Does adiposity status influence femoral cortical strength in rodent models of growth hormone deficiency?

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INTRODUCTION

To determine whether adiposity status influences femoral cortical strength in rodents, we assessed cortical strength in a range of rodent models of GH deficiency with divergent degrees of adiposity. In rats, skeletal growth differs from that in humans in that the epiphyseal plates never fully close (24), but similarity in the processes of bone growth and remodeling justifies the wide use of young rats to model the growing human skeleton (reviewed in Ref. 38). Therefore, we have compared the pubertal development of impaired cortical strength in the femori of transgenic growth-retarded (Tgr) and dwarf (dw/dw) rats, relating total bone and material strength to endocrine status, and parameters of morphology and mineralization. These two rat models differ in that the Tgr rat displays moderate GH deficiency (17, 40) combined with profound hyperleptinemia (11, 15) similar to that seen most frequently in childhood-onset GH-deficient patients (Fig. 1) (19, 31, 33), whereas the dw/dw rat displays profound GH deficiency (8) accompanied by a reduction in fat deposition and hyperleptinemia (Fig. 1) (11).

To determine whether the relative impairment of bone strength in these rat models of GH deficiency is independent of the degree of reduction in GH signaling, we also quantified bone strength in two murine models with either partial or complete loss of GH signaling. In these models, GH deficiency not only results in short stature but also leads to impairment of bone mineral density (BMD) (22) and an increased fracture risk in adults (34). However, GH deficiency is usually associated with elevated adiposity, hyperleptinemia, and increased fracture risk. Since leptin is thought to enhance bone strength; femoral morphology; leptin

IN ADDITION TO ITS ROLE AS THE PRIMARY REGULATOR of postnatal longitudinal growth in mammals, growth hormone (GH) contributes to the endocrine control of bone mineralization (4, 30). In humans, GH deficiency not only results in short stature but also leads to impairment of bone mineral density (BMD) (22) and an increased fracture risk in adults (34). However, GH deficiency is also associated with increased fat accumulation and elevated circulating leptin concentrations (16, 23), both of which appear to influence bone homeostasis. It is thought that increased muscular forces in obese adults promote the observed elevation in bone strength (18), potentially affording protection against osteoporotic fracture (13). However, the effect of leptin on bone is more equivocal. Central leptin treatment has been reported to impair trabecular bone formation (14), whereas peripheral leptin treatment has been shown to induce osteoblast formation (36) and enhance the accrual and mineralization of cortical bone (10, 20, 31). Thus, in the context of GH deficiency, the impairment of cortical bone strength should be ameliorated, at least in part, by the influence of the accompanying elevation in adiposity and circulating leptin.

To test this hypothesis, we have assessed cortical strength in a range of rodent models of GH deficiency with divergent degrees of adiposity. In rats, skeletal growth differs from that in humans in that the epiphyseal plates never fully close (24), but similarity in the processes of bone growth and remodeling justifies the wide use of young rats to model the growing human skeleton (reviewed in Ref. 38). Therefore, we have compared the pubertal development of impaired cortical strength in the femori of transgenic growth-retarded (Tgr) and dwarf (dw/dw) rats, relating total bone and material strength to endocrine status, and parameters of morphology and mineralization. These two rat models differ in that the Tgr rat displays moderate GH deficiency (17, 40) combined with profound hyperleptinemia (11, 15) similar to that seen most frequently in childhood-onset GH-deficient patients (Fig. 1) (19, 31, 33), whereas the dw/dw rat displays profound GH deficiency (8) accompanied by a reduction in fat deposition and hyperleptinemia (Fig. 1) (11).

To determine whether the relative impairment of bone strength in these rat models of GH deficiency is independent of the degree of reduction in GH signaling, we also quantified bone strength in two murine models with either partial or complete loss of GH signaling. In these models, GH receptor (GHR) antagonist G119KbGH-transgenic (GHA) mice (9, 25) and GHR/binding protein-null (GHR/BPnull) mice (44) mice. These models show broadly similar degrees of adiposity/hyperleptinemia (Fig. 1).

In addition, to determine whether the effect of GH deficiency on femoral strength is exacerbated by an obese/hyperleptinemic phenotype, we also measured femoral strength in...
**Materials and Methods**

**GH-Deficient Rodents**

The animal procedures described below, including those involving genetically modified animals, conformed to the institutional and national ethics guidelines for animal experimentation at the respective institutions and were specifically approved by local ethics review. Homozygous dw/dw rats bred on an Albino Swiss (AS) background, used in study 3, were housed in the Division of Biological Services, National Institute of Medical Research (NIMR; London, UK), under conditions of 12:12-h light-dark (lights on at 0600), with food and water available ad libitum. Mice were genotyped by PCR analysis of a tail biopsy at 28 days of age and were anesthetized with halothane and killed by decapitation. Left femurs were dissected and total length measured with a hand-held micrometer before being wrapped in isotonic saline-soaked gauze and stored at −20°C prior to measurements of bone mineral content, morphology, and strength.

**Study 1: Transpubertal Development of Bone Strength in Tgr and dw/dw Rats**

Groups of nonfasting 3-, 6-, and 9-wk-old male WT, Tgr, and dw/dw rats (n = 4–6) from the Cardiff colonies were weighed, anesthetized with halothane, and killed by decapitation. Left femurs were dissected and total length measured with a hand-held micrometer before being wrapped in isotonic saline-soaked gauze and stored at −20°C prior to measurements of bone mineral content, morphology, and strength.

**Study 2: Femoral Strength in Murine Models of GH Deficiency**

The contribution of reduced GH signaling to the impairment of femoral strength was determined in GHA and GHR/BP−/− mice. Male GHA and GHR/BP−/− mice together with their respective WT controls were killed at 10 wk of age by cervical dislocation, and left femori were excised and stored as above.

**Study 3: Effect of Neonatal MSG Treatment on Bone Strength and Adiposity Profiles in dw/dw Rats**

To determine the potential contribution of elevated adiposity on impaired bone strength in GH deficiency, bone strength and adiposity were measured in dw/dw rats following neonatal treatment with MSG. Three equally sized litters of dw/dw rats received intraperitoneal injections of either vehicle (50 μl of 0.9% sterile saline) or MSG (4 mg/g body wt in 50-μl vehicle) on postnatal days 2, 4, 6, 8, and 10 and were carefully monitored for potential adverse effects. This dose of MSG has previously been shown to destroy 70–90% of neuronal perikarya in the arcuate nuclei, including 90% of GH-releasing factor neurons (1, 3), and to cause a significant elevation in adiposity in normal (3, 6) and dw/dw rats (11). Vehicle- and MSG-treated male and female dw/dw rats were anesthetized with halothane and killed by decapitation at 8 wk of age, with left femori excised and stored as above.

**Study 4: The Effect of a High-Fat Diet on Bone Strength in dw/dw Rats**

To determine the potential contribution of elevated adiposity on impaired bone strength in GH deficiency, femoral strength was measured in dw/dw rats after the induction of elevated fat deposition by pubertal exposure to elevated dietary fat. Five- to seven-week-old female dw/dw and WT rats (n = 5/group for dw/dw rats, n = 6/group for WT rats) were fed normal chow or a 40% high-fat diet for 4 wk and weighed weekly. This procedure doubles abdominal fat in dw/dw rats without significantly elevating fat deposition in AS rats (11). After 4 wk the rats were stunned and decapitated, and left femori were excised and stored as above.
Tissue Analyses

Femoral morphometry. Middiaphyseal cortical mediolateral (ML) and anterior-posterior (AP) diameters and lateral, medial, anterior, and posterior wall thicknesses were measured following strength testing (see below) at the fracture site using a Pye Scientific travelling microscope.

Femoral mineralization. Bone mineral content (BMC) was measured by dual-energy X-ray absorptiometry using the Lunar Pixi small animal scanner, as described previously (15). Briefly, femori were thawed at room temperature for 30 min prior to measurement and aligned in an AP orientation relative to the scanning beam. Measurements of total BMC and bone surface area were used to calculate areal bone mineral density (aBMD; BMC/surface area).

Femoral strength. Femoral strength was determined as described previously (15). In brief, thawed femurs were loaded in three-point bending, with the middle roller positioned over the thinnest part of the femoral shaft to give a roughly posterior load direction. Each bone was loaded until failure, with load and displacement data recorded by a Lloyd LRX tensile testing machine with 100-N load cell (Lloyd Instruments, Segensworth, Hants, UK).

Using the failure load, morphometric measurements (see above), and simple beam theory, ultimate tensile stress (UTS) was calculated using

\[ \sigma = \frac{M y}{I} \]

where the bending moment, \( M \), is one-half the applied load multiplied by the distance from the central to the outer support, \( y \) is one-half the outside depth, and the second moment of area, \( I \), is given by

\[ I = \frac{\pi}{64}(b_o d_i^3 - b_i d_o^3) \]

where \( b \) and \( d \) are the breadth and depth, respectively, of the cross-section and the subscripts \( o \) and \( i \) indicate the outside and inside dimensions respectively.

Statistical Analysis

All data are expressed as means ± SE, with statistical comparisons being performed by either Student’s t-test or ANOVA plus Bonferroni’s post hoc test as appropriate.

RESULTS

Study 1: Transpubertal Development of Bone Strength in Tgr and dw/dw Rats

Femoral morphometry. Femoral length increased with age in all three strains (\( P < 0.001; \) Fig. 2A). There was no difference between the groups at 3 wk, but from 6 wk femoral length in Tgr rats was 12% shorter than that in the WT counterparts (\( P < 0.001 \)). In 6- to 9-wk-old dw/dw rats femoral length was in between that seen in WT and Tgr rats, being 10% shorter than in WT rats at 9 wk (\( P < 0.01 \)). The diameter of the middiaphyseal femoral cortex increased with age in all three strains in both AP (\( P < 0.001; \) Fig. 2B) and ML (\( P < 0.001; \) Fig. 2C) planes. Although not significantly different at 3 and 6 wk, AP diameters were ∼12% lower in Tgr (\( P < 0.01 \)) and dw/dw (\( P < 0.05 \)) rats at 9 wk. ML diameter was 20% lower in dw/dw
males at 3 wk ($P < 0.001$) but not different in Tgr rats. At 6 and 9 wk, ML diameter was similarly reduced (15–20% lower) in Tgr and $dw/dw$ rats ($P < 0.001$).

Morphometric analysis of the fracture site revealed progressive increases in anterior (WT and $dw/dw$ only, $P < 0.05$; Supplemental Fig. S1A), posterior ($P < 0.05$; Supplemental Fig. S1B), lateral (WT and Tgr only, $P < 0.01$; Supplemental Fig. S1C), and medial ($P < 0.05$; Supplemental Fig S1D) cortical wall thickness. By 9 wk, $dw/dw$ males showed significantly thinner anterior (35% less, $P < 0.05$) and medial (31% less, $P < 0.05$) wall thicknesses than their WT counterparts, whereas the same parameters in Tgr males were not significantly reduced. The thickness of the thicker lateral wall in $dw/dw$ rats, although only 64–68% of that in the WT controls at 6 and 9 wk, was not significantly different ($t = 2.433$ (6 wk) and 2.616 (9 wk)). Lateral wall thickness in Tgr males was similar to $dw/dw$ rats at 6 wk, but similar to WT males by 9 wk, being 74% higher than that in $dw/dw$ males ($P < 0.01$). Medial wall thickness was significantly thinner in Tgr males at 6 wk (36% thinner, $P < 0.01$) and in $dw/dw$ males at 9 wk (31% thinner, $P < 0.05$) compared with WT males.

Femoral mineralization. A rapid linear increase in total femoral BMC was observed in all three groups of rats ($P < 0.001$; Fig. 2D), but the rate of increase was significantly lower in Tgr and $dw/dw$ males so that by 9 wk total femoral BMC was 27% less in $dw/dw$ males ($P < 0.001$) and 38% less in Tgr males ($P < 0.001$). Tgr rats being 15% lower than their $dw/dw$ counterparts ($P < 0.001$). Calculated aBMD showed a broadly similar profile (Fig. 2E), except that at 6 wk aBMD in Tgr and $dw/dw$ males was significantly lower than in WT males ($P < 0.01$ and $P < 0.001$), and at 9 wk the mineralization of Tgr femur was significantly lower than in their WT counterparts (18% less, $P < 0.001$). aBMD in $dw/dw$ rats not significantly different from either group. Similar changes in the aBMD were seen in the highly cancellous bone immediately distal to the growth plate (data not shown).

Femoral strength. Cortical femoral strength was assessed by direct measurement of failure load (indicating the strength of the whole bone) with subsequent calculation of UTS (an index of the strength of the calcified tissue per se) and the second moment of area, I (an index of the geometric contribution to strength). The linear increase in failure load with age in WT rats ($P < 0.001$; Fig. 3A) was also seen in Tgr and $dw/dw$ rats until 6 wk of age. By 9 wk, femoral strength was compromised in both models of GH deficiency, failure load being 32 and 26% lower in Tgr ($P < 0.001$) and $dw/dw$ ($P < 0.001$) rats, respectively. Regression analysis demonstrated that there was no relationship between cortical strength and aBMD (WT: $r^2 = 0.249$, Tgr: $r^2 = 0.003$, $dw/dw$: $r^2 = 0.094$; Fig. 3B), and calculation of UTS revealed that, although the strength of the calcified tissue increased with age, there were no significant differences between the three strains (Fig. 3C). In contrast, given the positive correlation between failure load and AP diameter in all three strains (Fig. 3D), our determination of the second moment of area revealed a significant reduction in the geometric contribution to strength in both models of GH deficiency from 6 wk of age [Tgr: 42% lower ($P < 0.001$), $dw/dw$: 37% lower ($P < 0.001$) at 9 wk; Fig. 3E].

Regression analysis also revealed that failure load and second moment of area were positively correlated with body weight in all three strains (Fig. 3, F and H). Correction of failure load for the influence of body weight revealed that at 3 wk of age Tgr males had significantly stronger bones on a per gram body weight basis than either WT or $dw/dw$ rats ($P < 0.01$; Fig. 3G). However, by 9 wk of age, weight-corrected femoral strength was higher in $dw/dw$ males than in Tgr rats ($P < 0.05$). Correction of second moment of area for the influence of body weight revealed that the geometric contribution to strength on a per gram body weight basis was not significantly different between the strains (Fig. 3I).

Study 2: Femoral Strength in Murine Models of GH Deficiency

To establish whether these changes in femoral strength were determined by the degree of GH deficiency, we quantified similar femoral parameters in murine models with profound and moderately reduced GH signaling, GHR/BP$^{-/-}$ and GHA mice.

Femoral morphometry. In these models of GH-deficient dwarfism, cortical diameter was only significantly reduced in the complete absence of GH signaling [GHR/BP$^{-/-}$] AP diameter $17\%$ lower ($P < 0.05$), ML diameter $29\%$ lower ($P < 0.001$); Table 1]. In contrast, none of the measures of cortical wall thickness were significantly reduced (Supplemental Table S1).

Femoral mineralization. We have previously shown that in 3-mo-old male GHR/BP$^{-/-}$ mice aBMD was reduced by $32\%$ (35). Antagonism of GH in GHA-transgenic mice resulted in a $45\%$ reduction in total femoral BMC (WT: $17.0 \pm 1.2$ mg vs. GHA: $9.5 \pm 1.0$ mg, $P < 0.001$), which, when combined with a $30\%$ reduction in femoral area ($P < 0.01$), resulted in a $15\%$ reduction in aBMD (WT: $48.6 \pm 1.6$ mg/cm$^2$ vs. GHA: $40.6 \pm 1.3$ mg/cm$^2$, $P < 0.01$).

Femoral strength. The compromised femoral strength (ultimate moment is a more representative measurement of femoral strength than failure load in smaller bones) in these two models of reduced GH signaling was broadly similar, ultimate moment being halved [GHR/BP$^{-/-}$ $54\%$ lower ($P < 0.001$; Fig. 4A); GHA $42\%$ lower ($P < 0.001$; Fig. 4D)] without a significant reduction in UTS (Fig. 4, B and E). However, a reduction in the geometric contribution to strength, second moment of area, was observable only in the complete absence of GH signaling [GHR/BP$^{-/-}$ $57\%$ lower ($P < 0.01$; Fig. 4C)]. Neither ultimate moment nor second moment of area was significantly lower when corrected for body weight (Fig. 4, D and E). Thus, in contrast to the Tgr and $dw/dw$ rat models, the impairment of femoral strength in the murine models appears to be related to the degree of GH deficiency.

Study 3: The Effect of Neonatal MSG Treatment on Bone Strength and Adiposity Profiles in $dw/dw$ Rats

To determine whether the development of impaired bone strength in the $dw/dw$ rat could be exacerbated by obesity and hyperleptinemia, we investigated the effects of neonatal MSG treatment on femoral strength in this model.

Femoral morphometry. Neonatal MSG treatment, which doubled abdominal and marrow adiposity and elicited a 10-fold elevation in circulating leptin (11), reduced femoral length in...
Fig. 3. The development of femoral cortical strength in 3-, 6-, and 9-wk-old male W-T ( ), Tgr (half-filled squares), and dw/dw (■) rats. Regression analysis of failure load (A) against femoral aBMD (B), cortical AP diameter (APØ; D), and body weight (F) are presented, with ultimate tensile stress (C), 2nd moment of area (E), and failure load corrected for body weight (G) shown. In addition, regression analysis of 2nd moment of area against body weight (H) and 2nd moment of area corrected for body weight (I) are also presented. Values shown are means ± SE [n = 3 (3-wk Tgr), 4 (6- and 9-wk AS), 5 (3-wk AS), and 6 (6- and 9-wk Tgr; 3-, 6-, and 9-wk dw/dw)]; statistical comparisons were performed by 1-way ANOVA and Bonferroni selected pairs post hoc test, with significant differences described in the text.
impaired femoral strength in both male and female due to the reduction in the geometric contribution, second impaired bone strength in MSG-treated in vehicle-treated dwarves (Fig. 5B). 

Values shown are means ± SE and in mm [statistical comparisons made between the genetically modified and their respective wild-type (WT) controls using Student’s t-test, *P < 0.05, **P < 0.001 vs. respective WT controls]. GHR/BP−/−, growth hormone receptor/binding protein-null; GHA, GHR antagonist G13/KbGH-transgenic; AP, anterior-posterior diameter; ML, mediolateral diameter.

dw/dw rats by 7–8% (P < 0.01; Table 2) commensurate with a reduction in residual GH secretion (11). In contrast, MSG treatment had little effect on cortical diameter (Table 2) or wall thickness (Supplemental Table S2), with only AP diameter being significantly reduced in MSG-treated dw/dw females (7% lower, P < 0.05).

Femoral mineralization. MSG treatment reduced total femoral BMC in dw/dw rats by 21–22% (P < 0.01; Table 2), but this only equated to a significant reduction in aBMD in females (11% lower, P < 0.01; P = 0.066 in males).

Femoral strength. Neonatal MSG treatment significantly impaired femoral strength in both male and female dw/dw rats, ultimate moment being reduced by 20% (P < 0.01; Fig. 5A). UTS was not significantly reduced by MSG treatment but was 109 (males, t = 1.194) and 114% (females, t = 1.862) of that in vehicle-treated dwarves (Fig. 5B). The exacerbation of impaired bone strength in MSG-treated dw/dw rats was entirely due to the reduction in the geometric contribution, second moment of area being reduced by 33 and 35% in males and females, respectively (P < 0.01; Fig. 5C). These effects of MSG treatment were largely unaffected by correction for body weight (Fig. 5, D and E) or femoral length (data not shown) but were abolished by correcting for both body weight and femoral length (data not shown).

Study 4: The Effect of a High-Fat Diet on Bone Strength in dw/dw Rats

To determine the potential contribution of elevated adiposity on impaired bone strength in GH deficiency without the confounding influence of reduced residual GH secretion, femoral strength was measured in dw/dw rats after the induction of elevated fat deposition by pubertal exposure to elevated dietary fat.

Femoral morphometry. Consumption of elevated dietary fat had no effect on the parameters of adiposity in WT rats but doubled abdominal adiposity and circulating leptin in dw/dw rats (11). Although elevated dietary fat reduced tibial growth in WT rats (11), this diet had no effect on femoral length in either WT or dw/dw rats (Table 3). Similarly, consumption of elevated dietary fat did not influence any of the other parameters of femoral morphometry in either WT or dw/dw rats, the differences between the two strains being largely maintained (Table 3 and Supplemental Table S3).

Femoral mineralization. As shown above in male rats (Fig. 3), female dw/dw rats showed a significant impairment in both total BMC and aBMD compared with their WT counterparts (P < 0.01; Table 3). Neither measurement of femoral mineralization was affected by maintenance on the high-fat diet (Table 3).

### Table 1. Femoral morphometry in 10-wk-old male GHR/BP−/− and GHA mice

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 6)</th>
<th>GHR/BP−/− (n = 5)</th>
<th>WT (n = 6)</th>
<th>GHA (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Cortical AP</td>
<td>1.23 ± 0.06</td>
<td>1.02 ± 0.06*</td>
<td>1.22 ± 0.08</td>
<td>1.15 ± 0.11</td>
</tr>
<tr>
<td>Cortical ML</td>
<td>1.74 ± 0.04</td>
<td>1.24 ± 0.05**</td>
<td>2.01 ± 0.10</td>
<td>1.72 ± 0.12</td>
</tr>
</tbody>
</table>

Values shown are means ± SE and in mm [statistical comparisons made between the genetically modified and their respective wild-type (WT) controls using Student’s t-test, *P < 0.05, **P < 0.001 vs. respective WT controls]. GHR/BP−/−, growth hormone receptor/binding protein-null; GHA, GHR antagonist G13/KbGH-transgenic; AP, anterior-posterior diameter; ML, mediolateral diameter.

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Femoral strength. Despite doubling abdominal adiposity and circulating leptin (11), consumption of elevated dietary fat did not affect femoral strength in dw/dw rats, ultimate moment remaining significantly lower than in similarly fed WT rats (P<0.01; Fig. 6A). As with MSG treatment, there was no significant increase in the strength of the calcified tissue in rats maintained on elevated dietary fat [mean UTS in fat-fed dw/dw rats was 113% of that in dw/dw rats on standard chow, t = 1.168 (dw/dw rats); Fig. 6B]. Second moment of area was not significantly affected by consumption of the high-fat diet but remained lower than that in similarly fed WT rats (P<0.05; Fig. 6C). Neither ultimate moment nor second moment of area were significantly different after correction for body weight (Fig. 6, D and E).

DISCUSSION

GH deficiency is usually associated with an increased fracture risk and the accumulation of increased adipose tissue reserves. Since the adipokine leptin has been reported to increase osteoblast activity (36) and enhance the formation and mineralization of cortical bone (10, 20, 31), we have investigated whether the elevated adiposity that usually accompanies GH deficiency ameliorates the impaired biomechanical properties of cortical bone.

We have shown previously that the adult Tgr rat model of moderate GH deficiency [30% of normal circulating GH (17, 40)] displays an impairment of femoral geometry, resulting in compromised cortical strength (15). Our present study reveals that this impairment of strength becomes apparent between 6 and 9 wk of age, during the GH-dependent rapid growth phase. The reduction in second moment of area, but not ultimate tensile stress, confirmed that, although areal BMD is reduced, this impairment of bone strength is due primarily to a reduction in geometric determinants.

However, we were surprised to find that femoral strength was similarly impaired in the profoundly GH-deficient dw/dw [5% of normal circulating GH (7, 8, 28)] rat. As in the Tgr model, the impairment of femoral strength in the dw/dw rat was correlated with cortical diameter and appeared to be due to the alteration in geometric variables, as indicated by the second

Table 2. Femoral morphometry and mineralization in 8-wk-old MSG-treated dw/dw rats

<table>
<thead>
<tr>
<th></th>
<th>Vehicle/Male (n = 5)</th>
<th>MSG/Male (n = 6)</th>
<th>Vehicle/Female (n = 5)</th>
<th>MSG/Female (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral length, mm</td>
<td>24.72±0.22</td>
<td>22.88±0.47aa</td>
<td>23.84±0.15</td>
<td>21.95±0.35aa</td>
</tr>
<tr>
<td>Cortical AP, mm</td>
<td>2.45±0.04</td>
<td>2.31±0.06</td>
<td>2.33±0.04</td>
<td>2.16±0.06*</td>
</tr>
<tr>
<td>Cortical ML, mm</td>
<td>2.84±0.06</td>
<td>2.57±0.16</td>
<td>2.78±0.10</td>
<td>2.40±0.02</td>
</tr>
<tr>
<td>Total femoral BMC</td>
<td>102.4±3.2</td>
<td>81.0±5.5aa</td>
<td>97.8±2.8</td>
<td>76.2±4.0aa</td>
</tr>
<tr>
<td>Total femoral aBMD, mg/cm²</td>
<td>91.1±2.5</td>
<td>84.5±2.0</td>
<td>93.1±0.8</td>
<td>83.3±1.8aa</td>
</tr>
</tbody>
</table>

Values shown are means ± SE [statistical comparisons made using 1-way ANOVA and Bonferroni selected pairs post hoc test. *P < 0.05, **P < 0.01 vs. vehicle treated (same sex)]. MSG, monosodium glutamate; BMC, bone mineral content; aBMD, areal bone mineral density.

Fig. 5. Cortical strength of femori from 8-wk-old dw/dw rats treated neonatally with either vehicle (open bars) or MSG (black bars). Parameters presented are ultimate moment (A), ultimate tensile stress (B), and 2nd moment of area (C), with ultimate moment (D) and 2nd moment of area (E) corrected for body weight also shown. Values shown are means ± SE [n = 5–6; statistical comparisons made using 1-way ANOVA and Bonferroni selected pairs post hoc test; *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle-treated (same sex)].

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moment of area. In contrast, UTS, a measurement of tissue strength that results from the combination of mineralization and collagen fibril structure, was unaffected. These results differ from a previous report indicating that the halving of ultimate load in tibiae of 12-wk-old male \(dw/dw\) rats was accompanied by a 25% reduction in UTS and a disruption in the structure and organization of the collagen microfibrils (27). Interestingly, when we corrected failure load for the potential influence of body weight, femoral strength was higher in Tgr males at the youngest age studied [when plasma leptin is high (11)] and in \(dw/dw\) males at the oldest age in the current study. Although correction for body weight may not represent the most robust method for adjusting strength measurements for body mass (26), these data suggest that the impairment of strength is not determined solely by the reduction in the mechanical load.

In the context of the considerable difference in GH deficiency between these two models [manifested in a reduction in GH pulse amplitude (40, 28)], our current data suggested that the relationship between the degree of GH deficiency and the impairment of femoral strength may be nonlinear. To test this hypothesis, we measured femoral strength in two murine models with varying degrees of impaired GH receptor signaling, the GHA-transgenic mouse with partially reduced signaling and the GHR/BP-null mouse with complete absence of signaling. Our data confirmed a previous report (39) that compromised cortical strength in GHR/BP\(^{-/-}\) mice is also a geometric phenomenon resulting from a reduction in cortical diameter. Although ultimate moment was also reduced in GHA mice, this impairment was not as marked and did not equate with a reduction in second moment of area. Thus, in the murine models the impairment of femoral strength appears to be proportional to the degree of GH deficiency. Therefore, it is possible that another factor may contribute to the regulation of the bone phenotype in the two rat models used in the current study, either partially alleviating the effects of profound GH deficiency in the \(dw/dw\) rat or exacerbating the impairment in Tgr rats.

In the majority of GH-deficient states, including the Tgr rat (11, 15) and both murine models of reduced GH signaling (2, 12), femoral morphometry and mineralization following high-fat feeding in WT and \(dw/dw\) rats

<table>
<thead>
<tr>
<th></th>
<th>Standard/WT ((n = 6))</th>
<th>High Fat/WT ((n = 6))</th>
<th>Standard/(dw/dw) ((n = 6))</th>
<th>High Fat/(dw/dw) ((n = 6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral length, mm</td>
<td>27.86 ± 0.31</td>
<td>28.32 ± 0.26</td>
<td>26.66 ± 0.22</td>
<td>27.43 ± 0.45</td>
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<tr>
<td>Cortical AP, mm</td>
<td>2.59 ± 0.04</td>
<td>2.50 ± 0.08</td>
<td>2.47 ± 0.03</td>
<td>2.43 ± 0.05</td>
</tr>
<tr>
<td>Cortical ML, mm</td>
<td>3.71 ± 0.05</td>
<td>3.65 ± 0.11</td>
<td>3.03 ± 0.09(\text{aaa})</td>
<td>2.89 ± 0.07(\text{aaa})</td>
</tr>
<tr>
<td>Total femoral BMC, mg</td>
<td>168.2 ± 4.0</td>
<td>175.2 ± 5.1</td>
<td>138.0 ± 5.0(\text{aa})</td>
<td>138.6 ± 5.7(\text{aaa})</td>
</tr>
<tr>
<td>Total femoral aBMD, mg/cm(^2)</td>
<td>123.4 ± 1.6</td>
<td>126.9 ± 1.4</td>
<td>112.9 ± 3.3(\text{aa})</td>
<td>111.0 ± 2.1(\text{aaa})</td>
</tr>
</tbody>
</table>

Values shown are means ± SE [statistical comparisons made using 1-way ANOVA and Bonferroni selected pairs post hoc test. \(\text{a}P < 0.05, \text{aa}P < 0.01\) vs. wild-type controls (same diet)].

Fig. 6. Cortical strength of femori from 9- to 13-wk-old female W-T and \(dw/dw\) rats maintained on either standard laboratory chow (open and black bars) or a high-fat diet (hatched bars) for 4 wk. Parameters presented are ultimate moment (A), ultimate tensile stress (B), and 2nd moment of area (C), with ultimate moment (D) and 2nd moment of area (E) corrected for body weight also shown. Values shown are means ± SE \(\langle n = 5–6\rangle\) statistical comparisons made using 1-way ANOVA and Bonferroni selected pairs post hoc test; \(\text{a}P < 0.05, \text{aa}P < 0.01\) vs. W-T (same diet)].
mechanisms need to be considered. First, receptors for leptin may significantly alter either ultimate moment or second moment of area. However, a recent regression study has suggested that, at least in humans, body fat mass may not have a protective effect (43). However, we cannot exclude the potentially confounding influence of weight-bearing [abdominal adiposity was elevated by 2- to 3-fold in \( \text{dw/dw} \) females (11)] on femoral strength in fat-fed \( \text{dw/dw} \) rats. Indeed, it has been reported previously that maintenance on a cafeteria-style diet has a small positive effect on the biomechanical properties of the midcortical femur in weight-gaining male rats without influencing ultimate stress (43). However, a recent regression study has suggested that, at least in humans, body fat mass may not have a protective effect on bone mass (36), and our correction for body weight did not significantly alter either ultimate moment or second moment of area.

Since our data indicate that adiposity status and circulating leptin do not appear to exert a significant influence over cortical strength in \( \text{GH} \) deficiency, a number of alternative mechanisms need to be considered. First, receptors for leptin are expressed in bone marrow stromal cells, osteoblasts, and osteoclasts (20, 37), and therefore, alterations in marrow adiposity and marrow leptin production might have a more direct influence over the determinants of strength. However, at the ages studied, there were no significant differences in marrow adiposity between the two rat models of \( \text{GH} \)-deficiency (11). Alternatively, it is possible that another factor, regulated by the production of human \( \text{GH} \) in the arcuate nuclei of the Tgr rat, might exacerbate the bone phenotype in this model. For example, we have recently shown that the Tgr male displays a hypergonadotropic phenotype without any change in circulating testosterone (12). However, this phenomenon is unlikely to exert a significant influence on the bone phenotype in this model because removal of LH signaling is accompanied by bone loss (42). Thus, the underlying mechanism giving rise to the similar bone phenotype in the Tgr and \( \text{dw/dw} \) models despite their disparate GH status remains to be determined.

In summary, the peripubertal development of impaired femoral strength in the moderately \( \text{GH} \)-deficient Tgr rat and the profoundly \( \text{GH} \)-deficient \( \text{dw/dw} \) rat was surprisingly similar. In contrast, the murine models displayed an impairment of femoral strength in proportion to their reduced \( \text{GH} \) signaling. \( \text{MSG} \)-induced obesity and hyperleptinemia in the \( \text{dw/dw} \) rat were associated with further impairment of femoral strength, most likely due to the suppression of residual \( \text{GH} \) secretion. In contrast, elevating adiposity and circulating leptin in \( \text{dw/dw} \) rats by maintenance on a high-fat diet had no effect on femoral strength. Thus, our data do not support the hypothesis that elevated circulating leptin exerts a significant influence on cortical bone in conditions of \( \text{GH} \) deficiency. However, given the site-specific nature of the influence of leptin, this does not exclude a potential role for this adipokine in the regulation of trabecular bone.

**REFERENCES**


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