Impaired skeletal growth in mice with haploinsufficiency of IGF-I: genetic evidence that differences in IGF-I expression could contribute to peak bone mineral density differences

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Abstract

Although it is well established that there is considerable inter-individual variation in the circulating levels of IGF-I in normal, healthy individuals and that a genetic component contributes substantially to this variation, the direct evidence that inter-individual variation in IGF-I contributes to differences in peak bone mineral density (BMD) is lacking. To examine if differences in IGF-I expression could contribute to peak BMD differences, we measured skeletal changes at days 23 (prepubertal), 31 (pubertal) and 56 (postpubertal) in mice with haploinsufficiency of IGF-I (+/−) and corresponding control mice (+/+). Mice (MF1/DBA) heterozygous for the IGF-I knockout allele were bred to generate +/+ and +/− mice (n=18–20 per group). Serum IGF-I was decreased by 23% ($P<0\cdot001$) in mice with IGF-I haploinsufficiency (+/−) group at day 56 compared with the control (+/+) group. Femoral bone mineral content and BMD, as determined by dual energy X-ray absorptiometry, were reduced by 20% ($P<0\cdot001$) and 12% respectively in the IGF-I (+/−) group at day 56 compared with the control group. The peripheral quantitative computed tomography measurements at the femoral mid-diaphysis revealed that periosteal circumference (7%, $P<0\cdot01$) and total volumetric BMD (5%, $P<0\cdot05$) were decreased significantly in the +/− group compared with the +/+ group. Furthermore, serum IGF-I showed significant positive correlations with both areal BMD (r=0·55) and periosteal circumference (r=0·66) in the pooled data from the +/+ and +/− groups. Our findings that haploinsufficiency of IGF-I caused significant reductions in serum IGF-I level, BMD and bone size, together with the previous findings, are consistent with the notion that genetic variations in IGF-I expression could, in part, contribute to inter-individual differences in peak BMD among a normal population.

Introduction

The risk of developing senile osteoporosis in men and postmenopausal osteoporosis in women is, in large part, determined by the amount of bone mass accumulated during the active growth phases early in life. In terms of the potential regulatory molecules that contribute to the acquisition of peak bone mineral density (BMD) during postnatal growth,

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insulin-like growth factor-I (IGF-I) has received considerable attention for a number of reasons, including: (1) the effects of growth hormone (GH) on skeletal growth are largely mediated via IGF-I (Kasukawa *et al.* 2004); (2) mice lacking a functional IGF-I gene exhibit severe impairment in bone formation and a severe deficiency in peak BMD (Bikle *et al.* 2001, Mohan *et al.* 2003); (3) osteoblast-specific knockout of the IGF receptor gene exhibits a decrease in cancellous bone volume, connectivity and trabecular number (Zhang *et al.* 2002); (4) transgenic overexpression of GH or IGF-I leads to an increase in bone accretion (Saban *et al.* 1996, Zhao *et al.* 2000, Eckstein *et al.* 2004); (5) a congenic mouse containing a chromosome 6 serum IGF-I quantitative trait locus (QTL) region from a C3H/HeJ mouse into the C57BL/6J background exhibited a decrease in serum IGF-I and femoral BMD (Bouxsein *et al.*, 2002); (6) IGF-I is required for the anabolic actions of parathyroid hormone on mouse bone (Miyakoshi *et al.* 2001, Bikle *et al.* 2002); and (7) insulin receptor substrate-1 knockout (KO) mice exhibited an insufficient proliferation of chondrocytes, calcification of hypertrophic chondrocytes, acceleration of apoptosis and early closure of the growth plate (Hoshi *et al.* 2004). Thus, there is strong evidence that IGF-I production is a major regulator of bone mass in mice.

There is also evidence that IGF-I plays an important role in the regulation of peak bone mass in men and women. In this regard, an adolescent male lacking a functional IGF-I gene had a BMD of 5 S.D. less than corresponding age-matched normal children (Woods *et al.* 1997). Furthermore, we and others have shown that serum levels of IGF-I increase during puberty and correlate with BMD (Moreira-Andres *et al.* 1995, Libanati *et al.* 1999, Thorsen *et al.* 1999, Richman *et al.* 2001, Kasukawa *et al.* 2003). Although recent studies provide evidence that both the variation in peak BMD and circulating levels of IGF-I are largely determined genetically (Harrela *et al.* 1996, Recker & Deng 2002, Baldock & Eisman 2004), the direct experimental evidence for the hypothesis that genetic-dependent variation in IGF-I production is a major determinant of the variation in peak BMD seen in normal healthy individuals is lacking at the present time. If differences in IGF-I expression caused by genetic alterations do indeed influence peak bone mass, then haplo-insufficiency should lead to decreased IGF-I levels and a corresponding decrease in BMD. We therefore generated heterozygous IGF-I KO mice and corresponding control mice to evaluate the consequence(s) of half-normal gene expression on bone accretion. This study is an extension of a previous study (Mohan *et al.* 2003) which compared skeletal phenotypes of homozygous IGF-I KO mice with wild-type mice.

Materials and Methods

Animals

Heterozygous breeder MF1/DBA IGF-I KO mice (kindly provided by Dr Argiris Efstradiatis, Columbia University College of Physicans and Surgeons, New York, USA) were mated to generate heterozygous IGF-I KO and wild-type mice as described previously (Mohan *et al.* 2003). Heterozygous IGF-I KO and corresponding control littermate mice were killed at day 23 (before puberty), day 31 (at the end of puberty) and day 56 (post puberty) to collect bones for phenotypic measurements and serum for IGF-I measurements.

Bone densitometry

Femur BMD and bone mineral content (BMC) measurements were performed by dual energy X-ray absorptiometry, using the PIXImus instrument (LUNAR Corporation, Madison, WI, USA). The precision was ± 1% coefficients of variation (C.V.) *in vitro* and ± 2% C.V. *in vivo*.

Volumetric bone density and geometric parameters of the femur

The length of the femur was measured with calipers. Volumetric bone density and geometric parameters at the mid-diaphysis were determined by peripheral quantitative computed tomography as described (Mohan *et al.* 2003). The C.V. for total BMD and periosteal circumference for repeat measurements of four mouse femur (two to five measurements) were less than 3% and 1% respectively (Mohan *et al.* 2003).

IGF-I RIA

IGF-I was measured by specific RIA using rabbit poly-clonal antiserum and recombinant IGF-I as standard and tracer respectively. IGF-binding proteins (IGFBP) were removed from serum prior to RIA by an acid gel filtration protocol (Mohan & Baylink 1995).

Statistics

All values are expressed as means ± S.D. Statistical analyses of the data were performed by Student's unpaired *t*-test.

Results

Serum IGF-I levels were reduced by 23% ($P<0·001$) in heterozygous IGF-I KO mice compared with control mice (Fig. 1). Body weight was decreased by 14·5% ($P<0·01$) in IGF-I +/− mice compared with corresponding control mice at 8 weeks of age (data not shown). Accordingly, femur length was significantly reduced ($P<0·01$) in heterozygous IGF-I KO mice compared with control mice (Fig. 2). Both the body weight and femur length were reduced to a similar extent in the male and female heterozygous IGF-I KO mice compared with corresponding control mice. The rate of gain in femur length was reduced by 10% during puberty in the heterozygous IGF-I KO mice compared with control mice (data not shown).

Femoral BMC was reduced by 25% ($P<0·001$) at days 23 and 31 in heterozygous IGF-I KO mice compared with control mice. The reduction in femoral BMC was 20% ($P<0·001$) at day 56 in heterozygous IGF-I KO mice compared with control mice (Fig. 3). The rate of gain in femoral BMC was reduced by 25% in heterozygous IGF-I KO mice compared with control mice during puberty (data not shown), suggesting that IGF-I plays a critical role in regulating the bone accretion that occurs during puberty.

Femoral BMD was reduced by 7%, 12% and 11% respectively at days 23, 31 and 56 in the heterozygous IGF-I KO mice compared with control mice (Fig. 4). The rate of gain in areal BMD was reduced by 24% in heterozygous IGF-I KO mice compared with control mice during puberty (data not shown), suggesting that IGF-I plays an important role in regulating BMD during sexual maturation.

Total volumetric BMD (vBMD) showed no variation between heterozygous IGF-I KO and control mice at day 23. Total vBMD was decreased by 9% and 4% respectively in heterozygous IGF-I KO mice compared with control mice at days 31 and 56 (Fig. 5). The rate of gain in total vBMD was reduced by 34% in heterozygous IGF-I KO mice compared with control mice during puberty (data not shown). There was no gender difference in the total vBMD deficit of heterozygous IGF-I KO mice (data not shown).

Femoral periosteal circumference was reduced by 7% in heterozygous IGF-I KO mice compared with control mice at days 23, 31 and 56 (Fig. 6). The magnitude of reduction in periosteal circumference was similar in male and female heterozygous IGF-I KO mice compared with control mice (data not shown). The rate of gain in periosteal circumference

was not significantly different between the heterozygous IGF-I KO and control mice during puberty.

Serum levels of IGF-I showed a significant positive correlation with areal BMD of the femur in heterozygous IGF-I KO mice and control mice (Fig. 7). Serum levels of IGF-I also showed a significant positive correlation with the periosteal circumference of the femur in both the heterozygous IGF-I KO mice and control mice (Fig. 8).

Discussion

The findings of this study have demonstrated for the first time that haploinsufficiency of IGF-I caused a significant reduction in serum IGF-I level in mice. If both copies of the IGF-I gene contribute equally to the level of serum IGF-I, we would anticipate the serum IGF-I level to be reduced by 50% in the heterozygous IGF-I KO mice compared with control mice. However, the magnitude of reduction in IGF-I was only about 25% in the mice with haploinsufficiency compared with 100% in homozygous IGF-I KO mice. There are a number of potential explanations for the less-than-anticipated reduction in circulating IGF-I, which include: (1) the lower IGF-I production in the heterozygous mice may lead to diminished negative feedback on the production of GH-releasing hormone and GH by the hypothalamus and the pituitary respectively (Mohan & Baylink 1999); (2) increased proportion of circulating IGF-I may be bound to acid labile subunit (ALS) and IGFBP-3/ IGFBP-5 in the heterozygous mice which would increase the half-life of circulating IGF-I (Rajaram *et al.* 1997); and (3) two copies of the IGF-I gene may not produce twice the amount of IGF-I compared with a single copy of the IGF-I gene. Future studies are needed to establish the extent to which the expression of IGF-I is reduced in various tissues compared with serum levels of IGF-I in heterozygous mice.

This study also demonstrated for the first time that a 25% reduction in circulating IGF-I caused by haploinsufficiency of IGF-I led to significant reductions in total vBMD (4%) and bone size (7%) in the femur in mice at 8 weeks of age. Consistent with these data, Bouxsein *et al.* (2002) have recently shown that a congenic mouse strain with a donated segment from a C3H/HeJ mouse into a C57BL/6J mouse had 11–21% lower IGF-I levels and 2·3–4·8% lower total femoral vBMD. The magnitude of reductions in BMD and/or bone size in IGF-I heterozygous mice and congenic mice were far less compared with IGF-I KO mice in which both copies of the IGF-I gene were disrupted (32% and 43% reduction in BMD and bone size respectively). These data raise the possibility that while a small reduction in IGF-I expression (10–20%) produces a significant effect on both BMD and bone size, two important determinants of bone strength, a reduction in IGF-I expression below a certain threshold level may contribute to a much more dramatic effect on the skeletal phenotype. In this regard, it was recently reported that the lowering of serum IGF-I below a threshold by crossing liver-derived IGF-I KO mice with ALS KO mice produced a dramatic effect on bone size which was not seen in either liver-derived IGF-I KO mice or ALS KO mice alone (Yakar *et al.* 2002).

In previous studies, we and others have shown that circulating levels of IGF-I vary considerably in normal healthy men and women between 20–40 years of age (Rajaram *et al.* 1997). Although variation in circulating levels of IGF-I may be regulated by a number of variables, including diet and exercise, there is considerable evidence for the involvement of genetic regulation of variation in serum IGF-I levels. Rosen *et al.* (1998) first reported that serum IGF-I levels are related to a polymorphism in a microsatellite within the IGF-I gene in healthy Caucasian men and women. Several subsequent studies have confirmed the association between IGF-I polymorphisms and circulating IGF-I levels (Johnston *et al.* 2003, Rietveld *et al.* 2003, 2004, Nielsen *et al.* 2004). Based on these data, it can be

concluded that genetic variation in IGF-I expression caused by polymorphism in one or more genes involved in regulating IGF-I expression and/or its action could contribute to inter-individual variations in peak BMD among a normal population.

The conclusion that differences in IGF-I expression could contribute to peak BMD differences in humans should be viewed in the following context. (1) IGF-I heterozygous mice and corresponding control mice were developed in mixed genetic backgrounds. Therefore, one could argue that the observed phenotypic differences between the heterozygous and control mice may be due to differences in genetic backgrounds. However, this is unlikely since we used corresponding control littermates, but of the same mixed genetic background, mice from several litters and a large number of mice ($n = 18$–20) per group in our studies. (2) It is known that differences in bone size can influence BMD measurements. Because the bone size of IGF-I heterozygous mice is slightly smaller compared with wild-type mice, the estimated magnitude of reduction in BMD in IGF-I heterozygous mice may not be precise. (3) While the findings in this study that haploinsufficiency of IGF-I leads to a reduction in BMD are consistent with previous studies using various transgenic mouse models regarding an important role for IGF-I in the regulation of peak bone mass in mice, the issue of whether genetic-dependent variation in IGF-I production is a major determinant of variation in peak BMD in normal healthy humans is still to be resolved.

Acknowledgments

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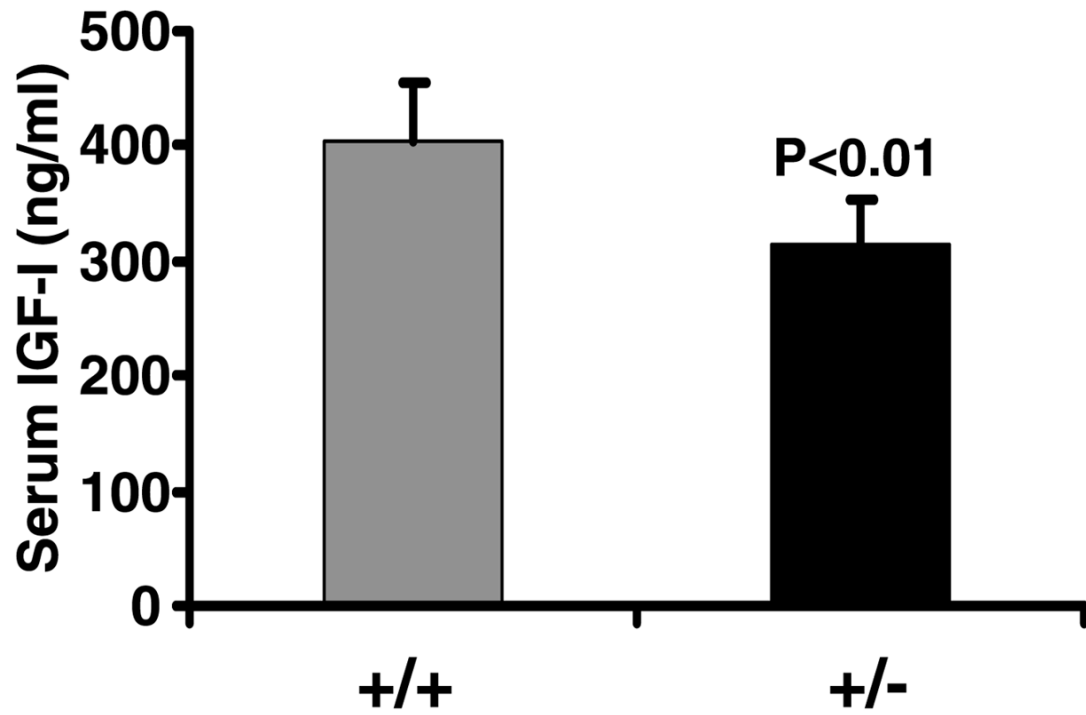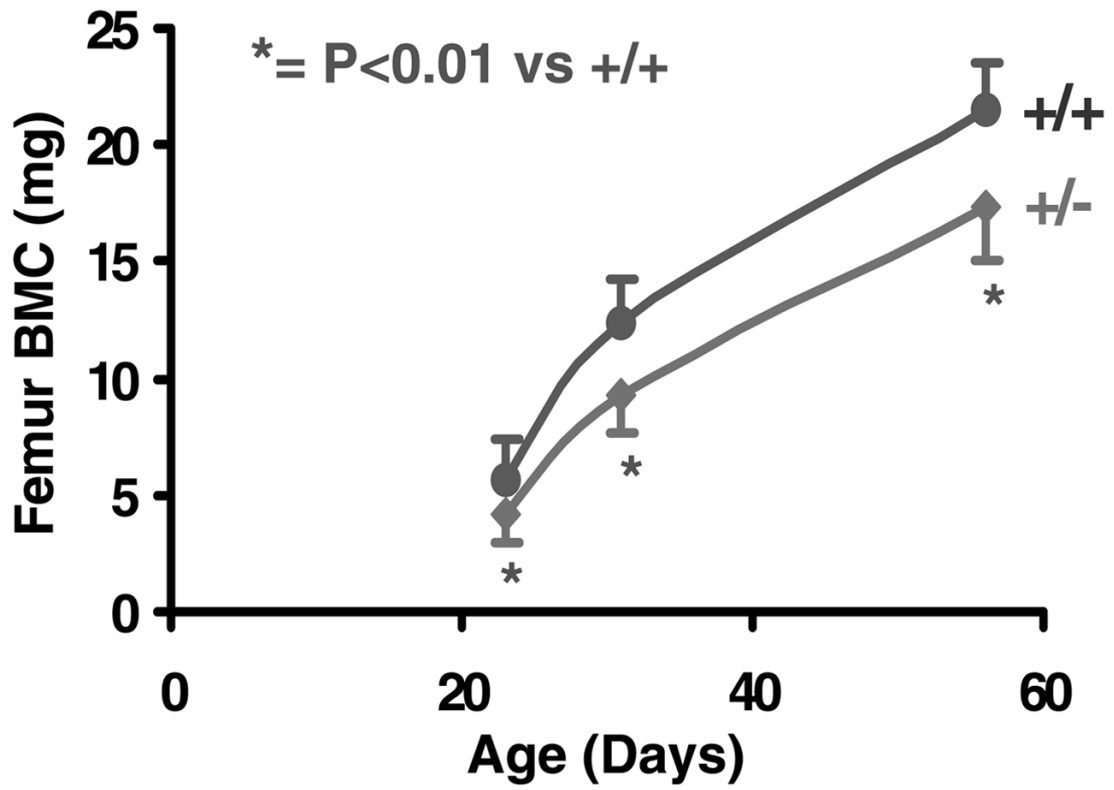
Figure 1.
Serum IGF-I levels in heterozygous IGF-I KO and control mice at 8 weeks of age. Values
are means ± S.D. and represent an *n* of 18–20 per group.
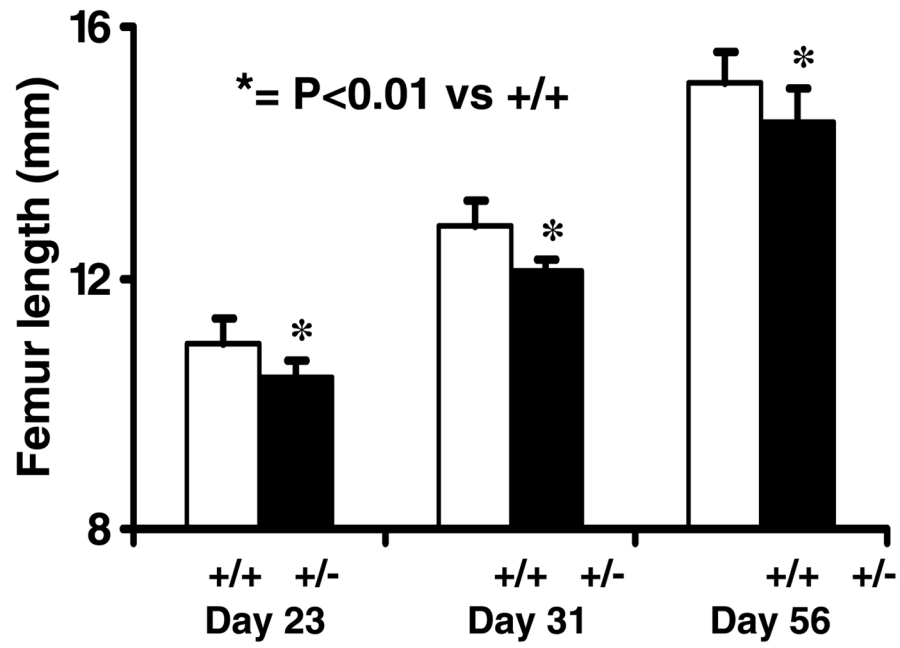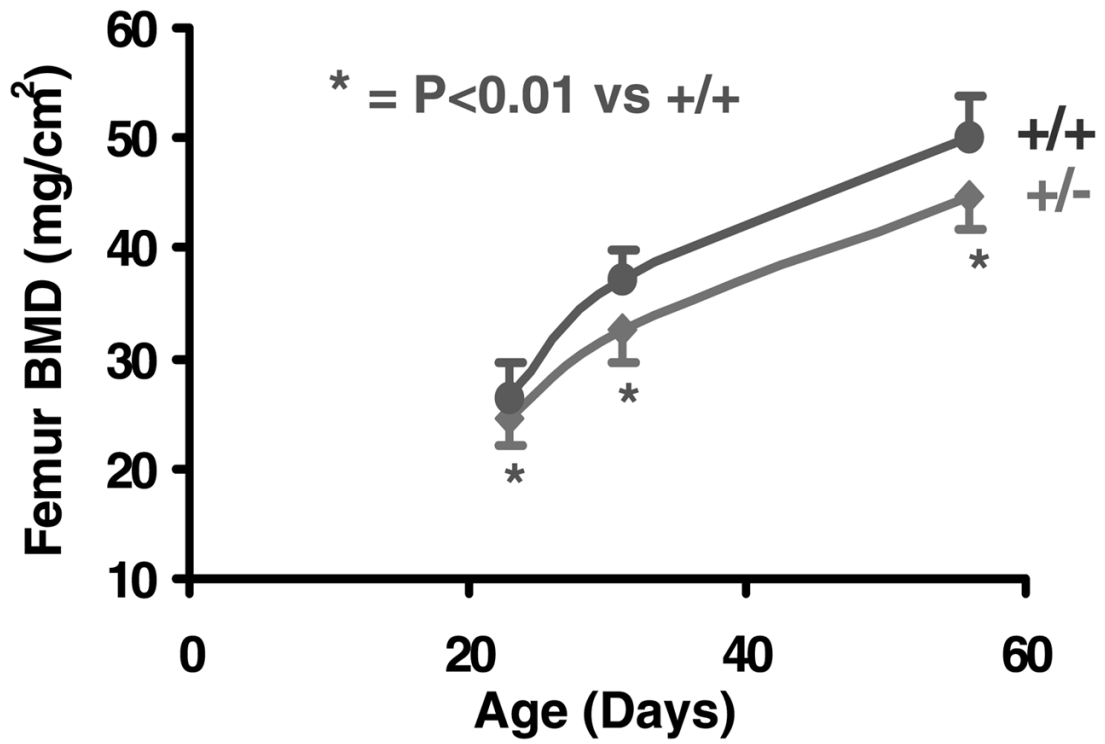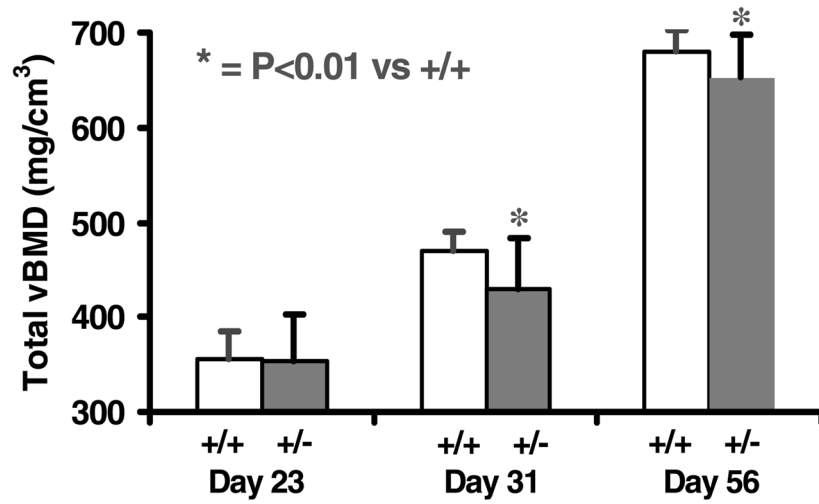
Figure 2.
Femur length in heterozygous IGF-I KO and control mice during postnatal growth. Values are means ± S.D. and represent an *n* of 18–20 per group.
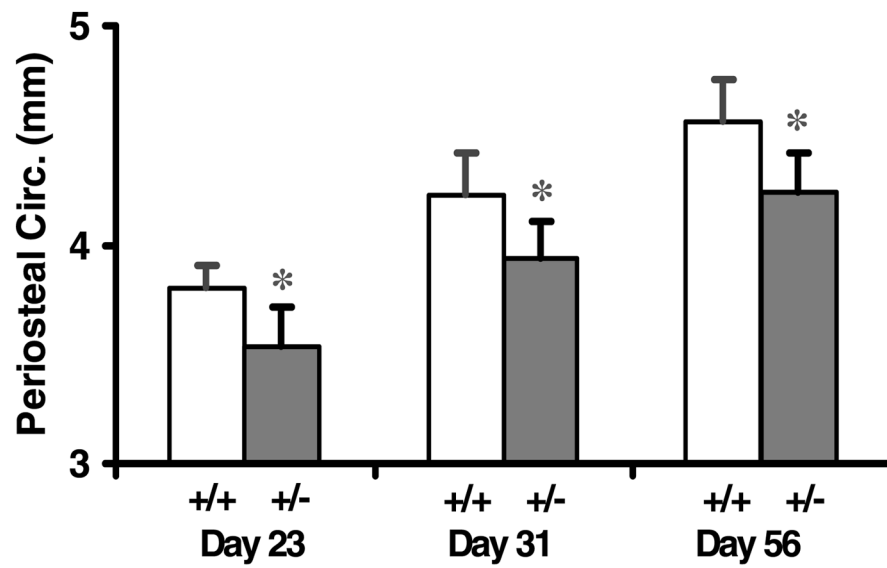
Figure 3.
Femoral BMC in heterozygous IGF-I KO and control mice during postnatal growth. Values are means ± S.D. and represent an *n* of 18–20 per group.
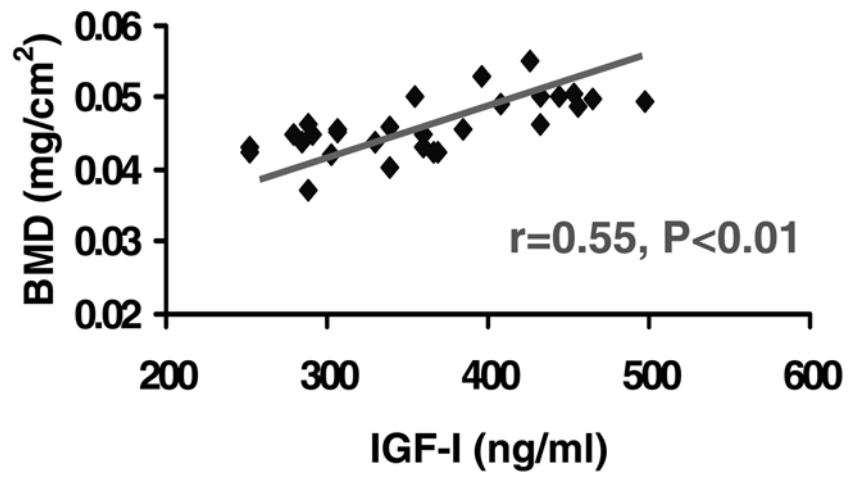
Figure 4.
Femur areal BMD in heterozygous IGF-I KO and control mice during postnatal growth.
Values are means ± S.D. and represent an *n* of 18–20 per group.

Figure 5.
Femur total vBMD in heterozygous IGF-I KO and control mice during postnatal growth.
Values are means ± S.D. and represent an *n* of 18–20 per group.

Figure 6.
Periosteal circumference (Circ.) of the femur in heterozygous IGF-I KO and control mice
during postnatal growth. Values are means ± S.D. and represent an *n* of 18–20 per group.

Figure 7.
Correlation between areal BMD of femur and serum levels of IGF-I in heterozygous IGF-I KO and control mice at 8 weeks of age.
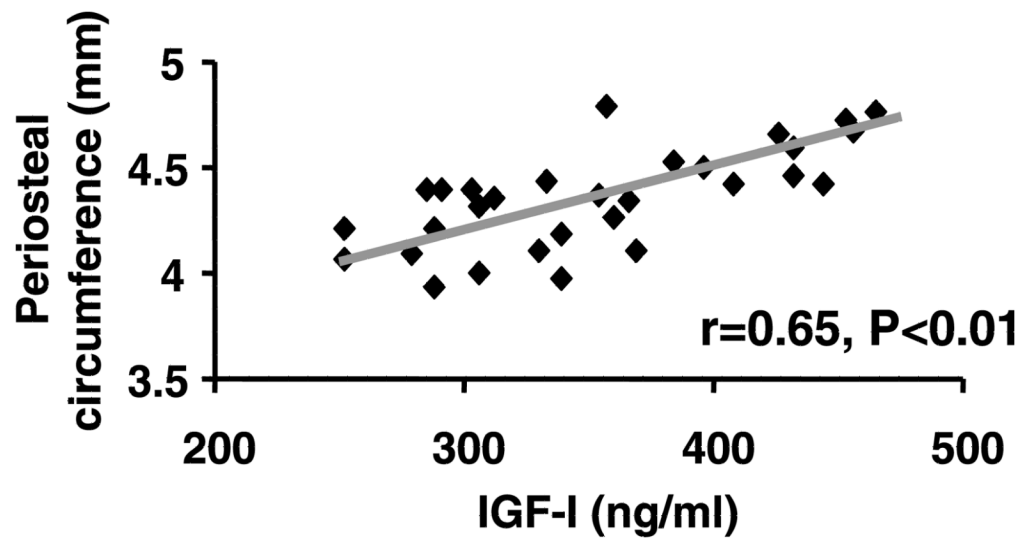
Figure 8.
Correlation between periosteal circumference of femur and serum levels of IGF-I in heterozygous IGF-I KO and control mice at 8 weeks of age.