Perinatal testosterone surge is required for normal adult bone size, but not for
normal bone remodelling

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Running head: Androgens in bone growth and remodelling

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Abstract

While testosterone has striking effects on mature skeletal size and structure, it is not clear whether this depends exclusively on adult circulating levels of testosterone or whether additional early life factors also play a role. We have compared the androgen-deficient hypogonadal (hpg) mutant mouse with intact, orchidectomised and testosterone-treated non-hpg mice to determine relative contributions of adult and perinatal testosterone to bone growth and development. At 3 weeks of age, while trabecular and cortical bone structure was normal, bone turnover was significantly altered in hpg male mice; osteoid volume (OV/BV) and osteoblast surface (ObS/BS) were significantly lower, and osteoclast surface (OcS/BS) significantly higher in hpg mice compared with age-matched non-hpg mice, pointing to a role for the perinatal testosterone surge in determining bone turnover levels before sexual maturity. At 9 weeks of age, the hpg bone phenotype mimicked closely that of age-matched non-hpg mice that had been orchidectomised at 3 weeks of age, including low trabecular bone mass and high bone turnover. These bone phenotypes of hpg and orchidectomised non-hpg mice were all prevented by replacement doses of testosterone (T) or dihydrotestosterone (DHT) suggesting that these are determined by adult sex steroid hormones. In contrast, a short bone phenotype that could not be prevented by T or DHT treatment was observed in 9 week old hpg mice yet not in intact or castrated non-hpg mice. This data suggests a role for the perinatal testosterone surge in determining adult bone length, and confirms that adult circulating testosterone determines adult bone density.

Keywords: testosterone, hypogonadism, skeleton
Introduction

Skeletal size, shape and internal structure are determined by three major processes: embryonic development, growth before sexual maturity, and remodeling of the trabecular network throughout life. These three pathways depend on complex intercellular communication between the cells within bone, particularly osteoblasts (bone forming cells), osteoclasts (bone resorbing cells) and chondrocytes (cartilage cells). Androgens are known to influence proliferation and function of all three of these cell types, and are required for both skeletal growth and maturation.

Androgens play an important role in trabecular remodeling. In most mammalian species, trabecular bone density is significantly higher in males (41). Low bone density is observed in men with androgen deficiency, regardless of whether the hormone deficit is congenital, e.g. idiopathic hypogonadotrophic hypogonadism (IHH) (5, 7, 8) or Klinefelter’s syndrome (KS) (5), or acquired during adult life, e.g. castration (6, 38, 39). While osteoporosis due to castration is associated with high bone turnover (6, 38, 39), this is not consistently reported in early onset (and sometimes less complete) androgen deficiency (5, 7, 8, 12, 17). By contrast, in rodent models of complete congenital androgen deficiency, such as androgen insensitivity due to androgen receptor (AR) mutations or orchidectomy, low trabecular bone mass due to high bone turnover is observed consistently (15, 41).

Bone size and geometry are also influenced by sex steroid hormone levels as reflected in sexual dimorphism in bone length and width in humans and rodents. Bone width and cortical bone growth are modified by defects in either estrogen or androgen actions (1, 15, 18, 22, 31, 35, 40). In contrast, bone length is unaltered in the absence of androgen receptors (15, 18, 40), but is modified by complete blockade of estrogen action due to either absence of estrogen receptors (ER) or aromatase in humans (1, 35) and mice (22, 31). Thus while both estradiol and testosterone regulate appositional bone growth,
it has been suggested that only estrogen, possibly acting via the GH/IGF-I axis, is required for normal longitudinal bone growth (24, 41).

While no sexual dimorphism is observed in bone size before sexual maturity, it is possible that differences in sex steroid secretion during development may determine adult bone size, shape and internal structure. The adult male-specific secretion pattern of GH is determined by a peak of testicular androgen secretion during the perinatal period (14), a process known as hormonal imprinting due to the perinatal androgen surge present in all male mammals. Since GH also regulates bone size and shape (29), perinatal testosterone may therefore also determine the quality and quantity of mature bone.

While studies in gonadectomised rodents provide much information about the need for androgens in bone growth and turnover during and after sexual maturation, any role of the perinatal androgen surge is overlooked in such models.

The androgen deficient (hpg) mouse is a naturally occurring mutant (2) with a large deletion in the GnRH gene (19). This produces complete permanent functional elimination of hypothalamic GnRH secretion so that male mice lack testosterone secretion from birth onwards, including both the perinatal testosterone surge and sexual maturation. As a result, hpg mice show persistence of an immature reproductive system with undetectable blood LH and FSH, very low blood testosterone (T), infantile testes and immature androgen dependent organs (33). The functional consequences of GnRH deficiency are fully rectified by transfer of the GnRH gene (20) and, to a lesser extent consistent with the later onset of functional GnRH replacement, by intracerebral transplantation of fetal GnRH neurons (16, 28) or GnRH-secreting tumour cells (27) or even frequent GnRH injections (3). The latter findings also confirm that, despite low level of expression of GnRH and/or its receptor in some extracranial sites, all physiological effects of GnRH are replicated by restoring GnRH action within the hypothalamo-pituitary unit. Although homozygous hpg mice have complete post-natal androgen
deficiency (21), they retain normal androgen sensitivity with testosterone replacement rectifying all androgen-dependent deficits in mature somatic organs and tissues (13, 26, 34, 36, 37). Hence, treatment of hpg mice with testosterone from weaning to emulate sexual maturation represents a model for loss of the perinatal androgen surge without loss of testosterone during sexual maturation.

We have utilized this mouse model to make a comparison between complete androgen deficiency either from birth (the hpg mouse) or after the perinatal period (orchidectomy), to determine the role of the perinatal androgen surge in determining adult bone size and trabecular structure. To do this, we examined the role of androgens in early skeletal growth by comparing immature 3 week old hpg and non-hpg male mice; the role of androgens in the determination in bone structure in early mature life by comparing 9 week old hpg and non-hpg male mice, and then determining whether exposure to the perinatal androgen surge makes a difference by comparing hpg mice with castrated non-hpg males. Finally, we determined whether androgen replacement using aromatisable testosterone or its non-aromatisable metabolite DHT could prevent the effects of congenital androgen deficiency, and thereby identify which effects of testosterone on the skeleton relate to loss of sexual maturation and which are due to lack of the perinatal testosterone surge.

**Materials and Methods**

The hpg mouse line is a naturally-occurring mutant (2) extensively used as a model of complete post-natal androgen deficiency with preservation of androgen sensitivity(10, 32, 37). The hpg colony is bred from fertile heterozygotes originally from F1 hybrids of two inbred strains C3H/HeH and 101/H as previously described (32). Animals with at least one wild type allele (non-hpg) were used as controls for all parts of the study as previously described (32). There was no significant difference in any marker of bone size, structure or turnover between heterozygous and homozygous non-hpg mice.
Animals were maintained under standard conditions at the ANZAC Research Institute and all experiments were approved by the Central Sydney Area Health Service Animal Welfare Committee within NHMRC guidelines for animal experimentation.

Skeletal specimens were collected from 3 and 9 week old male mice. Samples were fixed in 10% buffered formalin and embedded in methylmethacrylate (29). Double fluorochrome labeling was performed as described previously (29). 5 µm sections were stained with toluidine blue or stained with Xylenol orange for analysis of fluorochrome labels (11). Histomorphometry was carried out in the secondary spongiosa of the proximal tibia according to standard procedures using the Osteomeasure system (OsteoMetrics Inc, Decatur, GA). Tibial cortical thickness, periosteal mineral appositional rates and proximal tibial growth plate widths were measured as described previously (29). Femoral and tibial length and femoral mid-diaphyseal diameter were determined from contact X-rays that were scanned and measured using NIH Image 1.62 as described previously (31).

Femoral cortical (Ct) and trabecular (Tb) bone mineral density (BMD), femoral circumference and femoral cortical thickness were measured by peripheral quantitative computer tomography (Stratec X-CT Research SA+, version 5.5) using methods adapted from Schmidt et al (25). Metaphyseal scans of the distal femur were taken at a resolution of 70um; trabecular and cortical measurements (including circumference) were taken at a distance proximal to the distal growth plate of 5% and 25% of the length of the femur, respectively; Tb.BMD was determined as the inner 45% of the total area (peel mode 20). Interassay coefficients of variation were <1%.

Serum osteocalcin levels were measured by a mouse specific IRMA (Immutopics Inc., USA), which utilizes polyclonal goat-anti-mouse antibodies against mid-region, C and N terminal osteocalcin. The intra assay CV for this assay was 4.6%, and the inter assay CV was 5.2%. Circulating levels of mouse
tartrate-resistant acid phosphatase 5b (TRACP5b) were determined by ELISA based upon a polyclonal antibody raised against intact mouse osteoclast TRACP 5b (Suomen Bioanalytiikka Oy, Finland). This assay had an intra assay CV of <6%, and an inter assay CV of <8%. All measurements were done in duplicate.

To compare the effects of congenital hypogonadism to gonadectomy after the perinatal period, 3-week-old non-\textit{hpg} mice were sham operated or orchidectomised. To determine the effects of testosterone, non-\textit{hpg} mice were orchidectomised at 3 weeks of age, and both non-\textit{hpg} and \textit{hpg} mice were treated from 3 to 9 weeks of age with testosterone (T) or DHT by subdermal implantation of 1 cm silastic implants filled with crystalline steroids (Sigma) as described previously (9, 32). We have previously shown that these implants provide reproducible and predictable steady-state blood T or DHT levels for up to 8 weeks (33). Mice were killed by cardiac puncture under anaesthetic at 9 weeks of age, when tissues were collected for analysis.

Statistically significant effects of genotype or treatment were determined by one- or two-way ANOVA followed by Fisher’s post hoc test using the StatView software package. All data is presented as means ± SEM. A p<0.05 was considered statistically significant.

Results

\textit{Effect of postnatal androgen deficiency on bone growth and skeletal maturation in immature mice}

At 3 weeks of age, no significant difference was detected in body weight (mean g ± SEM: non-\textit{hpg}: 12.3±0.5, \textit{hpg}: 11.5±0.7), femoral circumference (FemCi), cortical thickness (CtTh), cortical BMD (CtBMD) or femoral length (FemL) between non-\textit{hpg} and \textit{hpg} mice (Figure1A,B,C), yet growth plate
width was significantly reduced (Figure 1D). Furthermore, there was no significant difference in trabecular bone structure or density, including trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N) or trabecular BMD (Tb.BMD) between non-\textit{hpg} and \textit{hpg} mice at 3 weeks of age (Figure 2A,B), confirming that androgens prior to sexual maturity do not determine bone size or trabecular structure at this age.

While there was no difference in trabecular structure between the non-\textit{hpg} and \textit{hpg} mice at 3 weeks of age, there was a striking difference in trabecular bone remodeling levels between the two mouse strains. In the 3 week old \textit{hpg} mice, osteoclast surface (Figure 2C) was significantly greater than in non-\textit{hpg} mice, yet this was not associated with a similar rise in bone formation, as usually occurs when bone resorption is increased. Rather, in the \textit{hpg} mice, histomorphometric indicators of bone formation including osteoblast surface (ObS/BS) and osteoid volume (OV/BV) were significantly lower than levels observed in non-\textit{hpg} mice (Figure 2C). A similar effect of the mutation on biochemical markers of bone turnover was observed, but this was not statistically significant (TRAP5b mean U/L ± SEM: non-\textit{hpg}: 26.4±1.5, \textit{hpg}: 30.5±1.6, \textit{p}=0.075); Osteocalcin mean ng/ml ± SEM: non-\textit{hpg}: 888±39, \textit{hpg}: 816±59, \textit{p}=0.312).). Thus, in \textit{hpg} mice there is disrupted coupling of osteoblast and osteoclast formation such that bone resorption is high and bone formation is low. At the 3-week time point, this imbalance in bone turnover has not yet resulted in a significant change in trabecular structure (Figure 2A,B).

In untreated 9-week-old animals, a significant difference in trabecular structure and density was observed between non-\textit{hpg} and \textit{hpg} mice (Figure 3A,B). In \textit{hpg} mice, TbBMD (Figure 3A) was significantly lower than in non-\textit{hpg} controls. Not only was the normal growth-associated increase in TbTh between 3 and 9 weeks inhibited, but there was also a significant reduction in TbN in the \textit{hpg} mice, resulting overall in significantly lower BV/TV at 9 weeks compared with age-matched non-\textit{hpg}
mice. This was also reflected in a significant reduction in TbBMD (Figure 3B). At 9 weeks of age, hpg mice also demonstrated significantly elevated bone turnover compared with non-hpgs (Figure 3C). Serum levels of tartrate-resistant acid phosphatase 5b (TRAP5b), a marker of bone resorption was also significantly elevated in 9 week old hpg mice (mean U/L ± SEM: non-hpg: 12.7±1.2, hpg: 19.7±2.0, p=0.007). In contrast, serum osteocalcin, a marker of bone formation, was significantly reduced compared to non-hpg mice (mean ng/ml ± SEM: non-hpg: 362±35, hpg: 203±18, p=0.0003). This phenotype is similar to the trabecular bone defect reported in orchidectomised mice.

Comparison of postnatal with post-weaning androgen-deficiency

To determine whether the phenotype of 9 week old hpg mice is exclusively due to the lack of testosterone during sexual maturation, these mice were compared with age-matched non-hpg mice that had been orchidectomised at 3 weeks of age (ORX).

In trabecular bone, the low Tb.BMD, BV/TV and TbN observed in 9 week old hpg mice was not significantly different from the structural defects observed in age matched non-hpg mice orchidectomised at 3 weeks of age (Figure 3A, B). The low trabecular bone volume and density induced by congenital androgen deficiency or orchidectomy at weaning was associated with a high level of both ObS/BS and OcS/BS (Figure 3C) as well as osteoid volume (OV/BV), osteoid surface (OS/BS), osteoid thickness (OTh), osteoblast number (NOb/BPm), and osteoclast number (NOc/BPm) (data not shown). We could detect no significant differences in trabecular structure (Figure 3A,B), bone remodeling parameters (Figure 3C), or bone appositional growth (Figure 4) between 9 week old hpg mice and age-matched ORX non-hpg mice, indicating that androgen-related effects on bone width and trabecular structure are determined by physiological events after 3 weeks of age.
In contrast, bone length and markers of longitudinal bone growth differed between orchidectomised non-\(hpg\) mice and age-matched \(hpg\) mice, suggesting a role for the perinatal testosterone surge in longitudinal bone growth. At 9 weeks of age, both femoral and tibial lengths were significantly lower in the \(hpg\) mice than in age-matched ORX non-\(hpg\) mice (Figure 5). This difference may relate to a lower level of chondrocyte proliferation, early in bone growth, suggested by reduced growth plate width at 3 weeks of age in the \(hpg\) mice (Figure 1C), but resolved by 9 weeks of age (Figure 5C).

*Androgen replacement in young orchidectomised and \(hpg\) mice*

A second method of determining whether perinatal or adult testosterone is the more major regulator of bone structure was to restore normal levels of testosterone in \(hpg\) mice and age-matched ORX non-\(hpg\) mice as described previously (13, 26, 34, 36, 37). Both testosterone (T) and dihydrotestosterone (DHT) treatments were used to separate out any difference between effects of a pure (non-aromatisable) androgen (DHT) from potential effects of T via its aromatized metabolite, estradiol.

Treatment with replacement doses of either T or DHT from 3 to 9 weeks of age prevented the reduction in Tb.BMD, BV/TV and TbN associated with maturation in \(hpg\) mice, and, at the same dose, prevented the bone loss observed in ORX non-\(hpg\) mice. The effects of T and DHT on trabecular bone in \(hpg\) mice were not significantly different (Figure 3A,B) and were both associated with an inhibition of bone turnover. In non-\(hpg\) mice, T inhibited the orchidectomy-induced increase in all markers of bone turnover (Figure 3C and data not shown). In \(hpg\) mice, T and DHT treatment significantly reduced bone formation, indicated by ObS/BS, OV/BV, OS/BS, OTh, NOb/BPm, mineral appositional rate (MAR), mineralizing surface (MS/BS) and bone formation rate (BFR/BS) to levels equivalent of that of 9 week old intact non-\(hpg\) mice (Figure 3C and data not shown). Similarly, with bone resorption, both T and DHT reduced NOc/BPm and OcS/BS until they were not significantly different from intact
non-\textit{hpg} mice at the same age (Figure 3C and data not shown). No significant differences between the effects of T or DHT were detected on trabecular density, structure or on histomorphometric markers of bone turnover.

In cortical bone, both T and DHT treatment from the age of 3 weeks completely prevented the low FemDi and CtTh (in both femora and tibia) associated with orchidectomy or postnatal androgen deficiency by maintaining periosteal bone growth (PsMAR) at a level similar to that observed in intact non-\textit{hpg} mice (Figure 4).

Supporting a role for the perinatal androgen surge in longitudinal bone growth, neither T nor DHT treatment was able to prevent the short bone phenotype observed in \textit{hpg} mice. Instead, T worsened this phenotype, and a further impairment of femoral longitudinal growth was observed in both \textit{hpg} and control mice (Figure 5A,B). This shortened both length was associated with a significantly narrower growth plate (Figure 5B), and a significant reduction in the proliferating compartment (Figure 5C), confirming the known anti-proliferative effect of both T and DHT on chondrocyte proliferation.

\textbf{Discussion}

The \textit{hpg} mouse, with complete postnatal androgen deficiency but preserved androgen sensitivity provides a unique model for studying the importance of sex steroids from the time of birth. This naturally-occurring mutant allows the study of sex steroid response without the complications of surgical or chemical gonadectomy and, in contrast to knockouts of androgen and estrogen receptors, these mice respond normally to natural ligands.
The perinatal androgen surge, which transiently results in mature male levels of testosterone, has been shown to be required for normal function of the mature mouse (32) and rat (23) prostate, and for longitudinal bone growth (14). However, its role in determining trabecular bone mass and bone turnover has not yet been established. A key difference between wild type and hpg mice from birth until 3 weeks of age, is the low (castrate equivalent) circulating testosterone levels in hpg male mice (33) whereas wild type mice demonstrate a surge in testicular testosterone secretion in the perinatal period reaching mature male levels in the non-hpg mice (4, 9). The shortened adult bone length and disordered bone turnover at 3 weeks of age in hpg mice appear to be a result of the lack of the perinatal androgen surge.

The reduced growth plate width at 3 weeks of age is consistent with the shortened bones observed in 9 week old hpg mice compared with intact non-hpg mice, and consistent with a role for the perinatal testosterone surge in determining growth hormone secretion in male mice (14). The shortened bone phenotype of the hpg mice was not observed in the absence of a functional androgen receptor (AR) (15), indicating that perinatal testosterone surge may affect longitudinal growth by aromatization of T as suggested by reduced bone length in adult male ERαβ−/− and aromatase KO mice (22, 31) or by the effects of estrogen or perinatal testosterone on the GH/IGF-I axis (14). The shortened bones, reduced chondrocyte proliferation and reduced GH secretion in the hpg mice are also consistent with reduced bone size in GHR deficient mice (29).

Adult hpg mice also demonstrated high bone turnover and low bone mass, which did not appear to relate to the lack of perinatal testosterone. While consistent with the bone phenotype of the adult male ARKO and aromatase KO mice (15, 22), this aspect of the phenotype is quite different to that observed in the absence of GHR, which demonstrated normal trabecular bone structure and turnover (29). Thus, while perinatal testosterone imprinting may regulate longitudinal growth via GH/IGF-I, this growth
factor mechanism does not appear to be the mediator of either the altered bone turnover at 3 weeks, or the high turnover osteopenia observed at 9 weeks in the hpg mice. Rather, the trabecular structure of hpg mice at 9 weeks of age is remarkably similar to that of age-matched non-hpg mice that were orchidectomised at 3 weeks of age. Notably, this phenotype is consistent with the effects of both early (5, 7, 8) and late onset (6, 38, 39) androgen deficiency on bone mass in men. The only significant difference in the bone phenotype between the hpg mice and orchidectomised mice is the reduced longitudinal growth in the former, which we attribute to a mild impairment in chondrocyte proliferation detected before orchidectomy (at 3 weeks). The similarities between the hpg and Orx non-hpg mice indicate that the trabecular and cortical bone phenotypes of the hpg mutation relate to the effects of the mutation on post-pubertal GnRH-dependent pituitary gonadotrophin and testicular androgen secretion associated with sexual maturation rather than the absence of the perinatal testosterone surge. Furthermore, the phenotype is distinct from the increased bone mass and low bone turnover observed in the male ERαβ−/− (31) suggesting that it is not the absence of aromatized T (i.e. estradiol) that has caused the osteopenia associated with the hpg mutation. These results confirm that testosterone itself through effects mediated via the AR plays a crucial role in determining the rate of juvenile bone turnover, which itself, determines adult cortical and trabecular structure.

Testosterone treatment is able to prevent orchidectomy-induced bone loss in the absence of estrogen receptor alpha (which is the only estrogen responsive receptor in male bone) (30), implying that aromatization is not required for the bone protective effects of testosterone on the trabecular bone. Here we also observed that the high bone turnover and low bone mass observed in adult hpg and Orx mice are prevented equally by either T or DHT treatment. This result, and the mechanism by which T and DHT prevent the bone loss via effects involving the AR are consistent with effects observed in orchidectomised rats (42) and the ERα+− (30, 31) and ARKO (15) mice. For example, in the absence of ERα, which is the only ER required for estrogen action in male bone, the effects of T and DHT on
orchidectomy-induced bone loss and the associated high level of bone remodeling are identical (30, 31), confirming that T does not require aromatization for its effect on trabecular bone.

The only effect of congenital androgen deficiency remaining uncorrected by T or DHT was the reduction in bone growth, which is exaggerated by this treatment protocol. This is consistent with our suggestion that the growth defect in hpg mice is largely due to the absence of the perinatal testosterone surge.

In conclusion, this study demonstrates that testosterone, via both its aromatisable, and non-aromatisable metabolites is able to prevent trabecular bone loss and partially reduce the impaired periosteal bone growth observed in growing congenitally androgen-deficient (hpg) mice, confirming a central role for testosterone in the regulation of skeletal growth and maturation. We also demonstrate that perinatal androgen imprinting via an AR dependent mechanism is a key determinant of mature bone length in males.

**Figure Legends**

**Figure 1:** Femoral diameter (Fem.D), Cortical width (Ct. Wi.) cortical BMD (Ct.BMD) and femoral length (Fem. L) were not significantly altered by the hpg mutation (black bars) at 3 weeks of age, yet growth plate width (G.Pl.Wi) was significantly reduced in the hpg mutant mice compared with non-hpg mice (ctrl). Values are means ± SEM on 8-10 mice per group. Effect of mutation: *, p<0.05 vs control of same age.

**Figure 2:** Comparison of trabecular bone structure and turnover in 3 week old hpg and control (ctrl) mice. A: Representative sections of proximal tibiae of control and hpg mutant mice at 3 weeks of
age stained with a modified Von Kossa stain (mineralised bone appears black). blue bar = 500µm. **B:** Trabecular bone volume (BV/TV), thickness (Tb.Th) and number (Tb.N) were not significantly different in *hpg* mice compared with non-*hpg* mice. **C:** Bone turnover was significantly altered by the *hpg* mutation. In *hpg* mice, osteoid volume (OV/BV) and osteoblast surface (ObS/BS) were significantly lower, and osteoclast surface (OcS/BS) significantly higher in *hpg* mice compared with age-matched non-*hpg* mice. Values are means ± SEM of 8-10 mice per group. Effect of mutation: *, p<0.05, **, p<0.01 vs control of same age.

**Figure 3:** Low bone mass and high turnover in 9 week old *hpg* mutant mice is similar to control mice orchidectomised at 3 weeks, and testosterone treatment is able to prevent this low bone mass. **A:** Representative Von Kossa stained proximal tibial sections from 9 week old control and *hpg* mice. Control mice were sham operated (Sham) or orchidectomised (Orx) at 3 weeks of age and treated with vehicle (Orx) or testosterone (Orx+T) from 3 weeks of age. *Hpg* mice were treated with vehicle (*hpg*), testosterone (*hpg*+T) or dihydrotestosterone (*hpg*+DHT) from 3 weeks of age; blue bar = 500µm. The structure of untreated *hpg* and Orx control mice appeared very similar. T and DHT treatment in both control (Orx+T) and *hpg* mice (*hpg*+T, *hpg*+DHT) prevented the low bone mass. **B:** Trabecular bone mineral density (TbBMD), trabecular bone volume (BV/TV), and trabecular number (TbN) were significantly lower in Orx and *hpg* mice compared with Sham control animals; T and DHT treatment in both *hpg* and control Orx mice prevented the low bone mass associated with Orx or *hpg* mutation. **C:** Osteoblast surface (ObS/BS) and osteoclast surface (OcS/BS) were both high in Orx and *hpg* mice. This elevation and mineral appositional rate (MAR) were all suppressed by T and DHT treatment in both Orx and *hpg* mice. Values are means ± SEM of 6-12 mice per group. Effect of sex steroid depletion: ++, p<0.01; ++++, p<0.001 in Orx or *hpg* vs. Sham. Effect of T or DHT treatment: *, p<0.05; ***, p<0.001 vs untreated Orx or *hpg*.
Figure 4: Effects of hpg mutation and sex steroid depletion or treatment on accrual of bone width and cortical thickness at 9 weeks of age. Femoral circumference (FemCi) and cortical thickness (FemCtTh), and tibial cortical thickness (TibCtTh) and tibial periosteal appositional rate (PsMAR) were significantly lower in hpg mice and Orx non-hpg mice compared with Sham operated non-hpg mice of the same age. T and DHT treatment of both hpg mice and Orx non-hpg mice prevented this reduction in bone width. Values are means ± SEM of 6-12 mice per group. Effect of sex steroid depletion: ++, p<0.01; ++++, p<0.001 in Orx or hpg vs. Sham. Effect of T or DHT treatment: *, p<0.05; ***, p<0.001 vs untreated Orx or hpg.

Figure 5: Effects of sex steroid depletion or treatment on bone length and longitudinal bone growth at 9 weeks of age. A: Representative contact X-rays of each treatment group. Bar = 500µm. B: Tibial and Femoral (Fem) lengths were significantly lower in hpg mice compared with both Sham operated and Orx non-hpg mice of the same age. T and DHT treatment did not prevent this reduction in bone length in hpg mice; even resulting in a further reduction in tibial length. C: Tibial growth plate width (G.P.Wi), but not proliferating zone width (Prol.Z.Wi) was significantly elevated in hpg mice compared with non-hpg mice. T and DHT treatment caused a reduction in both G.P.Wi and Prol.Z.Wi in both non-hpg and hpg mice. Values are means ± SEM of 6-12 mice per group. Effect of sex steroid depletion: ++, p<0.01; ++++, p<0.001 in Orx or hpg vs. Sham. Effect of T or DHT treatment: *, p<0.05; ***, p<0.001 vs untreated Orx or hpg.
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References


Figure 1:

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Figure 4:
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