localized to bone. Since mice with a targeted disruption of the TSP2 gene (TSP2-null) have increased bone formation, we hypothesized that mice lacking TSP2 would show an enhanced osteogenic response to mechanical loading. We addressed our hypothesis by subjecting wild-type (WT) and TSP2-null mice to mechanical loading using the non-invasive murine tibia loading device, and statistical comparisons were made between loaded and unloaded bones within genotype, between genotypes, and between the periosteal and endocortical surfaces within genotype. Right tibiae of WT and TSP2-null mice received 5 days of a low-magnitude loading protocol. This low-magnitude loading (inducing ~900 and 500 με at periosteal and endocortical surfaces of WT bones, respectively) affected neither periosteal nor endocortical bone formation rate (BFR/BS) when comparing loaded to intact bones in either WT or TSP2-null mice, nor did it result in any significant differences between WT and TSP2-null. As well, there was no difference between loaded endocortical and periosteal surfaces in WT mice; however, endocortical BFR/BS in TSP2-null loaded tibia was significantly elevated relative to the periosteal BFR/BS—despite peak periosteal strains being significantly greater than endocortical strains in TSP2-null mice (690 versus 460 με). To confirm this counterintuitive surface-specific response in TSP2-null mice and to induce significant periosteal bone formation, osteogenic potency of the loading protocol was amplified by doubling the number of loading bouts (10 loading days) and loading magnitude (1 Hz, resulting in 1400 and 900 με peak strain at the periosteal and endocortical surfaces, respectively). Under load, both WT and TSP2-null mice showed significantly increased periosteal mineralizing surface (by nearly three-fold and five-fold, respectively), but mineral apposition rate (MAR) was not statistically changed. The increased MS/BS resulted in a five-fold increase in WT periosteal BFR/BS, but the TSP2-null periosteal BFR/BS was unchanged. Furthermore, this increase in WT loaded periosteal BFR/BS was statistically greater than the WT endocortical BFR/BS. At the endocortical surface of WT mice, loading did not significantly increase bone formation parameters (versus intact). In contrast, at the endocortical surface of TSP2-null mice, loading induced a significant two-fold increase in BFR/BS (versus intact), that was also significantly greater than the endocortical BFR/BS of loaded WT mice. Thus, exogenous loading of TSP2-null mice resulted in highly variable responses that did not reflect the induced strains at the periosteal and endocortical surfaces. While in WT mice, loading resulted in increased periosteal BFR/BS that was greater than the endocortical BFR/BS, in TSP2-null mice loading resulted in endocortical (not periosteal) BFR/BS that was elevated. This reversal in envelope-specific bone formation in TSP2-null mice occurred despite periosteal strains being significantly greater than endocortical (1290 versus 775 με) and strain distributions being similar to that of WT. These results show that the disruption of a single gene can lead to a reversal in normal pattern of load induced bone formation, and more specifically, that the functional interaction of TSP2 with mechanical loading is highly contextual and specific to the cortical bone envelope examined.

Keywords: Matrix proteins; Cortical bone; Bone formation; Mechanical loading; Histomorphometry–mice

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Introduction

Osteocytes are mechanically responsive cells residing within cortical bone [1]. One potential mechanotransduction pathway is that osteocytes, in response to load, release soluble signals that are transmitted through canaliculi to osteoblast-lineage cells located at the periosteum and endosteum. These soluble factors are not well defined but could include growth factors, such as VEGF, as well as inflammatory mediators, such as prostaglandins [2,3]. These signals are likely modulated by other extracellular molecules, either systemically or locally derived; by the transmitting state of the transducing cells; or by the physiological status of the responding cell. For instance, responsiveness to mechanical load is influenced by estrogen, as revealed in ovariectomized rats [4]. Additionally, Gross et al. have shown that mice overexpressing IGF-1 from an osteocalcin promoter show an enhanced response to mechanical loading [5], while systemic administration of PTH increased bone responses to mechanical stimuli [6].

The influence of extracellular matrix (ECM) proteins on load responsiveness has not been extensively explored. Osteocytes, as well as pre-osteoblasts and osteoblasts, exist in an extensive ECM milieu. The ECM may directly influence the activity of a soluble signal by binding to a factor and either promoting or interfering with the factor. In this manner, the ECM may enhance the transfer of a soluble signal through the canalicular network, or it may modulate receptor interactions at the site of the effector cells. As an example, mice lacking biglycan, an extracellular proteoglycan that binds to TGFβ, have reduced bone formation because of reduction in TGFβ signaling [7]; however, the influence of load in this model has not been determined. Alternatively, since cell adhesion to the ECM is required for proper growth factor signaling in vitro, alterations in ECM–cell interactions could alter the cells' response to a soluble, mechanically induced signal [8]. For example, the overexpression of a dominant negative β1 integrin in mice leads to an altered pattern of periosteal bone formation, but this model has not been studied in response to load [9].

One ECM protein that could play a role in regulating responsiveness to mechanical loading is thrombospondin 2 (TSP2). TSP2 is a multifunctional, trimeric protein produced by osteoblast-lineage cells. In vitro, TSP2 regulates cell cycle progression of marrow-derived osteoprogenitors [10]. TSP2 function in vivo appears to be highly contextual, dependent upon its temporal and spatial expression [11]. Mice with a targeted disruption of the TSP2 gene (TSP2-null) have an increase in osteoprogenitor number and an increase in endocortical bone formation [12]. Given the known functions of TSP2, the presence or absence of this protein could impact mechanically induced signals in bone.

For this study, we hypothesized that mice lacking TSP2 would have an enhanced osteogenic response to mechanical loading. We examined our hypothesis utilizing a non-invasive murine tibia loading device [5] and found that the absence of TSP2 leads to a novel pattern of bone formation in response to load. In contrast to loading of WT mice, loading of TSP2-null mice did not influence bone formation at the periosteal surface but enhanced bone formation at the endocortical surface, despite a lower induced strain at the endocortical surface, relative to the periosteal surface. This counterintuitive adaptive response, reported herein for the first time, represents an altered pattern of bone accretion secondary to mechanical loading and induced strain distributions.

Methods

In vivo mechanical loading

Congenic 129/SvEms-+^{Ter} wild-type (WT) (n = 11) and TSP2-null (n = 13) adult female mice underwent external loading of the right tibia using the non-invasive murine tibia loading device [5]. All experimental procedures were approved by the University of Washington animal care and use committee and were done blinded to mouse genotype.

Briefly, with a mouse anesthetized (2% isofluorane), the in vivo loading device secured the proximal tibia metaphysis from motion via a brass gripping cup. The tibia diaphysis was then placed under 'cantilever' bending in the medial–lateral plane by applying force to the lateral distal tibia metaphysis via a computer controlled linear force actuator. To examine whether a lack of TSP2 enhances bone adaptation induced by exogenous mechanical loading, we used two different loading regimens: (1) low-magnitude, short-duration loading and (2) higher magnitude, longer duration loading.

For low-magnitude loading, 5-month-old WT (n = 6) and TSP2-null mice (n = 5) underwent 100 cycles/day of mechanical loading (0.15 N, 1 Hz, 0.02/s strain rate) for 5 days. The animals were permitted food and water ad libitum and allowed 14 additional days of free cage activity. To determine alterations in bone growth induced by mechanical loading, animals received double calcein labeling (200 μl, i.p., 10 mg/kg) on days 0 and 18 and were euthanized on day 20.

Upon sacrifice, the animals were weighed, and the right (loaded) and left (intact contralateral) tibiae were freed of soft tissue, tibial lengths measured, and 200 μm thick cross-sections obtained at the tibia mid-shaft (2.5 mm proximal to the tibiofibular junction) utilizing a Struers minitom. Sections were hand ground to 100 μm thickness and mounted unstained for evaluation of dynamic indices of bone formation.

A Nikon epifluorescent microscope was used to image (200×) the sections with identity blinded, and composite images of the whole cross-section were obtained post-imaging. As described previously [13], custom written software within PV-Wave (VNI Inc., Boulder, CO) was used to determine standard static and dynamic histomorphometry measures. Briefly, s.LS, d.LS, Ir.L.Th, and BS were determined at endocortical and periosteal surfaces. Surface referent MS, MAR, and BFR were then determined from the measured quantities as previously described [14], using the following calculations: MS/BS = [d.LS + 0.5 × s.LS], MAR = [Ir.L.Th/Ir.L.t], and BFR/BS = [MAR × MS/BS]. As well, static measures such as cortical area (Ct.Ar), endocortical envelope area (Ec.Ar), periosteal envelope area (Ps.Ar), and cortical thickness (Ct.Th) were also measured in intact contralateral tibiae to examine structural difference accrued during the process of growth in the presence (WT) or absence of TSP2 (Null). WT and TSP2-null mice had equivalent body weights at harvest.

Based upon the findings with low-magnitude loading, we attempted to amplify osteogenic potency with a higher magnitude, longer duration loading protocol. Specifically, the right tibiae of WT (n = 5) and TSP2-null (n = 8, age = 6 months) underwent 100 cycles/day of high-magnitude loading (0.3 N, 1 Hz, 0.04/s strain rate) for 5-days/week for 2 weeks (i.e., total of 10 loading bouts, at double the loading magnitude and strain rate as the previous experiment). Animals received double calcein labeling on days 3 and 12 and were euthanized on day 15, 3 days after the 2nd label. As in the first experiment, the right (loaded) and left (intact, contralateral) were processed, and bone formation parameters and cortical bone properties were determined at the tibia mid-shaft. WT and TSP2-null mice had equivalent body weights at harvest.

Animal-specific strain distributions and peak strain magnitudes were determined at the periosteal and endocortical surfaces of the tibia mid-shaft in WT and TSP2-null mice [14]. Briefly, based on force and moment boundary conditions determined at the tibia mid-shaft from calibration studies, longitudinal