Continuous infusion of insulin-like growth factor-I into the epiphysis of the tibia

Aziz Abbaspour · Shinjiro Takata · Yoshito Matsui ·
Shinsuke Katoh · Mitsuhiro Takahashi · Natsuo Yasui

Received: 20 November 2006 / Revised: 25 December 2006 / Accepted: 8 January 2007 / Published online: 13 March 2007
© Springer-Verlag 2007

Abstract We have developed a method to promote longitudinal bone growth at the level of a specific growth-plate (GP) in young rabbits. Insulin-like growth factor-I (IGF-I) was continuously infused by means of an osmotic pump into the bone marrow cavity of the proximal epiphysis of the tibia. Radiological measurement showed a 2-mm overgrowth of the tibia after 4 weeks of treatment, while histological analysis demonstrated a 15% increase in the thickness of the selected GP. The local infusion of IGF-I increased the numbers of both proliferative and hypertrophic chondrocytes and promoted hyperplasia of bony trabeculae within the epiphysis. The distribution of material infused locally into the epiphysis was simulated by the infusion of Indian ink using the same methodology (osmotic pump) as that for IGF-I. Most of the dye remained within the bone marrow cavity of the epiphysis, but a portion infiltrated into the GP, reaching the deep layer of the physal chondrocytes and primary spongiosa of the metaphysis. These results suggest that the method reported here is a valid one for delivering cytokines or growth factors to the selected GP and for controlling the growth and differentiation of physal chondrocytes.

Résumé Nous avons mis au point une méthode pour évaluer la croissance longitudinale du cartilage de croissance chez les jeunes lapins. Nous avons utilisé

pour cela une pompe osmotique avec utilisation d'un facteur de croissance (IGF1) insuline like growth factor en injection continue au niveau de la cavité médullaire à proximité de l'épiphyse du tibia. Radiologiquement, les mesures ont montré 2 mm de croissance supplémentaire sur le tibia après 4 semaines de traitement. L'analyse histologique a également montré que le cartilage s'était épaissi de 15%, le nombre de chaque type de chondrocyte étant augmenté. Par ailleurs cette perfusion d'IGF1 entraîne une hyperplasie de l'os trabéculaire au voisinage de l'épiphyse. La diffusion du produit a été visualisée à l'aide d'un marqueur à base d'encre de chine injecté en même temps dans la pompe osmotique. La plupart du liquide reste au niveau de la cavité médullaire de l'épiphyse et une partie de ce liquide s'infiltre au niveau de la plaque de croissance atteignant les couches profondes et l'os spongieux primaire de la métaphyse. Ces résultats nous permettent de penser que cette méthode est utile pour apporter des cytokines et des facteurs de croissance de façon sélective au niveau de la plaque de croissance et permet également de contrôler la croissance et la différenciation cellulaire des chondrocytes.

Introduction

In childhood, the ends of the long bones, called the epiphyses, are separated from the diaphysis by the cartilaginous growth-plates (GPs). These GPs are exclusively responsible for the longitudinal growth of bone. Traumatic injury [16], infection [1] or tumour [5] involving the GP may cause impairment of the longitudinal growth of the bone, resulting in various deformities and inequality in the length of the affected limb. Fractures of the diaphysis in children, on the other hand, and inflammatory lesions

A. Abbaspour · S. Takata · Y. Matsui · S. Katoh · M. Takahashi ·
N. Yasui (✉)
Department of Orthopedics, Institute of Health Biosciences,
the University of Tokushima Graduate School,
3-18-15 Kuramoto,
Tokushima 770-8503, Japan
e-mail: nyasui@clin.med.tokushima-u.ac.jp

near the GP may cause overgrowth of the bones [2]. Limb length discrepancy (LLD) caused by the above conditions is currently corrected by surgery if the final difference is predicted to be more than 2 cm [7]. Limb lengthening by distraction osteogenesis [9, 23, 24] is yet another option of surgical treatment.

The GP is an avascular tissue that comprises resting, proliferating and hypertrophic chondrocytes in columnar arrangement. Various cytokines and growth factors are known to affect the growth and differentiation of chondrocytes. Of these, insulin-like growth factor-I (IGF-I) is the most prominent growth factor, mediating growth hormone (GH) effects either by endocrine [3] or by autocrine/paracrine mechanisms [13]. The lack of GH or IGF-I may cause a short stature [21], while their overproduction may cause gigantism or acromegaly.

Subcutaneous or intravenous administration of GH or IGF-I enhances systemic bone growth [8, 22], and recombinant human GH and IGF-I are now available to treat the GH/IGF-I-deficient patients. To date, however, no standard method has been established for controlling the growth of a particular bone by such a humeral growth factor. The patient with LLD has no choice other than undergoing surgical lengthening of the short bone, which

usually requires the long-term application of a bulky external fixation device. We report here a technique for enhancing local bone growth at a selected GP by the continuous infusion of IGF-I into the epiphysis. This method does not require major surgery and is applicable for treating patients with LLD.

Materials and methods

Animals

Thirty-three male Japanese white rabbits (age: 4 weeks; weight: 650–800 g) were purchased from Oriental Yeast Co. (Tokyo, Japan). The animals were housed under standardised environmental conditions and fed standard rabbit chow (RC4, Oriental Yeast Co.). The control group consisted of five rabbits whose body weight and bone growth were observed but which were not operated on (intact tibia). The remaining 28 rabbits were anaesthetized with an intravenous injection of ketamine hydrochloride and xylazine at doses of 20 mg/kg and 5 mg/kg body weight, respectively. A small hole (diameter: 0.35 mm) was then drilled into the epiphysis of both proximal tibia. An L-

Fig. 1 **a** A young male rabbit bearing two osmotic pumps on his back, **b** a radiograph of the animal. Each osmotic pump was connected to a radiolucent plastic catheter and aluminium canula

inserted into the proximal epiphysis of the tibia. The right pump was filled with normal saline (*N/S*) and the left pump with insulin-like growth factor-I (*IGF-I*)

Fig. 2 A radiograph of rabbit tibiae treated for 4 weeks with normal saline (N/S) and insulin-like growth factor-I (IGF-I). The IGF-I-treated tibia was 2 mm longer than the N/S-treated tibia. Note that the fibula did not show overgrowth

shaped cannula of a 28-gauge needle was inserted through the hole, and the surrounding tissues were sutured to stabilize the needle. Two ALZET osmotic pumps were implanted subcutaneously on the back of the rabbit (Fig. 1a). The left tibia was infused with IGF-I at 150 $\mu\text{g/kg}$ per day through a polyethylene catheter connected to the cannula, and the right tibia was infused with normal saline (N/S) (Fig. 1b). The recombinant human IGF-I was a generous gift of Fujisawa Chemical Co. (Osaka, Japan).

The rabbits were divided into three groups based on the length of time infused with the IGF-I and N/S solutions: 1-week treatment group ($n=5$), 2-week treatment group ($n=5$) and 4-week treatment group ($n=14$). Following the treatment period, the animals were killed by sodium pentobarbitone injection (60 mg/kg body weight), and the tibiae were harvested to measure the bone mineral density (BMD) and to carry out radiological and histological analyses. This protocol was approved of by the local animal protection agency and the Ethics Committee of the University of Tokushima.

In a subset of this experiment, we performed a continuous local infusion of Indian ink at 2.5 $\mu\text{l/h}$ into the proximal epiphysis of the tibia of each of four animals with the aim of analysing the distribution of the infused material. After 48 h all four rabbits were killed, and the harvested tibiae were fixed with 10% neutral formalin for 5 days and then decalcified in 20% aqueous EDTA for 4 weeks. Specimens were cut coronally (5 mm thick) for macroscopic photographs and then embedded in paraffin. The embedded sections were then cut coronally (3 μm thick) for microscopic observation.

Radiological analysis

Soft X-rays of the tibiae were taken using a Model CMB-2T (Softex Co., Kanagawa, Japan). Bone length was measured using SCION Image software.

Histological analysis

All specimens from the harvested tibiae were fixed with 10% neutral formalin for 5 days, decalcified in 20% aqueous EDTA for 4 weeks and then embedded in paraffin. Sections 3 μm in thickness were cut coronally and stained with haematoxylin and eosin. The numbers of chondrocytes per column in the proliferative (PZ) and hypertrophic (HZ) zones of the GP were counted under a microscope with a 20 $\times$ objective. All well-orientated “full or intact” cell columns (i.e. columns sectioned in a plane showing consecutive cells extending the entire thickness of each zone) were included in the analysis. The numbers of the cells residing within each zone in these columns were counted, expressed as numbers of cells per column and averaged for each zone of each animal [22]. The total height of the GP was measured as the distance between the two chondroosseous junctions. To measure the heights of the PZ and HZ, their borders were delineated on the basis of morphological criteria [10].

Table 1 Effect of continuous local infusion of insulin-like growth factor-I (IGF-I) on the length of tibia bone (NS not significant)

Tibia length (mm)					
Before infusion	n^a	Duration of infusion (weeks)	Normal saline (N/S)	IGF-I	p value
59.5 $\pm$ 0.9	5	One	67.4 $\pm$ 0.4	67.4 $\pm$ 0.4	NS
61.7 $\pm$ 1.8	5	Two	73.9 $\pm$ 1.6	74.9 $\pm$ 1.7	<0.01
61.1 $\pm$ 0.9	14	Four	88.2 $\pm$ 1.4	90.3 $\pm$ 1.3	<0.004

^a n , Number of animals

Fig. 3 Photomicrographs of the proximal tibial epiphysis treated for 4 weeks with IGF-I (a) and N/S (b). Stain: Haematoxylin-eosin. After continuous local infusion of IGF-I, new bone trabeculae were formed in the centre of the epiphysis. In the N/S-treated bone, there was an atrophy of bone trabeculae in the centre of the epiphysis. This atrophy was consistently observed in young rabbits even in intact bone (c)

Peripheral quantitative computerised tomography (pQCT)

Computerised tomography was performed with the Stratec pQCT apparatus (model XCT-960A; Norland/Stratec, Fort Atkinson/Pforzheim, USA/Germany). Metaphyseal pQCT scans of the proximal part of the tibiae were taken to measure trabecular BMD. Measurements were performed on 1-mm-thick slices at 5 mm distal to the proximal growth plate of the tibia.

Statistical analysis

Statistical analysis of the data was performed using Student's paired *t*-test for experimental animals. The results are shown as the mean $\pm$ the standard deviation (SD). Values of $p < 0.05$ were considered to be significant.

Results

Skeletal growth

Figure 2 shows a radiograph of IGF-I- and N/S-infused tibiae. After the 2-week infusion of IGF-I locally, the left tibia was approximately 1 mm longer than the right tibia, which had been infused with N/S ($p = 0.01$). After the 4-week infusion of IGF-I locally, the left tibia was approximately 2 mm longer than the right tibia, which had been infused with N/S ($p < 0.004$). To summarise, after the 2- and 4-week treatments with IGF-I, the length of the left tibia had increased by approximately 1 mm and 2 mm, respectively (Table 1). There was no overgrowth in the fibula of the injected tibiae. There was no difference in the length of

the intact tibiae of the control animals and that of the N/S infused tibiae.

Histological analysis

Structures of the epiphysis and adjacent GP were analysed histologically at various stages of the experiment. The intact tibia of a 4-week-old control rabbit showed characteristic histological features, i.e. the bone trabeculae of the epiphysis were thicker and coarser than those of the metaphysis, and the centre of the epiphysis contained only a few bone trabeculae. Intact tibiae of 6- and 8-week-old rabbits showed basically the same histological features, with the exception that the height of the GP had become spontaneously smaller in older animals. When N/S was infused continuously into the epiphysis for 2 or 4 weeks and the histological findings compared with those of age-matched intact tibiae, there was no significant change in the epiphysis and GP (Fig. 3b,c). When IGF-I was infused for 2 weeks or 4 weeks, however, there was a significant increase in the number of bone trabeculae in the centre of the epiphysis (Fig. 3a). In addition, there were significant increases in the height of the GP and in the number of proliferating and hypertrophic chondrocytes in each longitudinal column (Fig. 4a–c; Table 2) in IGF-I treated tibia as compared with N/S-treated tibiae. This result was consistently observed throughout the experiment.

In order to examine the distribution of infused materials, we infused Indian ink – instead of IGF-I – into the proximal epiphysis using the same methodology (osmotic pump). Macroscopically, Indian ink filled up the bone-marrow cavity of the epiphysis (Fig. 5a). Microscopically, some of the dye infiltrated into the GP

Fig. 4 Photomicrographs of the growth-plates (GPs) treated for 4 weeks with IGF-I (**a**) and N/S (**b**). Stain: Haematoxylin-eosin. Continuous local infusion of IGF-I into the epiphysis resulted in a 15% increase in the height of the adjacent GP. Note the significant

increase in number of the proliferative (PZ) and hypertrophic (HZ) chondrocytes in each column. **c** Photomicrographs of the growth plate of the intact tibia. There is no difference between the N/S-treated GP and the intact GP

and stained the proliferating and hypertrophic chondrocytes. The distribution of the Indian ink within the GP was not homogeneous, and an unstained area was distinguishable under low-power magnification. Under high-power magnification, however, the dye reached more or less into the deep layer of the GP. Some of the dye appeared to pass through the GP to reach into the primary spongiosa of the metaphysis (Fig. 5b).

pQCT data

Trabecular volumetric BMD (mg/cm^3) of the proximal metaphysis of IGF-I-infused tibiae was significantly higher than that of the control tibiae both in the 2-week group ($p=0.012$) and 4-week group ($p<0.001$) (Table 3). No

significant difference was seen in the 1-week treatment group ($p=0.18$).

Discussion

We describe herein a method for controlling local bone growth at the level of the individual GP. The continuous infusion of IGF-I into the proximal epiphysis of the tibia promoted longitudinal bone growth: 1 mm in the 2-week treatment and 2 mm in the 4-week treatment. This promotion of bone growth was associated with an increase in the number of bone trabeculae in the epiphysis, an increase in the thickness of the GP, an increase in the number of the proliferating and hypertrophic chondrocytes in each column

Table 2 Effect of the continuous local infusion of IGF-I on the growth-plate of the proximal tibia (NS not significant)

Experimental groups ^a	N/S	IGF-I	<i>p</i> value
One-week group			
Total height (μm)	714.6±14.9	715.6±17.3	NS
RZ height (μm)	78.1±20.6	78.5±22.8	NS
PZ height (μm)	382.8±21.9	382.8±22.7	NS
PZ cell number	30.0±1.6	30.5±2.4	NS
HZ height (μm)	253.8±12.8	254.2±16.5	NS
HZ cell number	12.0±1.4	12.7±0.9	NS
Two-week group			
Total height (μm)	549.6±15.2	586.9±8.1	<0.004
RZ height (μm)	49.9±1.8	49.7±1.9	NS
PZ height (μm)	290.1±15.2	316.4±8.1	<0.017
PZ cell number	29.6±1.4	33.6±1.5	<0.005
HZ height (μm)	209.5±5.4	220.7±5.4	<0.023
HZ cell number	9.4±0.9	11.6±0.9	<0.04
Four-week group			
Total height (μm)	511.2±8.4	556.6±20.7	<0.01
RZ height (μm)	22.5±1.4	21.2±1.2	NS
PZ height (μm)	279.6±8.2	304.6±16.9	<0.03
PZ cell number	26.8±1.6	30.6±0.9	<0.009
HZ height (μm)	209.2±3.6	230.9±4.9	<0.003
HZ cell number	9.4±1.1	12.2±1.3	<0.03

^a RZ, Resting zone; PZ, proliferating zone; HZ, hypertrophic zone. RZ = GP – (PZ + HZ)

and an increase in the BMD and bone mineral content (BMC) of the proximal metaphysis of the tibia bone. Our results are consistent with those of Ohlsson et al., who reported that in the hypophysectomised rat IGF-I increased the width of the epiphyseal GP by increasing the number of proliferating cells [14]. The results of our Indian ink study suggest that locally infused IGF-I mostly stayed within the epiphysis, although a portion did infiltrate into the GP, stimulating the growth and differentiation of physeal chondrocytes. The fibula in the same limb did not show any overgrowth, suggesting that the locally infused IGF-I acted only at the adjacent GP.

IGF-I, also called as somatomedin C, is a single-chain polypeptide of approximately 7.5 kDa that circulates in the plasma at a concentration of 20–80 nM in humans [17]. In the original ‘somatomedin hypothesis’, IGF-I was believed to be a mediator of pituitary GH. Although the mode of action of IGF-I remains open to discussion [20], it has been proposed that the primary effect of GH is to stimulate IGF-I production by the hepatocytes; as such, IGF-I is responsible for stimulating the longitudinal expansion of GPs in an endocrine fashion [3]. More recent work has suggested that GH may act directly on the GP to amplify the production of chondrocytes from germinal zone precursors and then to induce local IGF-I synthesis, which is thought to stimulate the clonal expansion of chondrocyte columns in an autocrine/paracrine manner [13].

The general administration of an over-dose of IGF-I should enhance systemic bone growth and may produce undesirable side effects. For example, high concentrations of serum IGF-I have been shown to be a major risk factor for the development

of breast, prostate and colon cancers [4, 6, 15]. We believe that the locally targeted injection of low-dose IGF-I is a more effective and appropriate method to avoid adverse events.

Previous studies in humans and experimental animals [11, 12, 18, 19, 25] have shown that systemic administration of IGF-I enhances not only systemic bone growth but also increases BMD in the metaphysis. There has been no report on the local effect IGF-I on the trabecular bone of the epiphysis and metaphysis. In our study, trabecular volumetric BMD of the proximal metaphysis increased following the continuous infusion of IGF-I into the epiphysis. The exact mechanism by which this effect is achieved is not known, but the Indian ink study suggests the possibility that IGF-I infused into the epiphysis passed through the GP and reached the metaphysis, where it acted on bone formation directly.

In conclusion, we have demonstrated that continuous local infusion of IGF-I into the epiphysis is a valid approach for delivering this protein into the selected GP and to control the growth rate and differentiation of physeal chondrocytes. In our preliminary experiment with this method, IGF-I was the most effective of the various growth factors and cytokines tested (data not shown). In terms of clinical application, this method does not require major surgery because human patients can use an extracorporeal pump instead of an osmotic pump. The patients also can avoid osteotomy and the long-term application of a bulky external fixation apparatus. We believe that this method will soon be applied to treat LLD in the field of pediatric orthopaedics.

Fig. 5 **a** Frontal section of the proximal tibia after continuous infusion of Indian ink. The bone-marrow cavity of the epiphysis was almost completely filled with the dye. **b** Photomicrograph (40×) of the GP after continuous infusion of the Indian ink into the epiphysis. The dye infiltrated into the GP and stained the proliferating and hypertrophic chondrocytes. Some of the dye seemed to reach into the primary spongiosa of the metaphysis

Table 3 Trabecular bone mineral density (mg/cm³) of the proximal metaphysis of the tibiae as measured with peripheral quantitative computerised tomography

Group	Number	N/S	IGF-I	<i>p</i> value
One week	5	92.5±19.6	101.4±17.6	0.180
Two weeks	5	148.6±36.0	164.6±32.2	<0.012
Four weeks	14	146.0±35.1	159.0±35.7	<0.001

Acknowledgements This research was supported, in part, by a grant from The Uehara Memorial Foundation 2007. We thank Toshiaki Sano, Department of Human Pathology, The University of Tokushima Graduate School, for his help in preparing the histological sections, Takashi Watanabe, Fuji Memorial Research Institute, Otsuka Pharmaceutical Company for his help in making the bone mineral density measurements and the Fujisawa Chemical Co for the donation of IGF-I.

References

1. Belthur MV, Bradish CF, Gibbons PJ (2005) Late orthopaedic sequelae following meningococcal septicaemia. A multicentre study. *J Bone Joint Surg Br* 87:236–240
2. Buckley SL, Smith G, Sponseller PD, Thompson JD, Griffin PP (1990) Open fractures of the tibia in children. *J Bone Joint Surg Am* 72:1462–1469
3. Daughaday WH, Hall K, Raben MS, Salmon WD Jr, van den Brande JL, van Wyk JJ (1972) Somatomedin: proposed designation for sulphation factor. *Nature* 235:107
4. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M (1998) Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* 279:563–566
5. Grimer RJ, Belthur M, Carter SR, Tillman RM, Cool P (2000) Extendible replacements of the proximal tibia for bone tumours. *J Bone Joint Surg Br* 82:255–260
6. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 351:1393–1396
7. Herring JA, Tachdjian MO (2002) Tachdjian's pediatric orthopaedics, vol. 2, 3rd edn. Saunders, Philadelphia, pp 1058–1095
8. Hunziker EB, Wagner J, Zapf J (1994) Differential effects of insulin-like growth factor I and growth hormone on developmental stages of rat growth plate chondrocytes in vivo. *J Clin Invest* 93:1078–1086
9. Kojimoto H, Yasui N, Goto T, Matsuda S, Shimomura Y (1988) Bone lengthening in rabbits by callus distraction. The role of periosteum and endosteum. *J Bone Joint Surg Br* 70:543–549
10. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A (2001) Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol* 229:141–162
11. Mohan S, Richman C, Guo R, Amaar Y, Donahue LR, Wergedal J, Baylink DJ (2003) Insulin-like growth factor regulates peak bone mineral density in mice by both growth hormone-dependent and -independent mechanisms. *Endocrinology* 144:929–936
12. Mueller K, Cortesi R, Modrowski D, Marie PJ (1994) Stimulation of trabecular bone formation by insulin-like growth factor I in adult ovariectomized rats. *Am J Physiol* 267:E1–E6
13. Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT, Słotweg MC (1998) Growth hormone and bone. *Endocr Rev* 19:55–79
14. Ohlsson C, Nilsson A, Isaksson O, Lindahl A (1992) Growth hormone induces multiplication of the slowly cycling germinal cells of the rat tibial growth plate. *Proc Natl Acad Sci USA* 89:9826–9830
15. Palmqvist R, Hallmans G, Rinaldi S, Biessy C, Stenling R, Riboli E, Kaaks R (2002) Plasma insulin-like growth factor 1, insulin-like growth factor binding protein 3, and risk of colorectal cancer: a prospective study in northern Sweden. *Gut* 50:642–646

16. Reynolds DA (1981) Growth changes in fractured long-bones: a study of 126 children. *J Bone Joint Surg Br* 63:83–88
17. Rosen CJ (1999) Serum insulin-like growth factors and insulin-like growth factor-binding proteins: clinical implications. *Clin Chem* 45–48:1384–1390
18. Spencer EM, Liu CC, Si EC, Howard GA (1991) In vivo actions of insulin-like growth factor-I (IGF-I) on bone formation and resorption in rats. *Bone* 12:21–26
19. Wakisaka A, Tanaka H, Barnes J, Liang CT (1998) Effect of locally infused IGF-I on femoral gene expression and bone turnover activity in old rats. *J Bone Miner Res* 13:13–19
20. Wang J, Zhou J, Bondy CA (1999) Igfl promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. *Faseb J* 13–14:1985–1990
21. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ (1996) Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 335:1363–1367
22. Xian CJ, Howarth GS, Cool JC, Foster BK (2004) Effects of acute 5-fluorouracil chemotherapy and insulin-like growth factor-I pretreatment on growth plate cartilage and metaphyseal bone in rats. *Bone* 35:739–749
23. Yasui N, Sato M, Ochi T, Kimura T, Kawahata H, Kitamura Y, Nomura S (1997) Three modes of ossification during distraction osteogenesis in the rat. *J Bone Joint Surg Br* 79:824–830
24. Yasui N, Kojimoto H, Sasaki K, Kitada A, Shimizu H, Shimomura Y (1993) Factors affecting callus distraction in limb lengthening. *Clin Orthop* 293:55–60
25. Zhao G, Monier-Faugere MC, Langub MC, Geng Z, Nakayama T, Pike JW, Chernausk SD, Rosen CJ, Donahue LR, Malluche HH, Fagin JA, Clemens TL (2000) Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology* 141:2674–2682