Accelerated Hypertrophic Chondrocyte Kinetics in GDF-7 Deficient Murine Tibial Growth Plates

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ABSTRACT: The Growth/Differentiation Factors (GDFs) are a subgroup of the Bone Morphogenetic Proteins (BMPs) well known for their role in joint formation and chondrogenesis. Mice deficient in one of these signaling molecules, GDF-5, have recently been shown to exhibit a decreased rate of endochondral bone growth in the proximal tibia due to a significantly longer hypertrophic phase duration. GDF-7 is a related family member, which exhibits a high degree of sequence identity with GDF-5. The purpose of the present study was to determine whether GDF-7 deficiency also alters the endochondral bone growth rate in mice and, if so, how this is achieved. Stereologic and cell kinetic parameters in proximal tibial growth plates from 5-week-old female GDF-7 −/− mice and wild type control littermates were examined. GDF-7 deficiency resulted in a statistically significant increase in growth rate (+26%; $p = 0.0084$) and rate of cell loss at the chondrosseous junction (+25%; $p = 0.0217$). Cells from GDF-7 deficient mice also exhibited a significantly shorter hypertrophic phase duration compared to wild type controls (−27%; $p = 0.0326$). These data demonstrate that, in the absence of GDF-7, the rate of endochondral bone growth is affected through the modulation of hypertrophic phase duration in growth plate chondrocytes. These findings further support a growing body of evidence implicating the GDFs in the formation, maturation, and maintenance of healthy cartilage. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

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The Growth/Differentiation Factors (GDFs) are a subgroup of the Bone Morphogenetic Proteins (BMPs) well known for their role in joint formation and chondrogenesis.[1–7] Five-week-old female mice deficient in one of these molecules, GDF-5, have additionally been shown to exhibit a significant reduction in proximal tibial growth rate due to a lengthened hypertrophic phase duration.[8] Most recently, Miyamoto and colleagues have demonstrated that *Gdf5* appears to be a susceptibility gene for osteoarthritis, and that decreased *Gdf5* expression may be associated with the development of this degenerative joint disease.[9] Clearly, GDF5 plays an important role in the formation, maturation, and maintenance of healthy cartilage.

GDFs 5, 6, and 7 share 80%–86% sequence identity with each other in the C-terminal signaling region (with the exception of a 26-amino-acid glycine-rich insert in GDF-7),[10,11] thus potentially rendering them capable of similar functions. GDF-7 (the murine homolog of human BMP-12), is the subject of a small, but growing body of literature in which a number of diverse roles for the protein have been established. These include effects on long bone mechanics,[12] tendon maintenance and repair,[13–16] neural tissue maturation and maintenance,[17,18] dental development,[19,20] and reproductive organ formation.[21] A line of GDF-7 deficient knockout mice have been engineered[21] and the phenotype of these animals includes abnormal seminal vesicle development in males,[21] hydrocephaly and premature death in a small percentage of knockouts,[21] subtle tendon abnormalities,[15] and altered cortical bone material properties[12]; however, no detailed investigations have yet been performed on the cartilage phenotype of these animals.

Given the known role of GDF-5 in chondrogenesis, and the documented effect of GDF-5 deficiency on murine tibial growth rate, we were interested in examining whether mice deficient in the related protein, GDF-7, exhibit a growth plate phenotype. Specifically, the purpose of this study was to examine growth plate stereology and cell kinetics to determine whether GDF-7 deficiency in mice would alter the endochondral bone growth rate in proximal tibial growth plates, and if so, how this is achieved.

METHODS

Animal Model

Five-week-old healthy female mice were used to assess growth plate stereologic parameters and chondrocyte kinetics in the proximal tibia. This site provides the largest growth plate available for analysis in the mouse, and was chosen to allow fewer total animals to be used to obtain statistically significant results using systematic random sampling methods. The experimental animals consisted of 10 GDF-7 −/− mice obtained from an in-house colony. Heterozygous breeder pairs were obtained from the Kingsley lab and the original knockout characterization is described elsewhere.[21] Knockout animals were initially generated by replacing a 1.1 kb fragment containing the mature signaling domain of the GDF-7 gene with a positive selectable neomycin resistance cassette.[21] Control animals for this study consisted of 10 wild type (+/+) littermates, and genotyping was performed to distinguish −/−, +/−, and +/+ mice. Animals were housed under 12-h light/dark conditions. At 5 weeks of age, all animals were injected intraperitoneally with 10 mg/kg of calceine in sterile 0.9% saline solution (Sigma Aldrich, St. Louis, MO) at 9:00 AM and euthanized exactly 4 days later at 9:00 AM by CO_2 inhalation. The calceine label was used to determine the growth rate for each individual growth plate in microns per day as described below. Stereologic and cell kinetic measurements were based

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on the work of Bailón-Plaza et al.[22] and Mikic et al.[8]; detailed methods, as adapted for the present study, are described below. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Sample Preparation

Immediately after sacrifice, the left and right tibia were dissected free from all soft tissue and further cleaned under a dissecting microscope in fixative solution (2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, 330 mOsm). The proximal bone ends were cut with a sharp razor blade below the metaphysis. Specimens from the left limb were then cut in two along a mid-sagittal plane and specimens from the right limb were cut in two along a mid-frontal plane. One block from each side was placed in fixative solution containing 0.7% (w/v) ruthenium hexamine trichloride (RHT; Alfa Aesar, Ward Hill, MA) at room temperature for 3–5 h on a rocker to preserve the morphology of hypertrophic chondrocytes.[23] The second block from each side was placed in 40% ethanol and was not used further for the purposes of this study.

Samples were washed three times for 5 min each with 0.1 M cacodylate buffer, pH 7.4, 330 mOsm and then dehydrated through graded ethanol solutions. Infiltration in Epon plastic (Sigma Aldrich, St. Louis, MO) was performed with four changes of 15 min each in propylene oxide (PPO) followed by 24 h of PPO:Epon at 3:1, 24 h at 1:1, 72 h at 1:3, and 24 h in 100% Epon. Specimens were then allowed to polymerize for 7 days at 60°C. One-micron vertical sections were cut using a cutting plane of 0° or 1.5° rotation about the vertical axis (randomly assigned to right or left blocks) using an autocut ultramicrotome (Leica, Vienna, Austria). Sections included bone from both the epiphysis and the metaphysis of each block. Alternating serial sections were either mounted on microscope slides, stained with Toluidine blue and cover-slipped for stereologic measurements, or mounted on slides without staining for visualization of calceine labels. A total of 20 alternating serial sections were processed in this way per side (left or right) for each mouse. All subsequent analyses were performed by a single blinded investigator (M. P. F.).

Zone Heights

Twelve stained sections per growth plate were viewed at 20× magnification and images captured using a color digital video camera (Olympus DP70, Olympus America Inc., Central Valley, PA). Images were aligned so that the direction of growth was vertical on the computer screen prior to image capture. Using Adobe PhotoShop 9.0.2 (Adobe Systems, Palo Alto, CA), the heights of the growth plate and each growth plate zone were obtained by delineating the top of the growth plate, the junctions between the resting and proliferative zones, and between the proliferative and hypertrophic zones based on morphological characteristics of the chondrocytes, as well as the chondrosseous junction based on changes in matrix staining. The vertical height of each zone was then measured at four equidistant locations per section. Values for each individual mouse were obtained by averaging all 96 measurements [4 locations per section × 12 sections per growth plate × 2 growth plates (left and right)] per animal.

Cell Area Fraction and Mean Cell Volume

Using point counting methods on 17 high magnification sections per growth plate (34 sections per mouse at approximately 800×), cell area fraction was estimated in the proliferative and hypertrophic zones (area occupied by cells in the region of interest as a percentage of the total area within the region of interest). Mean cell volume (MCV) was estimated in the proliferative and hypertrophic zones using point-sampled intercepts measured on cells lying on grid intersection points.[24] For each growth plate, 17 sections were analyzed for MCV estimates to obtain a minimum of 300 intercepts per mouse, which were then averaged together. Each section was analyzed using a different grid angle (3°R, 7°L, 15°R, 22°L, 34°R, 46°L, 64°R, 82°L, 5°R, 9°L, 16°R, 28°L, 40°R, 52°L, 76°R, 94°L, 1°R).

Cell Numbers

Cell populations in the proliferative and hypertrophic zones were estimated by counting chondrocyte numbers in a reference volume of a cylinder of 1-mm diameter with height equal to the height of the growth plate zone being analyzed.[22,25] The number of cells per cylinder in a given zone was then calculated as:

$$[\text{cells/cylinder}]_{\text{zone}} = [\text{Cylinder Volume/Cell Volume}]_{\text{zone}} \times \text{Cell Vol. Fraction}_{\text{zone}}. \quad (1a)$$

Assuming that the cell volume fraction is proportional to cell area fraction in a given growth plate zone, Equation 1a can further be reduced to:

$$[\text{cells/cylinder}]_{\text{zone}} = [\pi R^2 H_{\text{zone}}]/\text{MCV}_{\text{zone}}] \times (\text{cell area fraction})_{\text{zone}} \quad (1b)$$

where R is the radius of the cylinder (0.5 mm), MCV is the mean cell volume in a given zone, and H is the zone height. Equation 1b can ultimately be expressed as:

$$[\text{cells/cylinder}]_{\text{zone}} = [0.25\pi \times (\text{zone height}) \times (\text{cell area fraction})_{\text{zone}}]/\text{MCV}_{\text{zone}} \quad (1c)$$

where area fraction, zone height, and mean cell volume (MCV) were obtained as previously described.

Growth Rate

The calceine label was visualized using an Olympus BX51 research microscope outfitted with dichroic mirror DM500, excitation filter BP450-80, and barrier filter BA515 (Olympus America Inc.). The center of the green label and the chondrosseous junction were outlined on six sections per growth plate (12 per mouse) and the vertical distance between the two curves was measured at four equidistant locations per image. Measurements (μm) were divided by 4 (days) and then averaged together to obtain a single value for the daily growth rate of each physis for each mouse.

Cells Lost per Day and Phase Durations

Wilsman et al. have shown that chondrocyte activity in the growth plate is in steady-state over a 24-h period.[25] Thus, the number of proliferative cells produced each day via differentiation from the resting zone is the same as the number of cells that differentiate into hypertrophic chondrocytes as well as the number of cells lost each day at the chondrosseous junction.[22,25] Consequently, the number of cells lost per day can be estimated as the number of cells lost per day in a reference volume cylinder of 1-mm diameter and height equal to the amount of growth that occurs in a 24-h period:

$$\text{cells lost/day} = [0.25\pi \times (\text{cell area fraction})_{\text{hyp}} \times (\text{growth rate})_{\text{hyp}}]/\text{MCV}_{\text{hyp}}. \quad (2)$$

The durations of the proliferative and hypertrophic cell phases can then be calculated as:

$$(\text{phase duration})_{\text{zone}} = (\text{cells/cylinder})_{\text{zone}}/(\text{cells lost/h}) \quad (3)$$

where $(\text{cells/cylinder})_{\text{zone}}$ is specific to a given zone and is given by Equation 1c.

Statistical Analysis

The following parameters were analyzed using a one-factor ANOVA with genotype as the independent factor: growth rate; total growth plate height; height of the resting, proliferative, and hypertrophic zones; mean cell volume of the proliferative and hypertrophic zones; number of cells per cylinder in the proliferative and hypertrophic zones; proliferative and hypertrophic phase durations; and number of cells lost per day. All analyses were performed using the statistical software Statview (Abacus Concepts, Berkeley, CA) with a cutoff level for statistical significance chosen as $p < 0.05$.

RESULTS

Samples from two wild type and one mutant animal were damaged during processing, thereby reducing the sample sizes accordingly. No grossly discernable abnormalities (e.g., disorganization, lack of columnation, cell death) were evident in the morphology of GDF-7 deficient growth plates when examined histologically (Fig. 1). The growth rate of the proximal tibia in GDF-7 −/− animals was significantly greater than that of wild type animals (Fig. 2; +26%, $p = 0.0084$). This faster growth rate was, in turn, associated with a greater number of cells lost per day at the chondrosseous junction (+25%, $p = 0.0217$) and a significantly shorter hypertrophic phase duration (−27%, $p = 0.0326$) in knockout mice (Table 1). No significant differences were found in total growth plate height, height of any individual physeal zone, nor in mean cell volume (Table 1). Although proliferative phase duration was also noticeably shorter in mutant animals (−36%), the difference between knockouts and controls was not statistically significant due to the high degree of data scatter in the wild type animals for this parameter (Table 1; $p = 0.1152$).

DISCUSSION

The aim of this study was to determine whether GDF-7 deficiency significantly affects endochondral bone growth rate, and if so, how this is achieved. We accomplished this by determining stereologic and cell kinetic parameters in the proximal tibial growth plates of 5-week-old female mice deficient in GDF-7 and their wild type littermates. Mice deficient in GDF-7 had a significantly elevated growth rate (+26%, $p = 0.0084$) that was associated with an accelerated hypertrophic phase: hypertrophic phase duration was 27% shorter in GDF-7 deficient animals compared to wild type controls ($p = 0.0326$). These data demonstrate that in the absence of GDF-7, (1) longitudinal bone growth rate is accelerated, and (2) this effect is achieved via modulation of the hypertrophic phase duration of physeal chondrocytes.

While a role for GDF-7/BMP-12 in chondrocyte biology and growth plate kinetics has, to our knowledge, not been reported in the literature, there is extensive evidence implicating the related molecule, GDF-5, in chondrocyte differentiation and maturation.[1–7] Most recently, 5-week-old female mice deficient in GDF-5 were shown to have the *opposite* phenotype in the proximal tibial growth plate relative to that seen in the present study with GDF-7 deficient mice of the same age and sex.[8] Compared to control growth plates, the physes from GDF-5 −/− animals had *slower* growth rates that were associated with a *longer* hypertrophic phase duration, whereas, in our current study, GDF-7 deficient growth plates had *faster* growth rates that were associated with a *shorter* hypertrophic phase duration. This opposite effect of two related GDF signaling molecules presents the intriguing possibility that GDFs 5 and 7 may have interrelated functions in the growth plate. Further molecular localization of mRNA and GDF proteins, along with BMP receptor localization, would be required to explore such a relationship in more detail.

Are the increased growth rate and accelerated hypertrophic phase duration seen in GDF-7 deficient mice due to a direct effect of the absence of GDF-7 on the growth plate, or could compensatory action by another, related GDF family member be at play? GDFs 5, 6, and 7 exhibit a high degree of sequence identity in the mature C-terminal signaling region of the molecule,[3] thus it is possible that these proteins could potentially substitute for one another. Indeed, such compensatory action has been seen with other knockout mouse models: the milder-than-expected bone phenotypes in estrogen receptor knockout mice appear to be due to compensation

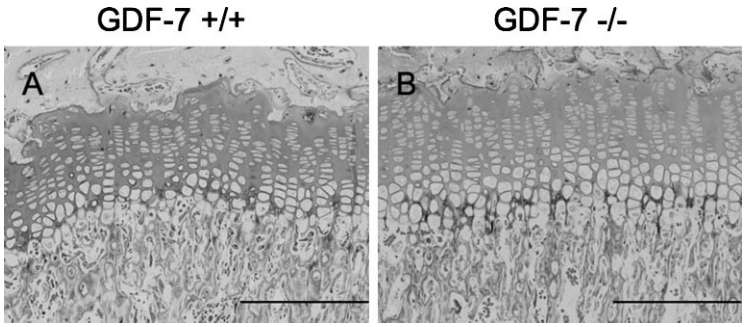

Figure 1. GDF-7 +/+ (A) and −/− (B) proximal tibial growth plates stained with Toluidine blue. Bars represent approximately 200 μm.

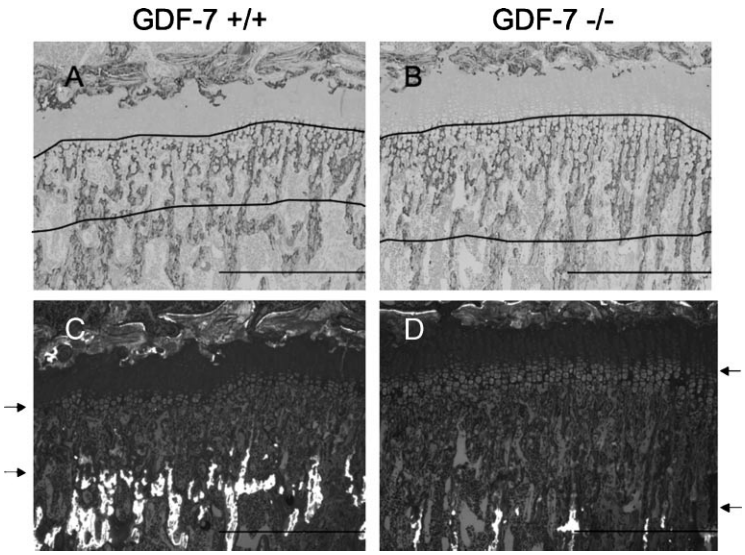

Figure 2. Measurement technique used to determine growth rates in GDF-7 +/+ (A,C) and −/− (B,D) proximal tibial growth plates. (A,B) Brightfield images. Upper curve delineates chondrosseous junction and lower curve indicates boundary of calceine label, with region between representing the total growth that has occurred in 4 days time. (Note that the calceine label itself is not shown on these figures.) (C,D) Same sections as shown in (A,B) but with calceine label shown on darkfield images. Upper arrows delineate the chondrosseous junction and lower arrows indicate the boundary of the calceine label, with the region between representing the total growth that has occurred in 4 days time. Bars indicate approximately 500 μm.

by other receptors and/or hormones[26]: for example, ERα expression is increased twofold in female ERKOβ bone.[27] Similarly, in the fibromodulin −/− mouse, elevated levels of the related small leucine rich proteoglycan, lumican, appear to partially rescue the tendon phenotype in this mouse model.[28] Previous studies on gene expression in tendons from the same line of GDF-7 deficient mice as were used in the present study have documented a twofold increase in *Gdf5* expression in both Achilles tendons and tail tendon fascicles.[15,29] Whether such compensatory action is at play in the growth plate of these mice remains to be determined, but it is an intriguing possibility given that the observed physeal phenotype in GDF7−/− mice is the opposite of that seen in GDF5 −/− mice. Further analysis of gene expression in growth plates from GDF knockout mice, as well as examination of the phenotype in double knockouts (e.g., GDF-5/GDF-7) may provide insight into the compensatory and synergistic actions of these molecules.

In summary, the results of this study demonstrate for the first time that 5-week-old female mice deficient in GDF-7 have a faster rate of endochondral bone growth in the proximal tibia due to an accelerated hypertrophic phase duration. Whether these observations are the result of a direct effect of the absence of GDF-7 on growth plate kinetics or the result of compensatory action by related GDF family members remains to be determined.

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Table 1. Tibial Growth Plate Stereologic Parameters from 5-Week-Old Female GDF-7 −/− and +/+ Mice [mean (SD)]

Parameters	GDF-7 (−/−) ($n=9$)	GDF-7 (+/+) ($n=8$)	Difference (%)	p-Value*
Growth rate (μm/day)	88 (20)	65 (10)	+26	**0.0084**
Growth plate height (μm)	209 (22)	200 (21)	+4	0.4097
Resting zone height (μm)	29 (3)	27 (6)	+7	0.6333
Proliferative zone height (μm)	88 (10)	91 (9)	−3	0.6193
Proliferative zone mean cell volume (μm^3)	560 (160)	620 (90)	−11	0.3977
Hypertrophic zone height (μm)	92 (13)	82 (13)	+11	0.1300
Hypertrophic zone mean cell volume (μm^3)	3,000 (630)	2,650 (370)	+12	0.1899
Cells lost/day	9,049 (1,850)	6,750 (1,840)	+25	**0.0217**
Proliferative zone cells/cylinder (mm^{-3})	31,600 (8,080)	29,600 (8,100)	+6	0.6056
Proliferative zone phase duration (h)	86 (22)	117 (50)	−36	0.1152
Hypertrophic zone cells/cylinder (mm^{-3})	9,500 (1,200)	8,850 (1,440)	+7	0.3236
Hypertrophic zone phase duration (h)	26 (4)	33 (8)	−27	**0.0326**

*Bold indicates statistical significance.

REFERENCES

1. Francis-West PH, Abdelfattah A, Chen P, et al. 1999. Mechanisms of GDF-5 action during skeletal development. Development 126:1305–1315.
2. Merino R, Macias D, Gañan Y, et al. 1999. Expression and function of GDF-5 during digit skeletogenesis in the embryonic chick leg bud. Dev Biol 26:33–34.
3. Storm EE, Kingsley DM. 1996. Joint patterning defects caused by single and double mutations in members of the bone morphogenetic protein (BMP) family. Development 122: 3969–3979.
4. Buxton P, Edwards C, Archer CW, et al. 2001. Growth/differentiation factor-5 (GDF-5) and skeletal development. J Bone Joint Surg [Am] 83-A: (Suppl 1, Pt 1):S23–S30.
5. Coleman CM, Tuan RS. 2003. Functional role of growth/differentiation factor 5 in chondrogenesis of limb mesenchymal cells. Mech Dev 120:823–836.
6. Coleman CM, Tuan RS. 2003. Growth/differentiation factor 5 enhances chondrocyte maturation. Dev Dyn 228:208–216.
7. Hatakeyama Y, Tuan RS, Shum L. 2004. Distinct functions of BMP4 and GDF5 in the regulation of chondrogenesis. J Cell Biochem 91:1204–1217.
8. Mikic B, Clark RT, Battaglia TC, et al. 2004. Altered hypertrophic chondrocyte kinetics in GDF-5 deficient murine tibial growth plates. J Orthop Res 22:552–556.
9. Miyamoto Y, Mabuchi A, Shi D, et al. 2007. A functional polymorphism in the 5′ UTR of GDF5 is associated with susceptibility to osteoarthritis. Nat Genet 39:529–533.
10. Davidson AJ, Postlethwait JH, Yan YL, et al. 1999. Isolation of zebrafish gdf7 and comparative genetic mapping of genes belonging to the growth/differentiation factor 5, 6,7 subgroup of the TGF-beta superfamily. Genome Res 9:121–129.
11. Storm EE, Huynh TV, Copeland NG, et al. 1994. Limb alterations in brachypodism mice due to mutations in a new member of the TGF-beta superfamily. Nature 368:639–643.
12. Maloul A, Rossmeier K, Pogue V, et al. 2006. Geometric and material contributions to whole bone structural behavior in GDF-7 deficient mice. Connect Tissue Res 47:157–162.
13. Lou J, Tu Y, Burns M, et al. 2001. BMP-12 gene transfer augmentation of lacerated tendon repair. J Orthop Res 19: 1199–1202.
14. Lou J, Tu Y, Ludwig FJ, et al. 1999. Effect of bone morphogenetic protein-12 gene transfer on mesenchymal progenitor cells. Clin Orthop Rel Res 369:333–339.
15. Mikic B, Bierwert LA, Tsou D. 2006. Achilles tendon characterization in GDF-7 deficient mice. J Orthop Res 24:831–841.
16. Wolfman NM, Hattersley G, Cox K, et al. 1997. Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6,and 7, members of the TGF-beta gene family. J Clin Invest 100:321–330.
17. Lee KJ, Mendelsohn M, Jessell TM. 1998. Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. Genes Dev 12:3394–3407.
18. Watakabe A, Fujita H, Hayashi M, et al. 2001. Growth/differentiation factor 7 is preferentially expressed in the primary motor area of the monkey neocortex. J Neurochem 76:1455–1464.
19. Morotome Y, Goseki-Sone M, Ishikawa I, et al. 1998. Gene expression of growth and differentiation factors-5, -6, and -7 in developing bovine tooth at the root forming stage. Biochem Biophys Res Commun 244:85–90.
20. Wikesjo UM, Sorensen RG, Kinoshita A, et al. 2004. Periodontal repair in dogs; effect of recombinant human bone morphogenetic protein-12 (rhBMP-12) on regeneration of alveolar bone and periodontal attachment. J Clin Periodontol 31:662–670.
21. Settle S, Marker P, Gurley K, et al. 2001. The BMP family member Gdf7 is required for seminal vesicle growth, morphogenesis and cytodifferentiation. Dev Biol 234:138–150.
22. Bailón-Plaza A, Lee AO, Veson EC, et al. 1999. BMP-5 deficiency alters chondrocyte activity in the mouse proximal tibial growth plate. Bone 24:211–216.
23. Hunziker EB, Herrman W, Schenk RK. 1982. Improved cartilage fixation by ruthenium hexamine trichloride (RHT): a prerequisite for morphometry in growth cartilage. J Ultrastruct Res 81:1–12.
24. Cruz-Orive LM, Hunziker EB. 1986. Stereology for anisotropic cells: application to growth cartilage. J Microsc 143: 47–80.
25. Wilsman NJ, Farnum CE, Leiferman EM, et al. 1996. Differential growth by growth plates as a function of multiple parameters of chondrocyte kinetics. J Orthop Res 14:927–936.
26. McCauley LK, Tözüm TF, Rosol TJ. 2002. Estrogen receptors in skeletal metabolism: lessons from genetically modified models of receptor function. Crit Rev Eukaryotic Gene Exp 12:89–100.
27. Windahl SH, Hollberg K, Vidal O, et al. 2001. Female estrogen receptor β –/– mice are partially protected against age-related trabecular bone loss. J Bone Miner Res 16:1388–1398.
28. Jepsen KJ, Wu F, Peragallo JH, et al. 2002. A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. J Biol Chem 277:35532–35540.
29. Mikic B, Entwistle R, Rossmeier K, Bierwert L. 2008. The effect of GDF-7 deficiency on tail tendon phenotype in mice. J Orthop Res (in press).