

Evidence supporting dual, IGF-I-independent and IGF-I-dependent, roles for GH in promoting longitudinal bone growth

J Wang, J Zhou, C M Cheng, J J Kopchick¹ and C A Bondy

Developmental Endocrinology Branch, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892, USA

¹Edison Biotechnology Institute and Department of Biomedical Sciences, Ohio University, Athens, Ohio 45701, USA

(Requests for offprints should be addressed to C A Bondy; Email: bondyc@exchange.nih.gov)

Abstract

The possibility that growth hormone (GH) has effects on long bone growth independent of insulin-like growth factor-I (IGF-I) has long been debated. If this is true, then long bone growth should be more profoundly affected by the absence of GH (since both GH and GH-stimulated IGF-I effects are absent) than by the absence of IGF-I alone (since GH is still present and actually elevated). To test this hypothesis, we compared long bone growth in mice with targeted deletions of *Igf1* vs *growth hormone receptor (Ghr)*. Tibial linear growth rate was reduced by approximately 35% in *Igf1* null mice and by about 65% in *Ghr* null mice between postnatal days 20 and 40, a time of peak GH effect during normal longitudinal growth. The *Igf1* null mouse growth plate demonstrated significant enlargement of the germinal zone; chondrocyte proliferation and numbers were normal but chondrocyte hypertrophy was significantly reduced. In contrast, the *Ghr* null mouse germinal zone was hypoplastic, chondrocyte proliferation and numbers were significantly reduced, and chondrocyte hypertrophy was also reduced. We have previously demonstrated that IGF-II is highly expressed in growth plate germinal and proliferative zones, so we

considered the possibility that GH-stimulated IGF-II production might promote germinal zone expansion and maintain normal proliferation in the *Igf1* null mouse growth plate. Supporting this view, IGF-II mRNA was increased in the *Igf1* null mouse and decreased in the *Ghr* null mouse growth plate.

Thus, in the complete absence of IGF-I but in the presence of elevated GH in the *Igf1* null mouse, reduction in chondrocyte hypertrophy appears to be the major defect in longitudinal bone growth. In the complete absence of a GH effect in the *Ghr* null mouse, however, both chondrocyte generation and hypertrophy are compromised, leading to a compound deficit in long bone growth. These observations support dual roles for GH in promoting longitudinal bone growth: an IGF-I-independent role in growth plate chondrocyte generation and an IGF-I-dependent role in promoting chondrocyte hypertrophy. The question of whether GH has direct effects on chondrocyte generation is still not settled, however, since it now appears that IGF-II may mediate some of these effects on the growth plate.

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Introduction

The interactions between growth hormone (GH) and insulin-like growth factor-I (IGF-I) in the regulation of somatic growth have been investigated for many years in many different model systems (reviewed in Ohlsson *et al.* 1998, Le Roith *et al.* 2001). Briefly, while IGF-I has long been regarded as the mediator of GH's systemic effects on growth (the 'somatomedin hypothesis'), evidence has accumulated in recent years suggesting that IGF-I has some GH-independent effects and that GH has some IGF-I-independent effects on somatic growth. The most compelling evidence for the former view is the finding that *Igf1* gene disruption results in significant intrauterine growth retardation beginning before the time of GH-regulated growth (Liu *et al.* 1993, Powell-Braxton *et al.* 1993, Woods *et al.* 1996). *Igf1* null mice and the single

human reported with *Igf1* gene deletion also exhibit impaired postnatal growth and elevated GH levels (Liu *et al.* 1993, Powell-Braxton *et al.* 1993, Woods *et al.* 1996).

The evidence for IGF-I-independent growth-promoting actions by GH has been less conclusive for a number of reasons. The finding that IGF-I administration to GH- or GH receptor (GHR)-deficient subjects results in less than optimal growth (Fielder *et al.* 1996, Ranke *et al.* 1999, Rosenbloom 1999) may reflect the absence of essential GH effects, or may be due to difficulty in delivering exogenous IGF-I to target tissues. GH's putative direct effects in cell or explant culture or after local injection *in vivo* could be attributed to autocrine or paracrine IGF-I activity, since GH stimulates IGF-I production in many different tissues and cell types and IGF-I receptor expression is nearly universal. For example, the fact that local injection of GH to the tibial growth plate

stimulates germinal zone expansion has been attributed to GH-induced local IGF-I production (Schlechter *et al.* 1986, Ohlsson *et al.* 1992, Hunziker *et al.* 1994). In support of a direct, IGF-I-independent effect by GH on germinal zone expansion, we recently reported that the tibial epiphyseal growth germinal is significantly enlarged in the *Igf1* null mouse (Wang *et al.* 1999). Since GH levels are chronically elevated while IGF-I is completely absent from all tissues as well as the circulation in the *Igf1* null mouse, this observation provides indirect evidence for an IGF-I-independent role for GH in stimulating chondrocyte generation and thus longitudinal bone growth. However, chondrocyte numbers and proliferation are maintained in the *Igf1* null mice growth plate (Wang *et al.* 1999), which does not fit the current view that GH-induced IGF-I production is responsible for these effects (reviewed in Ohlsson *et al.* 1998).

The rate of longitudinal bone growth is a product of the rate of production of new growth plate chondrocytes times the average size of the terminal hypertrophic chondrocytes which form the scaffold for bone elongation (Hunziker & Schenk 1989). The *Igf1* null mouse demonstrates deficits in chondrocyte metabolism and hypertrophy that account for moderately impaired long bone growth (Wang *et al.* 1999). If GH does indeed have IGF-I-independent effects on long bone growth, the linear growth deficit should be more severe in GHR-deficient mice. To further investigate this issue, in the present study we compared somatic and longitudinal bone growth parameters in *Igf1* and *Ghr* null mice. We have specifically focused our growth analyses on mice from postnatal day 20 to postnatal day 40 because this is a period of peak GH effect during normal murine growth and also because this is before sexual maturity. Since sexual maturation is delayed in *Ghr* null mice (Zhou *et al.* 1997) and may not occur at all in *Igf1* null mice (Baker *et al.* 1996), evaluation of growth patterns in older animals may be confounded by deficiencies in dwarf gonadal steroids as well as GH and/or IGF-I. In this study, the two deletions are in 2 different strains of mice, and thus to allow comparison across the strains, all data are expressed as a percent of littermate wild-type (WT) controls. Normalization to littermate wild-types is intended to control for genetic variation between the different strains as well as for uterine and postnatal environmental factors which strongly impact growth.

Materials and Methods

Mice

The *Igf1* deletion mouse line used in this study was provided by Lynn Powell-Braxton at Genentech, Inc., San Francisco, CA, USA (Powell-Braxton *et al.* 1993) and bred in our facility for more than 30 generations into a CD1 strain obtained from Taconic Farms (Germantown, NY, USA). The *Ghr* line was generated in Sv129 Ola/

Balb/c background (Zhou *et al.* 1997). Males (+/-) from this genetically heterogeneous line were backcrossed for over 12 generations to C57/6J females resulting in a line that was 99.61% congenic, and the line was maintained in C57/6J mice for over 10 generations at the National Institutes of Health to obtain the animals for this study. The mice were studied in a protocol approved by the NICHD Animal Use and Care Committee. Mice were genotyped by PCR of tail biopsy DNA as previously described (Powell-Braxton *et al.* 1993, Zhou *et al.* 1997) and killed by decapitation after CO₂ inhalation between 1200 and 1400 h at postnatal days 10, 20 or 40 (P10, P20, P40).

Cell proliferation

For cell proliferation studies of the tibial growth plate mice were injected intraperitoneally with [³H]thymidine (2 µCi/g) or bromodeoxyuridine (BrdU) (100 µl/g, Calbiochem HCS24; San Diego, CA, USA) one hour before death. Tissue sections were dipped in Kodak NTB2 nuclear emulsion and exposed at 4 °C for 3 weeks, developed in D19 (Kodak), fixed and counter-stained with hematoxylin for detection of nuclear [³H]thymidine incorporation. Positive cells had 5 or more grains/nucleus. BrdU incorporation was detected immunohistochemically as previously described (Wang *et al.* 1999). A blinded observer scored all proliferative zone chondrocytes in 3–4 tissue sections from each animal under 400 × magnification.

Growth plate morphometry

Tibias were dissected, fixed in formalin, decalcified in EDTA, embedded in paraffin and cut longitudinally into 5 µ sections, which were mounted onto poly-L-lysine-coated slides. The longitudinal dimensions of the tibia and of the proximal tibial growth plate were measured on photomicrographs of anatomically matched, mid-sagittal sections, taken at 2.5 × and 200 × magnification respectively. For the growth plate dimensions, three different measurements, clustered about the midline, were taken on each of two sections for each animal, as previously described (Wang *et al.* 1999). These data were meaned for each animal and then pooled and meaned for each group. The terminal chondrocyte longitudinal diameter was measured on micrographs taken at 400 × magnification. Measurements were made only on cells with intact superior and inferior transverse septa.

In situ hybridization

The clones used for riboprobe synthesis and the *in situ* hybridization protocol have been described previously (Wang *et al.* 1995). IGF-II mRNA was quantified in growth plate proliferative and germinal zones by counting

Table 1 Effects of *Igf1* and *Ghr* deletion on body weights at P20 and P40. Data represent means \pm S.E.M. with the number of mice for each observation indicated in parentheses

	<i>Igf1</i> WT	<i>Igf1</i> null	<i>Ghr</i> WT	<i>Ghr</i> null
P20 weight (g)	13 \pm 0.5(15)	8 \pm 0.4*(14)	11 \pm 0.4(10)	6 \pm 0.8*(5)
%WT		61 \pm 3		55 \pm 7
P40 weight (g)	26.3 \pm 1.2(11)	10.2 \pm 0.8*(14)	22.2 \pm 0.6 (5)	8.8 \pm 0.3*(5)
%WT		38 \pm 4		40 \pm 1

* $P < 0.0001$ compared with the relevant WT control group.

grains overlying 100–200 germinal zone cells and \sim 500 proliferative zone cells for each animal on masked slides under high magnification ($630\times$) microscopy. Background signal from the sense probe hybridization was subtracted from data prior to analysis.

Data analysis

Data for each group are expressed as means with standard errors. Genotyped, homozygous *Igf1* and *Ghr* null mice were compared with littermate WT mice at P20 and P40 with a minimum of 4 mice per group. Comparisons between the different strains were made only after normalization to the relevant WT mean. Differences between groups were analyzed by ANOVA using Stat View 4.1 (SAS Institute, Cary, NC, USA) followed by Fisher's least significant difference tests. A P value < 0.05 was taken as significant.

Results

Total body weights of homozygous *Igf1* and *Ghr* null dwarf mice were compared with the corresponding WT littermate mice at P20 and P40 (Table 1). The weights of the *Igf1* null dwarfs were \sim 60% of WT mice at P20 and the weights of the *Ghr* null dwarfs were \sim 55% of their littermate WT group at this stage, with no statistically significant difference between the two dwarf groups. By P40, both *Igf1* and *Ghr* null mice were reduced in weight by \sim 60% compared with their respective WT groups. Note that the two WT strains (CD1 and Sv129 Ola/Balb/c) are significantly different in weight and therefore direct comparisons of the dwarf phenotypes without normalization to mice of the same genetic backgrounds would be misleading.

Tibial morphology during postnatal development was grossly normal in both *Igf1* and *Ghr* null mice (Fig. 1). Tibial growth rate was assessed in *Igf1* and *Ghr* null mice between P20 and P40, a time of peak GH-induced longitudinal bone growth in normal mice. Tibial growth rate between P20 and P40 was reduced by approximately 37% in *Igf1* null mice and by 65% in *Ghr* null mice (Table 2). Note that tibial growth is substantially slower in the

Sv129 Ola/Balb/c strain compared with the CD1 mice, again showing the necessity of evaluating the mutant data against the relevant genetic background.

To elucidate the cause of this marked differential in growth rate, we compared proximal tibial epiphyseal growth plate characteristics in the two dwarf phenotypes (Fig. 2 and Table 3). We evaluated the extent of proximal tibial epiphyseal ossification in P20 dwarf mice by comparing the area occupied by the secondary ossification center to the entire area of the epiphysis using image analysis. In the *Igf1* null mice the tibial secondary ossification center occupied $21 \pm 1.2\%$ while in the WT it occupied $30 \pm 1.4\%$ of the total epiphysis ($P < 0.05$). In the *Ghr* null mice the secondary ossification center occupied $25 \pm 1.4\%$ and in the the WT it occupied $34.4 \pm 4\%$ of the total epiphysis ($P < 0.05$). Thus in both dwarfs, the area of the epiphysis occupied by a secondary ossification center was reduced by about 30%.

As previously reported, the *Igf1* null mouse growth plate demonstrates an enlarged germinal zone, which is the region at the top of the growth plate thought to produce chondrocyte precursors. Chondrocyte numbers and proliferation are not significantly different in *Igf1* null and littermate WT proliferative zones (Figs 3 and 4; Table 3). Chondrocyte hypertrophy is significantly reduced in these mice, however, the reduction in height of *Igf1* null mice terminal hypertrophic chondrocytes is proportionate to the reduction in *Igf1* null mice long bone growth. In contrast, the *Ghr* null mouse growth plate germinal zone was diminished and the proliferative zone was reduced in cell number and proliferative index (Figs 3 and 4; Table 3). Finally, the *Ghr* null mouse hypertrophic zone was also significantly attenuated, with hypertrophic chondrocytes reduced both in number and size. Thus the *Ghr* null mouse epiphyseal growth plate demonstrates a compound deficit in chondrocyte number and hypertrophy contributing to the pronounced linear growth failure.

Since IGF-II is expressed by growth plate germinal and proliferative zone cells (Shinar *et al.* 1993, Wang *et al.* 1995), we hypothesized that GH-enhanced IGF-II expression might be responsible for the effects of GH on chondrocyte generation/proliferation, which is preserved in the *Igf1* null mouse. Therefore, we compared IGF-II mRNA levels in *Igf1* and *Ghr* null mice and their

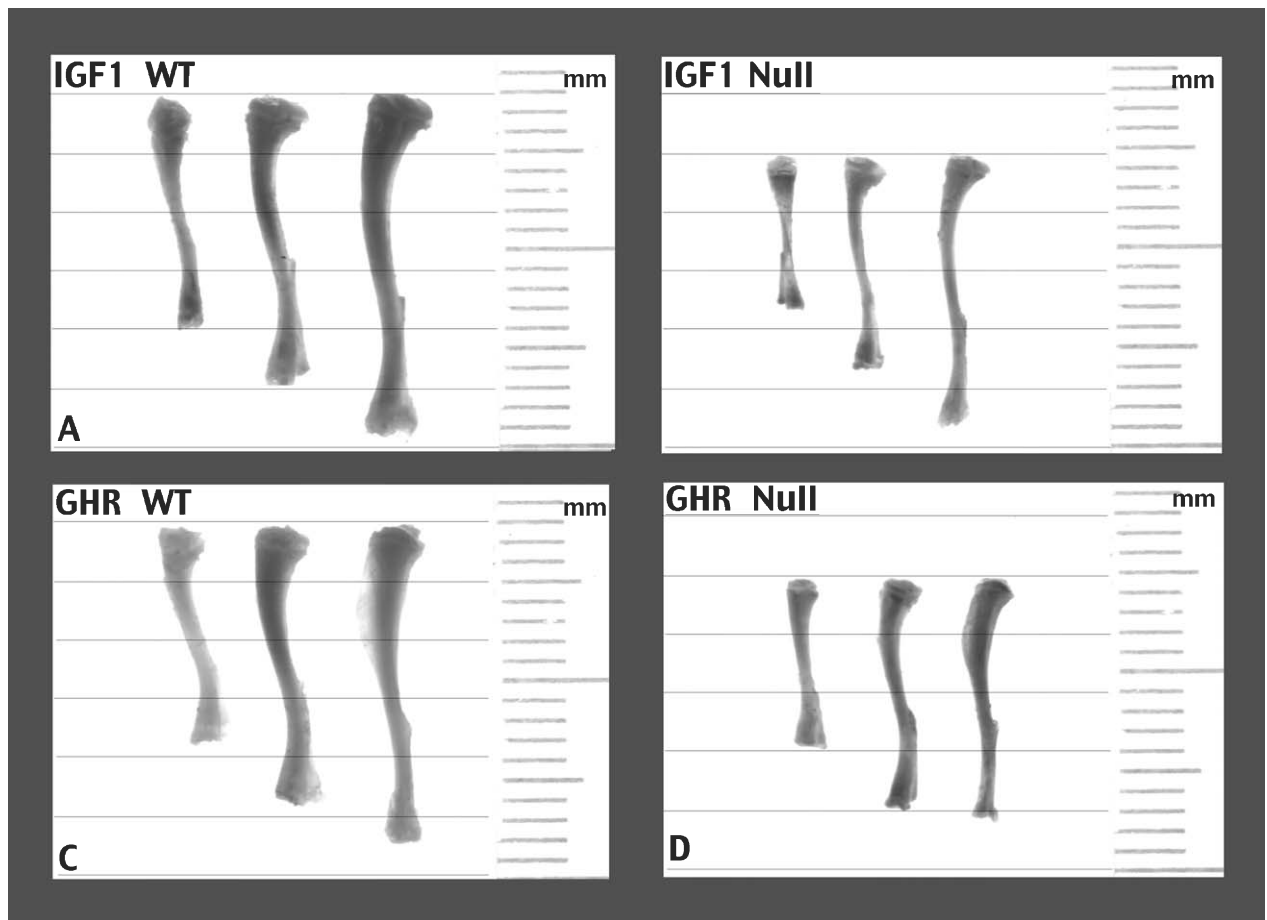


Figure 1 Tibial longitudinal growth in *Igf1* null and *Ghr* null mice. Representative tibias from postnatal day 10 (P10), P20 and P40 *Igf1* null and *Ghr* null mice are compared with tibias from their respective WT littermates.

respective WT littermates using *in situ* hybridization. Cellular patterns for IGF-II gene expression were exactly as previously reported (Wang *et al.* 1995). IGF-II mRNA levels were significantly increased in the germinal and proliferative zones of the *Igf1* null mouse and reduced in both zones of the *Ghr* null mouse (Table 3), supporting the possibility that GH enhances growth plate IGF-II expression.

Discussion

This study shows that longitudinal bone growth is significantly more retarded by *Ghr* deletion than by *Igf1* deletion, with *Ghr* null mice demonstrating attenuation of both numbers and size of growth plate chondrocytes, while *Igf1* null mice exhibit only reduced chondrocyte size. The fact that the germinal zone is enlarged and chondrocyte

Table 2 Comparison of tibial lengths and growth rates. Data represent means \pm S.E.M. with 4–5 mice in each group

	<i>Igf1</i> WT	<i>Igf1</i> null	<i>Ghr</i> WT	<i>Ghr</i> null
P20 tibial length (mm)	13.4 \pm 0.07	10.5 \pm 0.2*	12.5 \pm 0.07	10.4 \pm 0.2*
P40 tibial length (mm)	19.3 \pm 0.6	14.3 \pm 0.4*	15.7 \pm 0.07	11.6 \pm 0.2*
Growth rate μ /d	295	187	161	57
%WT		63%		35%

* $P < 0.001$ compared with the relevant WT control group.

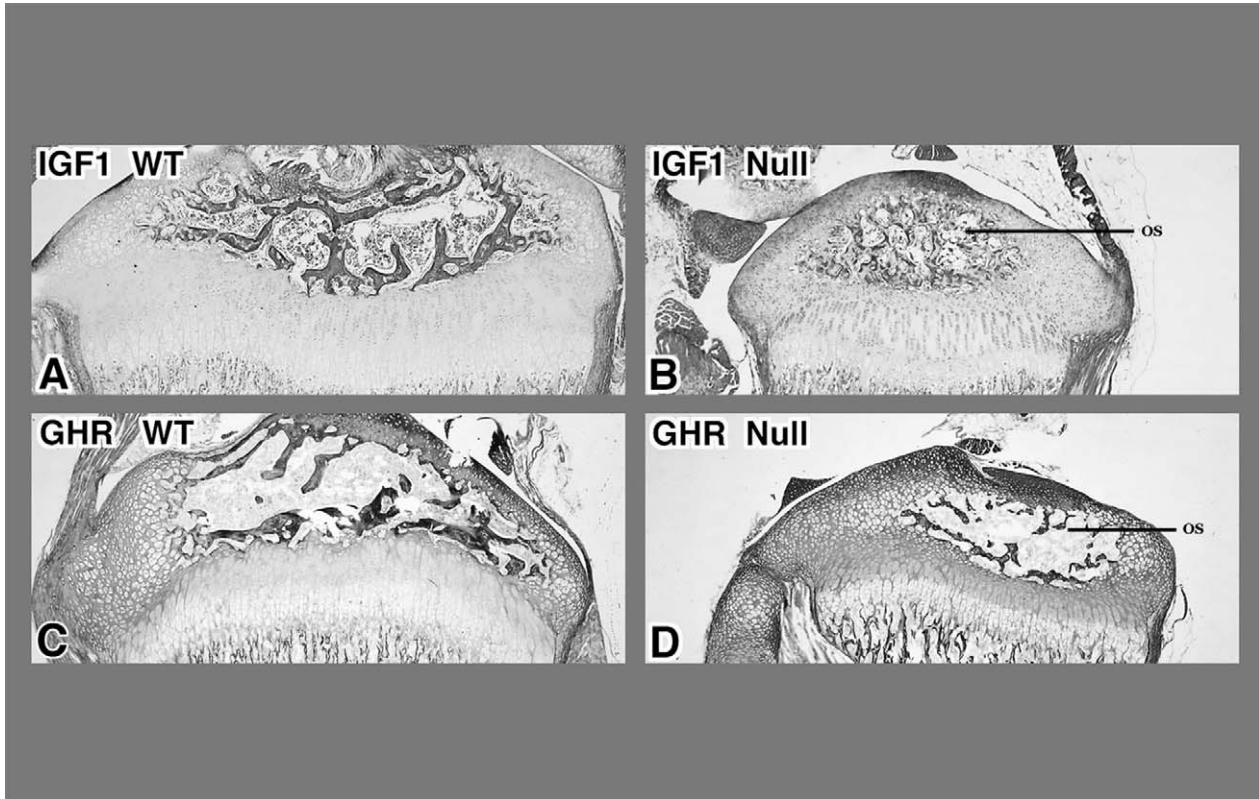


Figure 2 The development of secondary ossification centers in *Igf1* and *Ghr* null dwarf mice. The Figure illustrates representative proximal tibial epiphyses from WT and dwarf mice at P20. The secondary ossification centers (OS) are well-developed in both *Igf1* null and *Ghr* null dwarfs, but occupy a smaller proportion of the epiphysis than in the WT in each case.

proliferation is normal in the *Igf1* null mouse growth plate supports the view that GH enhances chondrocyte generation and proliferation independent of IGF-I, since GH is elevated while IGF-I is completely absent in these animals. It has been proposed that GH acts directly on the growth plate germinal zone to promote generation of chondrocyte precursors, and indirectly via IGF-I to promote chondrocyte proliferation (reviewed in Ohlsson *et al.* 1998). The present data, however, show that growth plate chondrocyte proliferation is maintained, even in the complete absence of IGF-I, suggesting that GH may have direct effects upon chondrocyte proliferation as well as on the germinal zone. Alternatively, since IGF-II mRNA is increased in *Igf1* null mice growth plates and reduced in *Ghr* null mice growth plates, GH may, in the absence of IGF-I, promote chondrocyte proliferation by stimulating IGF-II gene expression. IGF-I receptor expression is abundant in all growth plate cells (Wang *et al.* 1995), and it is likely that both the effects of IGF-II on chondrocyte proliferation and of IGF-I on chondrocyte hypertrophy are mediated by this receptor. Thus experiments involving IGF-I injected directly into the growth plate or elevated systemic IGF-I levels may enhance chondrocyte generation/proliferation, but these effects may normally be

due to locally produced IGF-I or IGF-II. Supporting a role for IGF-II in longitudinal bone growth, the linear growth rate of the femur is reduced by 10–15% in the *Igf2* null mouse (Mohan *et al.* 2003).

Another study compared growth in independent *Igf1* and *Ghr* deletions in different strains of mice (Lupu *et al.* 2001). They did not analyze long bone growth rates in the different groups (which included a small group of double *Igf1/Ghr* null dwarfs), but did report tibial length at different postnatal stages. Analysis of the data in Table 3 in Lupu *et al.* (2001) shows that the *Igf1* null mouse tibia increases in length by 3.4 mm while the *Ghr* null mouse tibia grows only 1.74 mm between P22 and P130 (a reduction of almost 60% compared with the WT growth of 4 mm). There were few observations on the double knockouts and no statistical analyses in this study, but tibial growth was ~1.3 mm between P22 and P130 in this group. Another study reported that femoral growth rate is reduced by approximately 60% in *Ghr* null mice (Fig. 1 in Sims *et al.* 2000), consistent with the reduced growth rate found in the present study. A very recent study compared growth in *Igf1* null mice to the GH-deficient *lit/lit* dwarf mice (Mohan *et al.* 2003). While the major focus of that work was on bone mineral density, the authors also

Table 3 Data on growth plate composition from the proximal tibia of P20 mice. IGF-II hybridizations were conducted in separate experiments — all the *Igf1* null and WT in one batch and the *Ghr* null and WT in another batch. Since probe-specific activity and exposure times may vary somewhat between experiments, it is not valid to directly compare the results for the *Igf1* with the *Ghr* mice. All data are means \pm S.E.M. for 4–6 mice/group

	WT	<i>Igf1</i> null	P	WT	<i>Ghr</i> null	P
Tibia (mm)	13.3 \pm 0.6	10.5 \pm 0.2	0.0001	12.5 \pm 0.1	10.5 \pm 0.2	0.0001
Growth plate (μ m)	366.9 \pm 4.3	318.9 \pm 8.1	0.002	296.4 \pm 10.1	208.5 \pm 17.8	0.0027
GZ (μ m)	29.9 \pm 2.8	44.8 \pm 2.8	0.01	21.4 \pm 1.0	9.4 \pm 0.6	0.0001
GZ cell number	2.06 \pm 0.1	2.9 \pm 0.2	0.04	2.5 \pm 0.2	0.8 \pm 0.1	0.0001
IGF-II mRNA (g/cell)	6.3 \pm 0.5	9.0 \pm 0.5	0.02	6.5 \pm 0.3	4.3 \pm 0.3	0.02
PZ (μ m)	118.4 \pm 5.8	131.0 \pm 10.3	NS	120.6 \pm 4.7	104.9 \pm 8.8	NS
PZ cells/50 μ	8.8 \pm 0.3	8.2 \pm 0.7	NS	8.8 \pm 0.3	6.8 \pm 0.2	0.04
PZ LI (%)	17.2 \pm 0.9	16.4 \pm 1.3	NS	18.9 \pm 1.5	12.3 \pm 1.0	0.011
IGF-II mRNA (g/cell)	38.3 \pm 1.5	47.3 \pm 2.8	0.03	51.9 \pm 3.4	37.7 \pm 2.8	0.01
HZ (μ m)	221.0 \pm 10.1	143.2 \pm 4.3	0.0004	154.3 \pm 10.8	94.3 \pm 16.5	0.016
HZ cell number	13.0 \pm 0.5	12.6 \pm 0.5	NS	8.2 \pm 0.5	5.6 \pm 0.1	0.0011
HZ cell height (μ m)	32.5 \pm 1.2	23.0 \pm 2.4	0.003	29.5 \pm 0.3	18.4 \pm 0.6	0.0001

Measurements given in mm or μ m represent the longitudinal dimension of the structure parallel to the long axis of the bone. The cell number data for the germinal (GZ) and hypertrophic zones (HZ) represent the mean number of cells in single columns spanning the entire longitudinal diameter of the zone, while the data for the proliferative zone (PZ) represents the number of cells in 50 μ m in a closely packed column. The DNA labeling index (LI) was determined by scoring the number of BrdU-positive nuclei per total nuclei in the PZ. The data for IGF-II mRNA are given as grains/cell (g/cell). The data on the *Igf1* null growth were published in Wang *et al.* (1999). NS, not significant.

reported on longitudinal growth of the femur. They showed that the rate of femoral growth for the *Igf1* null mouse was reduced by about 35% while the *lit/lit* femoral growth rate was reduced by \sim 55–60% of *Lit/+* controls between P31 and P56 (Fig. 2 and Tables 1 and 3 in Mohan *et al.* 2003). Thus four independent studies, encompassing independent targeted or naturally occurring deletions, in different murine strains, all show a marked \sim 60% reduction in longitudinal bone growth in GH-deficient mice, in contrast to a significantly lower, $<$ 40% reduction in *Igf1* null mice, even though in general, *Igf1* null mice show a more pronounced dwarfism in terms of body weight.

Our findings of compound defects in the *Ghr* null mouse growth plate, i.e. a reduced germinal zone, reduced chondrocyte proliferation and reduced chondrocyte hypertrophy provide a mechanistic explanation for the greater linear growth deficit in the *Ghr* null mice and provide support for the view that GH has important, IGF-I-independent effects on long bone growth. Lupu *et al.* (2001) confirm, in a new mouse line, our previously reported observations of germinal zone expansion and reduced chondrocyte hypertrophy in *Igf1* null dwarfs (Wang *et al.* 1999). However, they appear to categorically reject the notion of GH as an independent effector at the growth plate, and attribute the germinal zone expansion to delayed ossification rather than to GH excess (Lupu *et al.* 2001). In addition, they report that the *Ghr* null mouse growth plate germinal zone is not enlarged, a finding we confirmed in the present study, and also that the *Ghr* null mouse tibia does not exhibit delayed ossification (Lupu

et al. 2001). However, Sjogren *et al.* (2000) report that the *Ghr* null mouse proximal tibia demonstrates extremely delayed ossification. These conflicting views may be due to problems in obtaining sections from the tiny bones of dwarf mice that are properly anatomically matched to the larger specimens from WT mice, and to qualitative histological data. We examined serial sections through each growth plate to obtain perfectly matched anatomical sections for image analysis and to be sure that we appreciated the full thickness of the growth plate. We found that tibial epiphysal ossification was very similar in *Igf1* null and *Ghr* null mice and that secondary ossification centers were about 30% smaller in area relative to the entire epiphysis in both dwarf lines compared with their respective WT at P20, indicating a mild delay in bone age. However, since both dwarf phenotypes exhibit the same secondary ossification delay, while the germinal zone is enlarged in *Igf1* null mice but reduced in *Ghr* null mice, the germinal zone alterations cannot be attributed to ossification delay. It is important to note that these concordant observations on germinal zone enlargement were made in independent *Igf1* targeted deletions maintained in different strains of mice. The present data from CD1 mice together with that of Lupu *et al.* (2001) in MF1/DBA mice clearly establish that *Igf1* deletion results in enlargement of the growth plate germinal zone, irrespective of genetic background. A likely explanation for this finding is that GH, which is elevated in both strains of *Igf1* null mice, stimulates germinal zone expansion.

While Lupu *et al.* (2001) confirmed our observations on germinal zone expansion and decreased chondrocyte

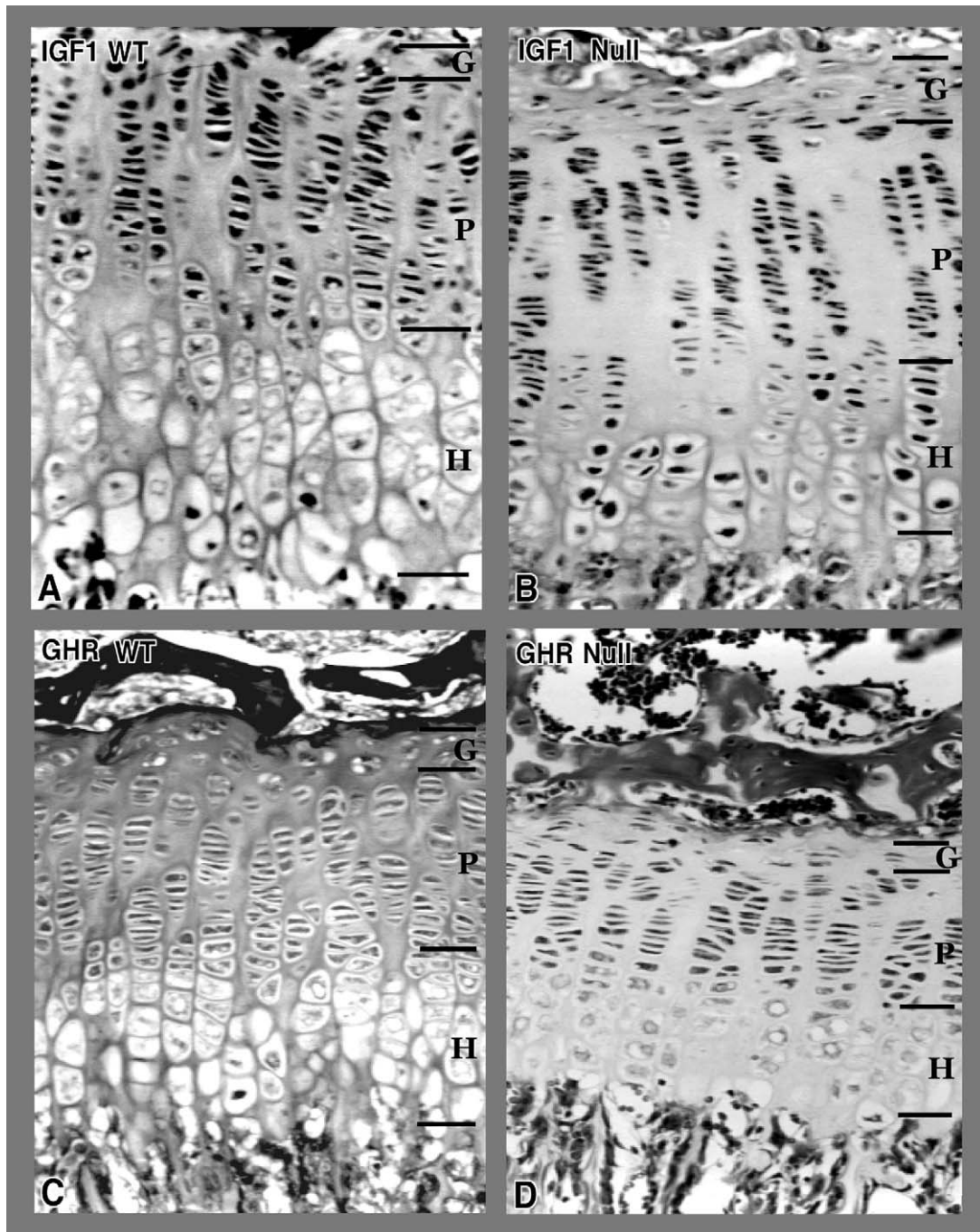


Figure 3 Effects of *Igf1* and *Chr* deletion on epiphyseal growth plate cell populations. The Figure illustrates representative proximal tibial growth plates from WT and dwarf mice. The germinal zone (G) is expanded in the *Igf1* null mice growth plate but reduced in the *Chr* null mice growth plate. The number of chondrocytes is equal in *Igf1* null and WT mice (A and B) but reduced in *Chr* null mice (C and D). The size of hypertrophic chondrocytes is reduced in both dwarfs. P, proliferative zone; H, hypertrophic zone.

hypertrophy in the *Igf1* null mouse growth plate, they do not agree with our finding of normal chondrocyte number and proliferation in the *Igf1* null mouse growth plate

(Wang *et al.* 1999). Lupu *et al.* (2001) report reduced proliferation and cell numbers in both dwarf growth plates. These observations, however, are not supported by

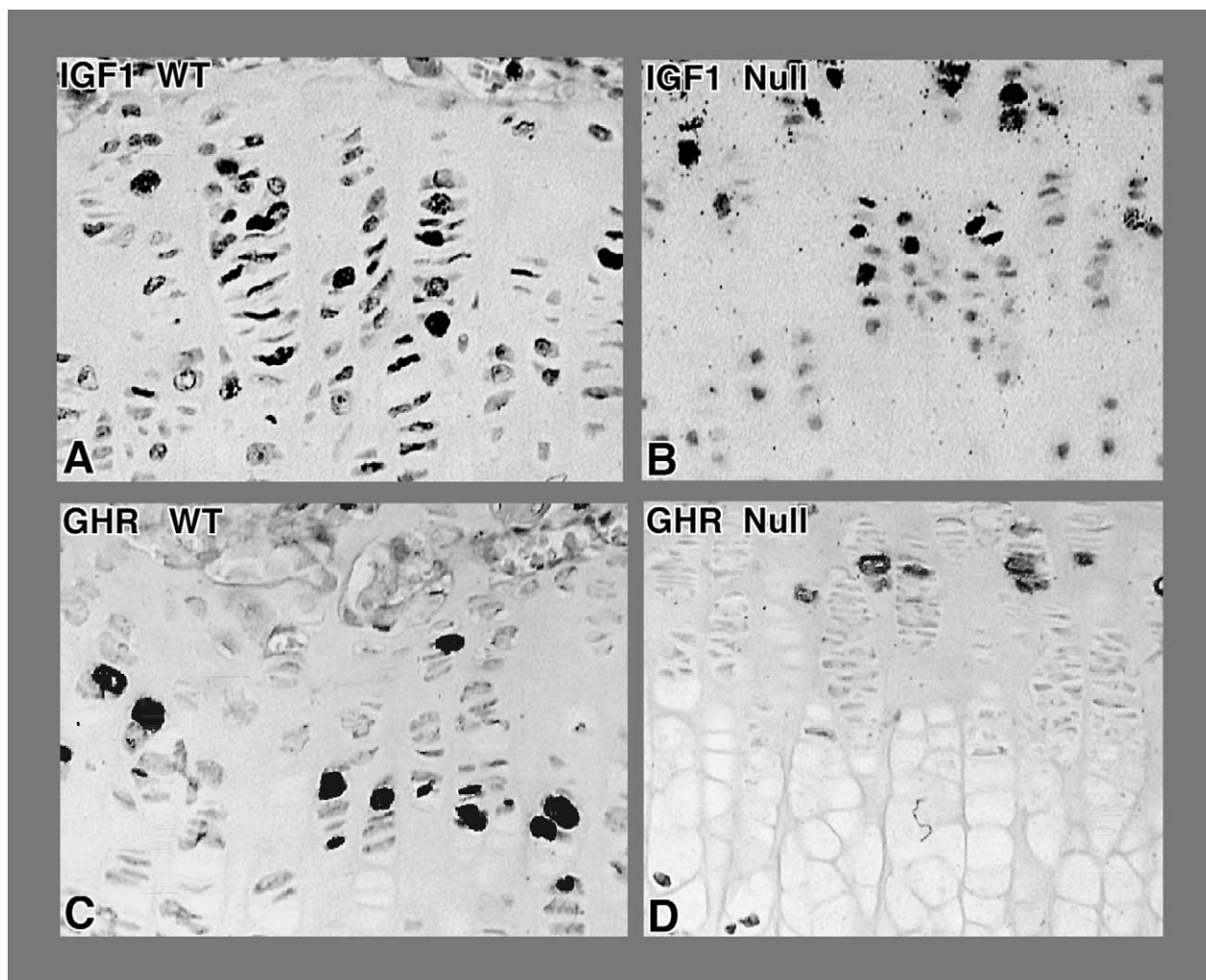


Figure 4 Chondrocyte proliferation is reduced in the *Ghr* null mice growth plate but not in the *Igf1* null mice growth plate. BrdU incorporation in growth plate chondrocytes from WT and dwarf mice at P20 is detected by immunohistochemistry. The micrographs show a high magnification view of the proliferative zone, wherein the dark-stained, rounded nuclei are BrdU-positive cells engaged in the mitotic cycle.

quantitative or statistical analyses and provide no explanation for the greater reduction in long bone growth rate in *Ghr* mice noted in four recent studies.

This analysis of growth plate dynamics provides a plausible explanation for the differences in longitudinal bone growth seen in *Igf1* null and *Ghr* null dwarfs which fits with previous experimental data bearing on the subject (reviewed in Ohlsson *et al.* 1998). The fact that *Igf1* and *Ghr* deletions each produce consistent tibial growth phenotypes in different strains of mice shows that these deficits are independent of the genetic background and relate specifically to the roles of GH and IGF-I in the promotion of longitudinal bone growth. The dual reduction in chondrocyte generation and hypertrophy accounts for the more profound deficit in the *Ghr* null mouse, while the reduction only in chondrocyte hypertrophy accounts

for a milder effect in the *Igf1* null mouse. However, this study does not resolve the issue of whether GH acts 'directly' on chondrocyte generation, leaving the door open to mediation by another somatomedin, IGF-II.

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