Elevated serum IGF-1 levels synergize PTH action on the skeleton only when the tissue IGF-1 axis is intact

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Elevated serum IGF-1 levels synergize PTH action on the skeleton only when the tissue IGF-1 axis is intact

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Abstract

There is growing evidence that IGF-1 and PTH have synergistic actions on bone and that part of the anabolic effects of PTH are mediated by local production of IGF-1. In this study we analyzed the skeletal response to PTH in mouse models with manipulated endocrine or autocrine/paracrine IGF-1. We utilized mice carrying a hepatic IGF-1 transgene (HIT), which results in a 3-fold increase in serum IGF-1 levels and normal tissue IGF-1 expression, and IGF-1 null mice with blunted IGF-1 expression in tissues but 3-fold increases in serum IGF-1 levels (KO-HIT). Evaluation of skeletal growth showed that elevations in serum IGF-1 in mice with igf-1 gene ablation in all tissues except the liver (KO-HIT) resulted in a restoration of skeletal morphology and mechanical properties by adulthood. Intermittent PTH treatment of adult HIT mice resulted in increases in serum osteocalcin levels, femoral total cross-sectional area, cortical bone area and cortical bone thickness, as well as bone mechanical properties. We found that the skeletal response of HIT mice to PTH was significantly higher than that of control mice, suggesting synergy between IGF-1 and PTH on bone. In sharp contrast, although PTH-treated KO-HIT mice demonstrated an anabolic response in cortical and trabecular bone compartments compared to vehicle treated KO-HITs, their response was identical to that of PTH-treated control mice. We conclude that 1) in the presence of elevated serum IGF-1 levels, PTH can exert an anabolic response in bone even in the total absence of tissue IGF-1 and, 2) elevations in serum IGF-1 levels synergize PTH action on bone only if the tissue IGF-1 axis is intact, thus enhancement of PTH anabolic actions is tissue IGF-1-dependent.

Keywords

IGF-1; Bone; transgenic mice; igf-1ko; micro-computed tomography; endocrine IGF-1; intermittent PTH

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is an important regulator of skeletal growth and development. IGF-1 acts in an endocrine/autocrine/paracrine fashion. Studies with transgenic and knockout mice have proven that IGF-1 modulates linear and transverse bone growth, as well as bone mineralization. Endocrine (serum) IGF-1 is mainly secreted by the

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liver and largely regulated by pituitary growth hormone (GH), while autocrine/paracrine (tissue) IGF-1 is regulated by a number of tissue factors as well as by GH. In our previous studies we performed skeletal characterization of the liver IGF-1 deficient (LID) mice, which exhibit 75% reductions in serum IGF-1 levels but have otherwise normal skeletal expression of IGF-1 (1). We found that ***reduced*** serum levels of IGF-1 in male LID mice were associated with the development of slender bones during growth. The slender phenotype resulted mostly from inhibition of transverse bone growth (sub-periosteal expansion); there were only minimal affects on linear growth. In a subsequent study we assessed how ***elevated*** serum IGF-1 affected skeletal development of females in the presence or absence of tissue IGF-1 expression (2). We studied mice carrying a hepatic IGF-1 transgene (HIT), which exhibit 3-fold increases in serum IGF-1 levels and normal tissue IGF-1 expression, as well as IGF-1 null mice with blunted IGF-1 expression in tissues but 3-fold increases in serum IGF-1 levels (KO-HIT). We found that increased serum IGF-1 in the presence of normal tissue IGF-1 levels (HIT mice) led to enhancement of morphological and mechanical properties of bone during development, such that at 16 weeks of age bones were longer, cortices were thicker, and tissue-level mineralization increased. Strikingly, we demonstrated that in the total absence of tissue IGF-1 gene expression, elevated serum levels of IGF-1 (KO-HIT mice) restored body weight, augmented growth rate, and led to normal skeletal development (2). Thus, an increase in serum IGF-1 was able to counteract the deficits in body size and skeletal development associated with an absence of tissue IGF-1.

Parathyroid hormone (PTH) exerts both anabolic and catabolic (3) effects on the skeleton in humans and animals. Upon binding to its receptor on osteoblasts, PTH initiates a signaling cascade leading to activation of cAMP protein kinase A (PKA) and phosphoinositide protein kinase C (PKC) (3,4). These in turn, eventually lead to transcriptional activation of genes involved in osteoblast differentiation and activity, such as RUNX2, osteocalcin, ALP, or in osteoclast activity i.e. RANKL (3,4). There is growing evidence that IGF-1 and PTH have synergistic actions on bone and that part of the anabolic effects of PTH are in fact mediated by local production of IGF-1 (5-9).

Intermittent administration of PTH has been shown to activate anabolic pathways in bone, and previous studies have shown that these pathways are mediated in part through the insulin-like growth factor-1 signaling system (IGF-1) (6,8,9). As such, the anabolic effects of PTH on the skeleton of the IGF-1 null mice were suppressed compared to controls (5,7). Similarly, PTH effects on the skeleton of mice with osteoblast-specific IGF-1 receptor gene ablation (IGF-1RobKO) were blunted in cortical bone and partially inhibited in trabecular bone (10). Despite the valuable insights gained from those studies, the mechanisms by which IGF-1 mediates PTH anabolic effects on the skeleton are still obscure. Moreover, in humans there is significant heterogeneity in the skeletal response to PTH. Previously, in a randomized controlled study, we noted that baseline IGF-1 levels did not predict the changes in trabecular bone mass in response to PTH, nor did the change in circulating IGF-1 (11). Thus, the role of circulating and skeletal IGF-1 in the skeletal response to PTH remains unclear. In the present study we set out to determine if PTH exerts its anabolic effects on bone in the total absence of autocrine/paracrine IGF-1, and to evaluate if PTH, in the presence of elevated serum IGF-1, can exert synergistic anabolic effects on the adult skeleton.

MATERIALS AND METHODS

1. Animals

Male HIT and KO-HIT mice (on FVB/N background) were generated as previously described (12). Male mice were housed 4 per cage in a clean mouse facility, fed standard

mouse chow (Purina Laboratory Chow 5001; Purina Mills) and water ad libitum, and kept on a 12 hour light:dark cycle. Animal care and maintenance were provided through the Mount Sinai School of Medicine's AAALAC Accredited Animal Facility. All procedures were approved by the Animal Care and Use Committee of the Mount Sinai School of Medicine.

2. Serum hormones

Mice were bled through the mandibular vein and serum samples were collected between 7-9 AM on a fed state at the indicated ages. Serum IGF-1 and osteocalcin levels were determined using commercial radio-immunoassays as previously described (13-15).

3. Micro-Computed Tomography

Cortical bone morphology at the mid-femoral diaphysis, and trabecular bone volume fraction and microarchitecture in the excised distal femoral metaphysis were assessed as previously described (1). Femora were reconstructed at an 8.7 micron voxel resolution. For trabecular bone regions, we assessed the bone volume fraction (BV/TV), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N), and trabecular spacing (Tb.Sp, μm). For cortical bone at the femoral midshaft, we measured the average total cross-sectional area inside the periosteal envelope (Tt.Ar, mm^2), the cortical bone and medullary area within this same envelope (Ct.Ar, mm^2 and Ma.Ar mm^2, respectively), the relative cortical area (RCA; Ct.Ar/Tt.Ar), the average cortical thickness (Ct.Th, μm) and the polar moment of inertia (J_o). Robustness was defined as Tt.Ar/Le; a higher ratio denotes a more robust, less slender bone. All regions of analysis were standardized according to anatomical landmarks. Tissue mineral density (TMD) was defined as the average mineral value of the bone voxels and expressed in hydroxyapatite density equivalents (HA mg/cm^3).

4. Mechanical testing

Mouse femora from 16 week-old control, HIT and KO-HIT mice were tested to failure by 4-point bending using a servohydraulic materials testing system (Instron Corp., Canton, MA, USA). From these tests measurements of whole-bone stiffness, maximum load, post-yield deflection and work-to-failure were calculated. Femora were placed with the anterior surface down on two lower supports. The two lower and two upper supports were set apart by 6.35 and 2.2 mm, respectively. Loading was centered over the midshaft, at a displacement of 0.05 mm/s until failure. All mechanical properties were calculated from the load-displacement curves, as described previously (16).

5. *PTH treatment*

Twelve-week old male mice of all genotypes were treated for 4 weeks with PTH (50 ng/g body weight, Bachem Bioscience, Inc.). PTH was injected intra-peritoneally (5mg/mL in saline solution with 2% heat inactivated mouse serum (Innovative Research C57BL6 Mouse Serum) 7 days a week for 4 weeks.

6. *Histomorphometry*

Animals were injected with calcein (20mg/kg) 12 and 2 days before sacrifice. Right femora from PTH treated male mice of all genotypes were fixed in 10% neutral buffered formalin and embedded in polymethylmethacrylate. Thick sections (~120 um) were cut using a low-speed band saw and diamond-coated wafering blade (Buehler, Inc., Lake Bluff, Illinois). Sections were adhered to glass slides and polished to 50 um for fluorescent imaging. Histomorphometric parameters were measured on the periosteal surface at the femoral midshaft. Measurements of labeled perimeter (L.Pm), bone formation rate (BFR) and

mineral apposition rate (MAR) were made using an OsteoMeasure system (Osteometrics, Atlanta, GA, USA) (17).

7. Statistical analysis

All bone traits, body weight (BW), serum hormones, and micro-computed tomography (micro-CT) measurements are presented as means ± SEM. One-way analysis of variance (ANOVA) was used to test for differences among groups at each age (Statview software version 5.0, SAS Institute Inc.). If ANOVA revealed significant effects, the means were compared by Fisher's test, considering $p<0.05$ as significant.

RESULTS

Elevated serum IGF-1 levels promote skeletal acquisition independent of local IGF-1 production

Male HIT and KO-HIT mice were generated as described previously (18). Both HIT and KO-HIT mice show 2 fold increases in serum IGF-1 during growth and development (Figure 1A), owing to the rat IGF-1 transgene expressed specifically in liver. Similar to our findings in females (2), male HIT mice, with elevated serum IGF-1 but otherwise normal autocrine/paracrine IGF-1 expression, exhibited greater body weights (Figure 1B) than controls: +4.6% at 4 weeks of age ($p<0.05$), +22.3% by 8 weeks ($p<0.0001$) and +25.4% by 16 weeks ($p<0.0001$). On the other hand, KO-HIT male mice, with elevated serum IGF-1 but no autocrine/paracrine IGF-1 gene expression, were smaller (−18%) at 4 weeks of age, but exhibited a "catch-up" growth phenotype, such that by 8 weeks their body weight reached its plateau and by 16 weeks did not differ significantly from that of controls. Body length (Figure 1C) and femoral length (Figure 1D) in HIT and KO-HIT mice followed body weight, such that HIT mice were longer than control mice at 8 and 16 weeks of age, while KO-HIT mice, were smaller than controls, and reached adult control length by 8 weeks of age.

To understand how modulations in serum and tissue IGF-1 affect skeletal morphology in male mice, we performed a longitudinal analysis of the femoral mid-diaphyseal cortical bone from 4 to 16 weeks of age. Similar to what we found in females (2), elevations in serum IGF-1 in HIT male mice led to increases in cross-sectional total area (Tt.Ar) (+16% at 8 and +19% at 16 weeks of age), cortical area (Ct.Ar) (+29% at 8 and +16% at 16 weeks of age), and cortical thickness (Ct.Th) (+19% at 8 and +7.5% at 16 weeks of age) at 8 and 16 weeks of age (Figure 2). Tissue mineral density (TMD) increased similarly with age in all groups until 16 weeks when KO-HIT mice showed significant increases (+3.3%) as compared to controls. Despite 2-fold increases in serum IGF-1 levels KO-HIT male mice exhibited reduced Tt.Ar (−19 %) and Ct.Ar (−18 %) at 4 weeks, indicating that tissue IGF-1 is essential for establishment of skeletal morphology during early postnatal growth. Nonetheless, KO-HIT male mice showed rapid increase in linear (+1.8 fold) and transverse (+5.9 fold) growth rates between 4 to 8 weeks of age, such that by 16 weeks their cortical bone morphology was similar to controls (Figure 3).

Trabecular architecture was assessed at the femoral distal metaphysis of 4, 8 and 16 weeks old mice and revealed minor changes between groups. We found that trabecular bone volume / total volume (BV/TV) peaked at 8 weeks of age for all groups, and decreased by ~13-27% with age (Figure 4). At 16 weeks of age both HIT and KO-HIT mice exhibited decreases in trabecular number (Tb.N) and subsequent increases in trabecular spacing (Tb.Sp). However, while trabecular thickness (Tb.Th) in HIT mice did not differ from controls, KO-HIT male mice showed significant decreases in Tb.Th at 16 weeks of age.

Whole-bone mechanical properties examined by 4-point bending tests of cortical bone at the femoral midshaft, showed a significant increase in maximum load (+19%) and stiffness (+18%) in HIT mice at 16 weeks of age (Figure 5), as expected from the enhanced cortical architecture of these mice. KO-HIT mice, however, showed increases in maximum load only at 8 weeks (+28%), but otherwise were similar to controls (data not shown).

Elevated levels of serum IGF-1 synergize PTH anabolic actions on bone

To understand how modulation of serum and tissue IGF-1 affects the anabolic actions of intermittent PTH on the skeleton, we treated control, HIT and KO-HIT mice from 12-16 weeks of age with PTH. As shown in Table 1, body weight and femoral length were not affected by PTH treatment in all groups. Similarly, serum IGF-1 levels were not affected by 4 weeks of intermittent PTH treatment. On the other hand, serum osteocalcin levels increased significantly in all groups treated with PTH (Table 1).

Analysis of cortical bone following intermittent PTH treatment revealed increases in total cross-sectional area (Tt.Ar), Ct.Ar and Ct.Th in both HIT and KO-HIT mice, and an increase in relative cortical area (RCA) in HIT mice (+10.5%) as compared to vehicle treated mice. The analysis of bone robustness (Tt.Ar/length) and polar moment of inertia also revealed that PTH treatment increased bone robusticity in both HIT and KO-HIT mice (+10%) as compared to vehicle treated mice. These increases in cortical bone traits in response to PTH treatment were in accordance with increased mechanical properties.

HIT and KO-HIT male mice showed increases in maximum load (~20%) and stiffness (~24%) (Table 1), suggesting that PTH anabolic actions on cortical bone in the absence of tissue IGF-1 can be compensated by elevations in serum IGF-1 levels. It is important to note that bone traits of PTH-treated HIT mice were significantly higher than PTH-treated control mice (Table 1, significance labeled with c), while no differences were detected between PTH-treated KO-HIT and control mice.

Analysis of trabecular bone response to intermittent PTH revealed significant increases in BV/TV in both HIT and KO-HIT mice (+22%, and +25%, respectively), accompanied by an increase in Tb.N (+20%, and +18%, respectively) and a decrease in Tb.Sp (−21%, and −20%, respectively) (Table 1). Unlike the synergy between PTH and IGF-1 that was observed in the cortical envelope of HIT mice, trabecular bone exhibited no enhancement of morphological traits when comparing PTH-treated HIT and control mice. These data indicate that PTH response in the trabecular bone compartment is minimally mediated by serum/tissue IGF-1.

Histomorphometric analysis at the femoral mid-shaft was performed after 4 weeks of intermittent PTH treatment, at 16 weeks of age (Table 2). We found that % labeled perimeter (%L.Pm) and bone formation rates (BFR) at the periosteal surface increased in all groups treated with PTH. This was in accordance with increased serum osteocalcin levels following intermittent PTH treatment. These results, although not statistically significant, are in accordance with increased morphological traits assessed by micro-CT analyses.

DISCUSSION

In this study we used mouse models in order to manipulate the endocrine and autocrine/paracrine modes of IGF-1 action. We found that elevations in serum IGF-1 levels during growth in male mice lead to enhanced bone accrual and to the development of a robust, mechanically superior bone (HIT mice). However, elevations in serum IGF-1 in mice with *Igf1*gene ablation in all tissues except the liver (KO-HIT) demonstrated no morphological or mechanical gains beyond the normal skeletal morphology and mechanical properties

exhibited by adult control mice. These findings are in agreement with our previous study of the development of the female HIT and KO-HIT skeleton (2), which demonstrated augmentation of all skeletal properties of HIT females and normalization of KO-HIT females skeletal properties to those of controls females by adulthood. Together, data from male (current study) and female mice (2) suggest that acceleration of skeletal acquisition through increased endocrine IGF-1 requires autocrine/paracrine IGF-1. The fact that the linear and transverse femoral growth rates of mice with elevated serum IGF-1 levels (HIT and KO-HIT), were statistically indistinguishable between 4 and 8 weeks of age (~2-4-fold increases), indicates that the lack of skeletal acceleration in the absence of autocrine/paracrine IGF-1 is due to abrogation of early growth (before 4 weeks) processes, which result in an attenuated “start point” for enhanced skeletal acquisition by the hepatic IGF-1 transgene.

A second focus of this study was the interaction between serum/tissue IGF-1 and PTH on bone accrual. Here we show that increases in serum IGF-1 levels (HIT mice) synergized PTH anabolic effects on bone, such that PTH-treated HIT mice exhibited further increases in several skeletal phenotypes compared to PTH-treated control mice. Four weeks of treatment with intermittent PTH of adult mice did not alter body weight, femur length, or serum IGF-1 levels, but increased significantly serum osteocalcin (a marker of bone formation). This was accompanied by augmentation of cortical and trabecular bone morphological indices. HIT males showed increases in Tt.Ar., Ct.Ar., and Ct.Th., leading to increased whole bone stiffness and max load as evaluated by 4-point bending. Unlike the IGF-1 null mice, which had no skeletal response to PTH (5,7), KO-HIT mice showed an anabolic response in both cortical and trabecular bone compartments. However, this anabolic response did not differ from PTH-treated control mice, even though serum IGF-1 levels were similar to those of HIT mice. It should be noted that although histomorphometric indices of increased cortical bone accrual (L.Pm and BFR) were increased in PTH-treated HIT and KO-HIT mice, these differences were not statistically significant. This was not unexpected as significant cortical bone enhancement from PTH treatment, with no corresponding changes in histomorphometric indices, has been reported previously (10,19) and appears to arise from increased sample variation as a result of PTH action on cortical surfaces. Together, these observations and published data (5,7) suggest that: 1) PTH can exert anabolic effect in bone in the absence of tissue IGF-1 but only in the presence of elevated serum IGF-1 levels and, 2) elevations in serum IGF-1 levels synergize PTH action on bone only if the tissue IGF-1 axis is intact. Thus, enhancement of PTH anabolic actions is tissue IGF-1-dependent. It should be noted that PTH has significant activity in the kidney both to enhance tubular reabsorption of calcium and to stimulate production of 1,25 dihydroxyvitamin D. The latter directly promotes calcium absorption in the intestine. High circulating levels of IGF-1 also can increase glomerular filtration rates and independently IGF-I stimulates 1 alpha hydroxylase activity in the kidney (20-25). Therefore, it is conceivable that the PTH anabolic effects in HIT and KO-HIT mice are also mediated through improved calcium balance.

Clinical studies show significant heterogeneity in the skeletal response to intermittent PTH treatment, implying that local or systemic factors contribute to the variation in anabolic effects of this agent (reviewed in (3,4,26-30)). One such factor may be IGF-1. Indeed, PTH treatment of IGF-1 null mice failed to induce anabolic responses in bone (5,7). Similarly, PTH treatment of IGF-1RobKO mice with selective depletion of the IGF-1 receptor in mature osteoblasts (but normal levels of serum IGF-1) failed to increase bone formation or resorption markers (10). Furthermore, mice lacking the insulin-like receptor substrate-1 (IRS-1), a secondary messenger that transmits IGF-1R signaling, did not respond to 4 weeks intermittent PTH treatment (31). In contrast, PTH treatment of the LID mice produced skeletal anabolic responses that were similar or greater than controls (13), despite a 75%

reduction in serum IGF-1 levels. Together these studies suggest that autocrine/paracrine actions of IGF-1 (via the IGF-1 receptor) in bone play important roles in mediating PTH anabolic actions. The current study extends our understanding of these IGF-1/PTH interactions by demonstrating that PTH can in fact exert robust anabolic responses on bones in the absence of autocrine/paracrine IGF-1 (KO-HIT mice) as long as serum IGF-1 levels are high. The reasons for this endocrine effect are unclear at the present time. One possibility is that very high circulating concentrations of IGF-1 may lead to tissue redistribution, thereby compensating for the absence of local IGF-1 expression. It is also conceivable that the very high levels of hepatic and circulating IGF-1 in the KO-HIT mice induce expression of IGF-binding proteins (IGFBPs) in bone that might promote IGF-1 activity. For example, IGFBP-2 and IGFBP-5 are up-regulated in response to IGF-1, and IGFBP-2 has been shown to have anabolic properties in bone when coupled to IGF-1 (32-34). Notwithstanding, the interaction between circulating IGF-1 and the skeletal response to PTH administration in the HIT mice is impressive and suggests that both tissue IGF-1 and the circulating pool of IGF-1 contribute to a robust skeletal phenotype. Although our previous human study (11) was unable to demonstrate a relationship between the trabecular response to PTH by QCT, and circulating IGF-1, the animals models presented in this study differ dramatically in their relative serum IGF-1 levels. Indeed, the only time circulating IGF-1 concentrations reach levels attained by the HIT model, are during a short span of puberty. As such it would be interesting to determine whether the skeletal response to PTH during transient periods of very high circulating IGF-1 and rapid growth compare with the skeletal responsiveness to PTH administration in aging animals.

Finally, it is conceivable that in our mouse models there may be significant compensatory changes, particularly in response to very high circulating levels of IGF-I. Serum levels of IGF-I in these two strains are at least twice those observed normally in wild type mice and markedly greater than during maximal pubertal growth. Further studies are needed to define how these changes affect the skeletal response to PTH.

In summary, we have shown that the skeletal response to PTH is tissue IGF-1 dependent and that circulating levels of IGF-1 can only compensate for the absence of skeletal IGF-1 when concentrations are quite high. The mechanism for this compensation requires further study.

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References

1. Yakar S, Canalis E, Sun H, Mejia W, Kawashima Y, Nasser P, Courtland HW, Williams V, Bouxsein M, Rosen C, Jepsen KJ. Serum IGF-1 determines skeletal strength by regulating subperiosteal expansion and trait interactions. J Bone Miner Res. 2009; 24(8):1481–92. [PubMed: 19257833]
2. Elis, Sebastien, 1; C, H-W.; Wu, Yingjie, 1; Rosen, Clifford J., 2; Sun, Hui, 1; Jepsen, Karl J., 3; Majeska, Robert J., 4; Yakar, Shoshana, 1. Elevated serum levels of IGF-1 are sufficient to establish normal body size and skeletal properties, even in the absence of tissue IGF-1. JBMR. 2009 in press.

3. Poole KE, Reeve J. Parathyroid hormone - a bone anabolic and catabolic agent. Curr Opin Pharmacol. 2005; 5(6):612–7. [PubMed: 16181808]
4. Martin TJ, Quinn JM, Gillespie MT, Ng KW, Karsdal MA, Sims NA. Mechanisms involved in skeletal anabolic therapies. Ann N Y Acad Sci. 2006; 1068:458–70. [PubMed: 16831943]
5. Bikle DD, Sakata T, Leary C, Elalieh H, Ginzinger D, Rosen CJ, Beamer W, Majumdar S, Halloran BP. Insulin-like growth factor I is required for the anabolic actions of parathyroid hormone on mouse bone. J Bone Miner Res. 2002; 17(9):1570–8. [PubMed: 12211426]
6. Canalis E, Centrella M, Burch W, McCarthy TL. Insulin-like growth factor I mediates selective anabolic effects of parathyroid hormone in bone cultures. J Clin Invest. 1989; 83(1):60–5. [PubMed: 2910920]
7. Miyakoshi N, Kasukawa Y, Linkhart TA, Baylink DJ, Mohan S. Evidence that anabolic effects of PTH on bone require IGF-I in growing mice. Endocrinology. 2001; 142(10):4349–56. [PubMed: 11564695]
8. Pfeilschifter J, Laukhuf F, Muller-Beckmann B, Blum WF, Pfister T, Ziegler R. Parathyroid hormone increases the concentration of insulin-like growth factor-I and transforming growth factor beta 1 in rat bone. J Clin Invest. 1995; 96(2):767–74. [PubMed: 7635970]
9. Watson P, Lazowski D, Han V, Fraher L, Steer B, Hodsman A. Parathyroid hormone restores bone mass and enhances osteoblast insulinlike growth factor I gene expression in ovariectomized rats. Bone. 1995; 16(3):357–65. [PubMed: 7786639]
10. Wang Y, Nishida S, Boudignon BM, Burghardt A, Elalieh HZ, Hamilton MM, Majumdar S, Halloran BP, Clemens TL, Bikle DD. IGF-I receptor is required for the anabolic actions of parathyroid hormone on bone. J Bone Miner Res. 2007; 22(9):1329–37. [PubMed: 17539737]
11. Sellmeyer DE, Black DM, Palermo L, Greenspan S, Ensrud K, Bilezikian J, Rosen CJ. Hetereogeneity in skeletal response to full-length parathyroid hormone in the treatment of osteoporosis. Osteoporos Int. 2007; 18(7):973–9. [PubMed: 17333451]
12. Wu Y, Sun H, Yakar S, Leroith D. Elevated levels of IGF-1 in serum rescue the severe growth retardation of IGF-1 null mice. Endocrinology. 2009
13. Yakar S, Bouxsein ML, Canalis E, Sun H, Glatt V, Gundberg C, Cohen P, Hwang D, Boisclair Y, Leroith D, Rosen CJ. The ternary IGF complex influences postnatal bone acquisition and the skeletal response to intermittent parathyroid hormone. J Endocrinol. 2006; 189(2):289–99. [PubMed: 16648296]
14. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu JL, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-1 directly regulate bone growth and density. J Clin Invest. 2002; 110(6):771–81. [PubMed: 12235108]
15. Yakar S, Setser J, Zhao H, Stannard B, Haluzik M, Glatt V, Bouxsein ML, Kopchick JJ, LeRoith D. Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1-deficient mice. J Clin Invest. 2004; 113(1):96–105. [PubMed: 14702113]
16. Jepsen KJ, Hu B, Tommasini SM, Courtland HW, Price C, Terranova CJ, Nadeau JH. Genetic randomization reveals functional relationships among morphologic and tissue-quality traits that contribute to bone strength and fragility. Mamm Genome. 2007; 18(6-7):492–507. [PubMed: 17557179]
17. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res. 1987; 2(6):595–610. [PubMed: 3455637]
18. Wu Y, Sun H, Yakar S, LeRoith D. Elevated levels of insulin-like growth factor (IGF)-I in serum rescue the severe growth retardation of IGF-I null mice. Endocrinology. 2009; 150(9):4395–403. [PubMed: 19497975]
19. Iida-Klein A, Lu SS, Cosman F, Lindsay R, Dempster DW. Effects of cyclic vs. daily treatment with human parathyroid hormone (1-34) on murine bone structure and cellular activity. Bone. 2007; 40(2):391–8. [PubMed: 17056311]
20. Bianda T, Glatz Y, Bouillon R, Froesch ER, Schmid C. Effects of shortterm insulin-like growth factor-I (IGF-I) or growth hormone (GH) treatment on bone metabolism and on production of

1,25-dihydroxycholecalciferol in GH-deficient adults. J Clin Endocrinol Metab. 1998; 83(1):81–7. [PubMed: 9435420]

21. Menaa C, Vrtovsnik F, Friedlander G, Corvol M, Garabedian M. Insulinlike growth factor I, a unique calcium-dependent stimulator of 1,25-dihydroxyvitamin D3 production. Studies in cultured mouse kidney cells. J Biol Chem. 1995; 270(43):25461–7. [PubMed: 7592714]
22. Nesbitt T, Drezner MK. Insulin-like growth factor-I regulation of renal 25-hydroxyvitamin D-1-hydroxylase activity. Endocrinology. 1993; 132(1):133–8. [PubMed: 8419119]
23. Wong MS, Sriussadaporn S, Tembe VA, Favus MJ. Insulin-like growth factor I increases renal 1,25(OH)2D3 biosynthesis during low-P diet in adult rats. Am J Physiol. 1997; 272(6 Pt 2):F698–703. [PubMed: 9227629]
24. Wong MS, Tembe VA, Favus MJ. Insulin-like growth factor-I stimulates renal 1, 25-dihydroxycholecalciferol synthesis in old rats fed a low calcium diet. J Nutr. 2000; 130(5):1147–52. [PubMed: 10801911]
25. Zoidis E, Gosteli-Peter M, Ghirlanda-Keller C, Meinel L, Zapf J, Schmid C. IGF-I and GH stimulate Phex mRNA expression in lungs and bones and 1,25-dihydroxyvitamin D(3) production in hypophysectomized rats. Eur J Endocrinol. 2002; 146(1):97–105. [PubMed: 11751074]
26. Sato K. Therapeutic agents for disorders of bone and calcium metabolism. Development of nasal formulation of hPTH (1-34). Clin Calcium. 2007; 17(1):64–71. [PubMed: 17211095]
27. Compston JE. Skeletal actions of intermittent parathyroid hormone: effects on bone remodelling and structure. Bone. 2007; 40(6):1447–52. [PubMed: 17045858]
28. Thomas T. Intermittent parathyroid hormone therapy to increase bone formation. Joint Bone Spine. 2006; 73(3):262–9. [PubMed: 16563840]
29. Rosen CJ. The cellular and clinical parameters of anabolic therapy for osteoporosis. Crit Rev Eukaryot Gene Expr. 2003; 13(1):25–38. [PubMed: 12839095]
30. Swarthout JT, D'Alonzo RC, Selvamurugan N, Partridge NC. Parathyroid hormone-dependent signaling pathways regulating genes in bone cells. Gene. 2002; 282(1-2):1–17. [PubMed: 11814673]
31. Yamaguchi M, Ogata N, Shinoda Y, Akune T, Kamekura S, Terauchi Y, Kadowaki T, Hoshi K, Chung UI, Nakamura K, Kawaguchi H. Insulin receptor substrate-1 is required for bone anabolic function of parathyroid hormone in mice. Endocrinology. 2005; 146(6):2620–8. [PubMed: 15718274]
32. DeMambro VE, Clemmons DR, Horton LG, Bouxsein ML, Wood TL, Beamer WG, Canalis E, Rosen CJ. Gender-specific changes in bone turnover and skeletal architecture in igfbp-2-null mice. Endocrinology. 2008; 149(5):2051–61. [PubMed: 18276763]
33. Amin S, Riggs BL, Melton LJ 3rd, Achenbach SJ, Atkinson EJ, Khosla S. High serum IGFBP-2 is predictive of increased bone turnover in aging men and women. J Bone Miner Res. 2007; 22(6): 799–807. [PubMed: 17352648]
34. Conover CA, Johnstone EW, Turner RT, Evans GL, John Ballard FJ, Doran PM, Khosla S. Subcutaneous administration of insulin-like growth factor (IGF)-II/IGF binding protein-2 complex stimulates bone formation and prevents loss of bone mineral density in a rat model of disuse osteoporosis. Growth Horm IGF Res. 2002; 12(3):178–83. [PubMed: 12162999]

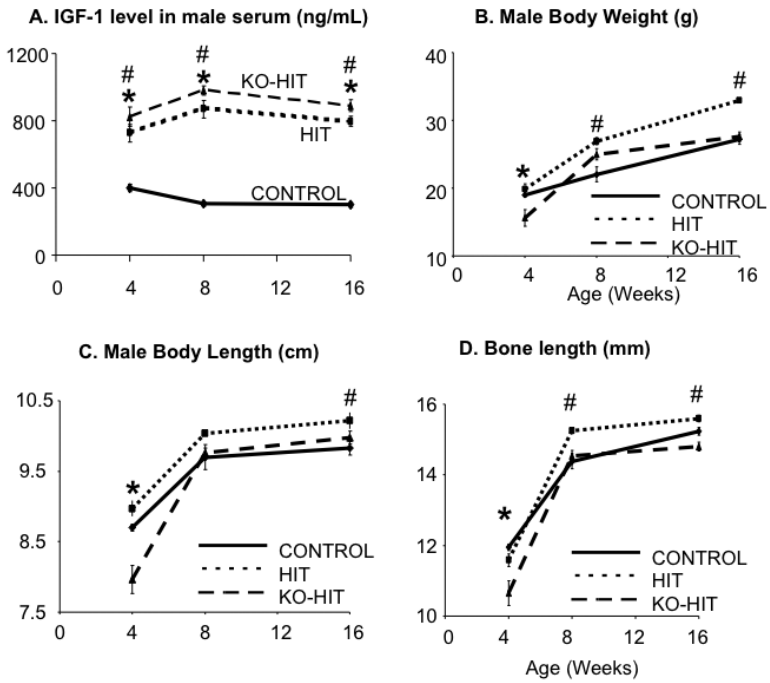

Figure 1. Growth characteristics of the HIT and KO-HIT male mice

(A) Serum IGF-1 levels were measured at 4, 8 and 16 weeks of age and show 2 fold increase in both HIT and KO-HIT mice as compared to controls. (B) Body weight of HIT mice increased significantly throughout growth as compared to control mice, while KO-HIT mice show reductions in body weight at 4 weeks but normalize (indistinguishable from controls) thereafter. (C) Body length (nose to anus) decreased significantly in 4 week old KO-HIT mice, but normalized at 8 weeks. HIT mice show significant increases in body length at 16 weeks of age. (D) Femur length (assessed by micro-CT) decreased significantly in 4 week-old KO-HIT mice, but normalized at 8 weeks. HIT mice show significant increases in bone length at 8 and 16 weeks of age. Data presented as mean +/− SEM of n=10-15 mice in each group at each time point. *-denotes $p<0.05$ comparing KO-HIT mice to Control. #-denotes $p<0.05$ comparing HIT mice to Control.

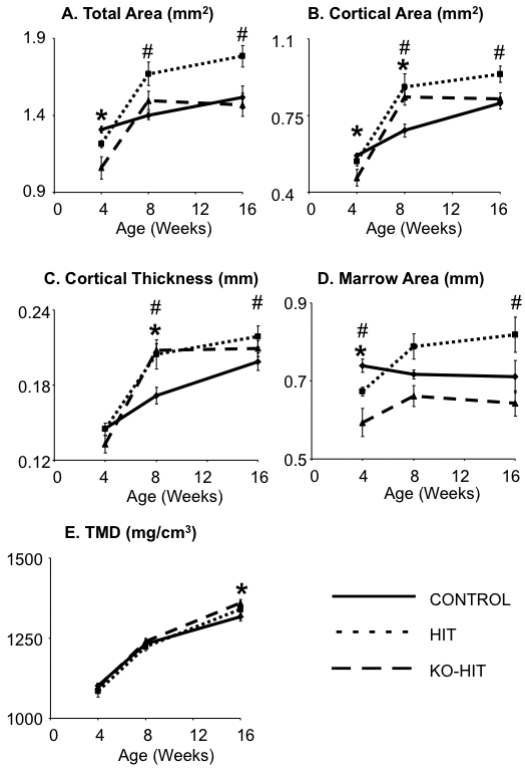

Figure 2.

Cortical bone morphology was analyzed at the femoral mid-shaft by micro-CT. (A), total cross-sectional area (Tt.Ar.) (B), cortical bone area (Ct.Ar.) (C), cortical bone thickness (Ct.Th.) (D), marrow area (Ma.Ar.) and (E), tissue mineral density (TMD) were measured at 4, 8, and 16 weeks of age. Data presented as mean +/− SEM of n=10-15 mice in each group at each time point. *-denotes $p<0.05$ comparing KO-HIT mice to Control. #-denotes $p<0.05$ comparing HIT mice to Control.

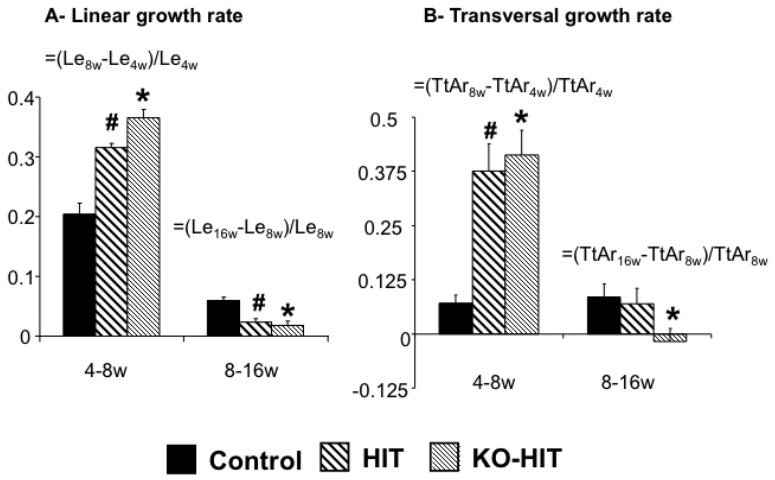

Figure 3. Linear and transverse bone growth rates

Linear growth rate indicated by increases in femur length (Le) (A) and transverse growth-rate indicated by increases in Tt.Ar (B) were analyzed over 2 growth periods (4-8 weeks and 8-16 weeks of age) and expressed as ratios (i.e., linear growth rate between 4 to 8 weeks $=(Le_{8w}-Le_{4w})/Le_{4w}$). Data presented as mean +/− SEM or n = 10-15 mice in each group over each period of time. *-denotes $p<0.05$ comparing KO-HIT mice to Control. #-denotes $p<0.05$ comparing HIT mice to Control.

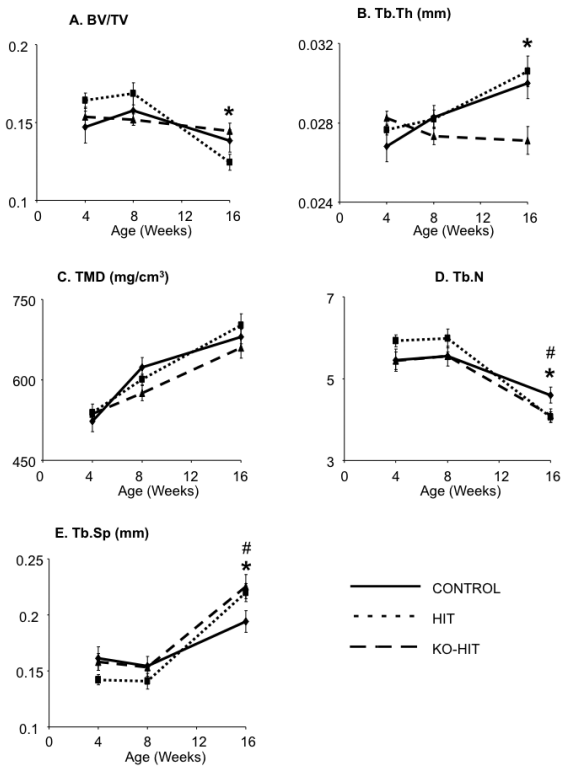

Figure 4.

Trabecular bone morphology was analyzed at the distal femur by micro-CT. Bone volume fraction (BV/TV) (A), trabecular thickness (Tb.Th.) (B), Tissue mineral density (TMD) (C), trabecular number (Tb.N) (D), and trabecular spacing (Tb.Sp.) (E), were measured at 4, 8, and 16 weeks of age. Data presented as mean +/− SEM of n=10-15 mice in each group at each time point. *-denotes $p<0.05$ comparing KO-HIT mice to Control. #-denotes $p<0.05$ comparing HIT mice to Control.

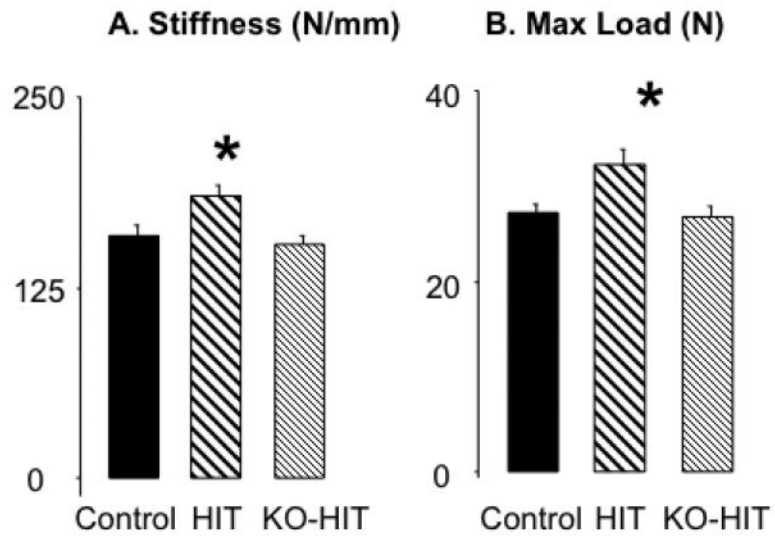

Figure 5.

Mechanical properties of femora were assessed by 4-point bending test on 16 week mice. Stiffness (A) indicates resistance to bending and max load (B)] indicates the maximum force the sample can bear before failure. Data presented as mean +/− SEM of n=10-15 mice in each group at each time point. *-denotes $p<0.05$ comparing KO-HIT mice to Control. #-denotes $p<0.05$ comparing HIT mice to Control.

Table 1

Changes in serum hormones and bone morphology following PTH treatment.

	Control		HIT		KO-HIT	
Treatment	**Vehicle**	**PTH**	**Vehicle**	**PTH**	**Vehicle**	**PTH**
Serum IGF-1 (ng/ml)	299 +/− 11	289 +/− 12	797 +/− 31 *b*	904 +/− 35 *c*	841 +/− 43 *b*	842 +/− 35 *c*
Serum osteocalcin (ng/ml)	76 +/− 6	134 +/− 11 *a*	95 +/− 5 *b*	179 +/− 16 *ac*	74 +/− 4	130 +/− 6 *a*
Body weight (g)	26.8 +/− 0.7	26.0 +/− 0.7	33.0 +/− 0.7 *b*	32.0 +/− 1.0 *c*	27.6 +/− 0.8	28.2 +/− 0.6
Femur length (cm)	15.2 +/− 0.1	15.2 +/− 0.1	15.6 +/− 0.1	15.7 +/− 0.2	14.8 +/− 0.1	15.1 +/− 0.1
Cortical bone morphology:						
Tt.Ar. (mm^2)	1.52 +/− 0.04	1.64 +/− 0.03	1.76 +/− 0.06 *b*	2.00 +/− 0.08 *ac*	1.46 +/− 0.05	1.64 +/− 0.05 *a*
Ct.Ar. (mm^2)	0.81 +/− 0.03	0.96 +/− 0.03 *a*	0.94 +/− 0.04 *b*	1.19 +/− 0.06 *ac*	0.83 +/− 0.03	0.97 +/− 0.04 *a*
Ma.Ar. (mm^2)	0.71 +/− 0.03	0.68 +/− 0.01	0.82 +/− 0.03 *b*	0.81 +/− 0.04 *c*	0.64 +/− 0.02	0.67 +/− 0.02
Ct.Th. (mm)	0.199 +/− 0.006	0.229 +/− 0.005 *a*	0.214 +/− 0.006	0.257 +/− 0.010 *ac*	0.209 +/− 0.004	0.234 +/− 0.008 *a*
Robustness (Tt.Ar./Le) (mm)	0.102 +/− 0.002	0.108 +/− 0.002	0.113 +/− 0.003 *b*	0.127 +/− 0.005 *ac*	0.099 +/− 0.003	0.109 +/− 0.003 *a*
Cortical TMD (mg/cm^3)	1316 +/− 10	1318 +/− 14	1339 +/− 9	1329 +/− 9	1359 +/− 15 *b*	1370 +/− 8 *c*
Trabecular bone morphology:						
BV/TV	0.137 +/− 0.007	0.155 +/− 0.009	0.122 +/− 0.005	0.148 +/− 0.007 *a*	0.114 +/− 0.006 *b*	0.145 +/− 0.008 *a*
Tb.Th. (μm)	29.8 +/− 0.7	30.1 +/− 1.0	30.3 +/− 0.8 *b*	30.3 +/− 1.3	27.2 +/− 0.8 *b*	29.3 +/− 1.1
Tb.N. (1/mm)	4.60 +/− 0.21	5.14 +/− 0.21	4.02 +/− 0.13 *b*	4.89 +/− 0.13 *a*	4.21 +/− 0.18	4.92 +/− 0.12 *a*
Tb.Sp. (mm)	0.195 +/− 0.010	0.167 +/− 0.011	0.223 +/− 0.008 *b*	0.176 +/− 0.006 *a*	0.217 +/− 0.011 *b*	0.175 +/− 0.005 *a*
Trabecular TMD (mg/cm^3)	676 +/− 15	687 +/− 22	694 +/− 21	663 +/− 33	662 +/− 23	709 +/− 21
Mechanical testing:						
Stiffness (N/mm)	158 +/− 8	162 +/− 7	186 +/− 7 *b*	232 +/− 15 *ac*	152 +/− 6	191 +/− 11 *ac*
Max load (N)	27.1 +/− 0.9	30.8 +/− 0.9	32.2 +/− 1.5 *b*	38.6 +/− 2.0 *ac*	26.6 +/− 1.1	32.5 +/− 1.7 *a*

a Different from Vehicle of the same genotype

b Different from Vehicle-treated control

c Different from PTH-treated control

Table 2

Mean histomorphometric parameters following PTH treatment.

	Control		HIT		KO-HIT	
	Vehicle	**PTH**	**Vehicle**	**PTH**	**Vehicle**	**PTH**
L.Pm (%)	70.9 +/− 0.8	80.5 +/− 4.8	61.7 +/− 11.5	73.2 +/− 6.5	53.4 +/− 6.1 [c]	62.3 +/− 6.2
MAR (um/day)	3.0 +/− 0.8	2.0 +/− 0.2	1.3 +/− 0.2	2.9 +/− 0.8	1.4 +/− 0.1	2.0 +/− 0.4
BFR/P.Pm (um/day*100)	226.4 +/− 83.6	165.9 +/− 20.0	81.6 +/− 24.4	229.3 +/− 75.8	78.0 +/− 13.4	131.4 +/− 29.7

[c]Different from PTH-treated control