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

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1School of Biosciences and 2Department of Child Health, School of Medicine, Cardiff University, Cardiff; 3Division of Molecular Neuroendocrinology, National Institute for Medical Research, London; 4Department of Anatomy, Bristol University Vet School, Bristol, United Kingdom; 5Department of Biomedical Sciences, College of Osteopathic Medicine, and 6Edison Biotechnology Institute, Ohio University, Athens, Ohio; and 7School of Engineering, Cardiff University, Cardiff, United Kingdom

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Stevenson AE, Evans BA, Gevers EF, Elford C, McLeod RW, Perry MJ, El-Kasti MM, Coschigano KT, Kopchick JJ, Evans SL, Wells T. Does adiposity status influence femoral cortical strength in rodent models of growth hormone deficiency? Am J Physiol Endocrinol Metab 296: E147–E156, 2009. First published November 11, 2008; doi:10.1152/ajpendo.90689.2008.—Growth hormone (GH)-deficiency is usually associated with elevated adiposity, hyperleptinemia, and increased fracture risk. Since leptin is thought to enhance cortical bone formation, we have investigated the contribution of elevated adiposity and hyperleptinemia on femoral strength in rodent models of GH deficiency. Quantification of the transpubertal development of femoral strength in the moderately GH-deficient/ hyperleptinemic Tgr rat and the profoundly GH-deficient/hyperleptinemic dw/dw rat revealed that the mechanical properties of cortical bone in these two models were similarly compromised, a 25–30% reduction in failure load being entirely due to impairment of geometric variables. In contrast, murine models of partial (GH antagonist transgenic) and complete (GH receptor-null) loss of GH signaling and elevated adiposity showed an impairment of femoral cortical strength proportionate to the reduction of GH signaling. To determine whether impaired femoral strength is exacerbated by obesity/hyperleptinemia, femoral strength was assessed in dw/dw rats following two developmental manipulations that elevate abdominal adiposity and circulating leptin, neonatal monosodium glutamate (MSG) treatment, and maintenance on an elevated fat diet. The additional impairment of femoral strength following MSG treatment is likely to have resulted from a reduction in residual activity of the hypothalamo-pituitary-GH-IGF-I axis, but consumption of elevated dietary fat, which did not reduce circulating IGF-I, failed to exacerbate the compromised femoral strength in dw/dw rats. Taken together, our data indicate that the obesity and hyperleptinemia usually associated with GH deficiency do not exert a significant influence over the strength of cortical bone.

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 (18) 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, GH receptor (GHR) antagonist G119KbGH-transgenic (GHA) mice (9, 25) and GHR/binding protein-null (GHR/BP−/−) (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. Tgr rats, wild-type (WT; AS) littersmates, and homozygous dw/dw rats used in studies 1 and 4 were derived from the original colonies at NIMR and were bred in the Transgenic Unit (School of Biosciences, Cardiff University) under conditions of 14:10-h light-dark (lights on at 0500), with food and water available ad libitum. Tgr rats were identified by PCR analysis of a tail biopsy. Standard chow diet (Cardiff) consisted of 4.0% fat, 14.2% protein, 4.5% fiber, 63.9% carbohydrate, and 4.7% ash (metabolizable energy: 12.99 MJ/kg, Rodent Maintenance Diet 2014; Harlan Teklad, Harlan, UK). Standard chow diet (NIMR) consisted of 3.4% fat, 18.8% protein, 3.7% fiber, 60.3% carbohydrate, and 3.8% ash (metabolizable energy: 15.6 MJ/kg). The high-fat diet used in study 3 was made by mixing normal rat chow (NIMR) with 60% fat containing chow (Special Diet Services, Witham, UK) to give a diet consisting of 41.1% fat, 19.6% protein, 29% carbohydrates, and 2.3% ash (metabolizable energy: 24.0 MJ/kg).

The GHR/BP+/− and GHR/BP−/− mice [together with their respective Ola/BalbC and C57BL/6J (WT) controls] were housed in a temperature-controlled room at 22°C with a light-dark cycle of 14:10-h light-dark in the mouse facility of Edison Biotechnology Institute at Ohio University (Athens, OH). Mice were weaned at 28 days of age onto a standard rodent chow diet (14% of calories from fat, 16% from protein, and 60% from carbohydrates; Prolab RMH 3000; PMI Nutrition International, Brentwood, NJ), with food and water supplied ad libitum. Mice were genotyped by PCR analysis of a tail biopsy.

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 mice (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 femurs 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 femurs 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

\[
\sigma = \frac{My}{I},
\]

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

\[
I = \frac{\pi}{64}(b_o d_o^4 - b_i d_i^4),
\]

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) (Supplemental Material for this article is available at the AJP-Endocrinology and Metabolism web site) middiaphyseal 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 femori 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 morphology.** In these models of GH-deficient dwarfish, 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: $17.0 \pm 1.2 \text{ mg/cm}^2$ vs. GHA: $9.5 \pm 1.0 \text{ mg/cm}^2$, $P < 0.001$), which, when combined with a 30% reduction in femoral area ($P < 0.01$), resulted in a 15% reduction in aBMD (WT: $48.6 \pm 1.6 \text{ mg/cm}^2$ vs. GHA: $40.6 \pm 1.3 \text{ mg/cm}^2$, $P < 0.01$).

**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 morphology.** 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)

UTS was not significantly reduced by MSG treatment but was

ultimate moment being reduced by 20% (\(0.11\) lower,

this only equated to a significant reduction in aBMD in females

7% lower, \(P < 0.05\).

Femoral mineralization. MSG treatment reduced total femoral BMC in \(d w / d w\) 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 \(d w / d w\) 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 \(d w / d w\) 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 \(d w / d w\) 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 \(d w / d w\) 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 \(d w / d w\) 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 \(d w / d w\) rats (Table 3). Similarly, consumption of elevated dietary fat did not influence any of the other parameters of femoral morphometry in either WT or \(d w / d w\) 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 \(d w / d w\) 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>
</thead>
<tbody>
<tr>
<td>Cortical AP</td>
<td>1.23 (\pm) 0.06</td>
<td>1.02 (\pm) 0.06*</td>
<td>1.22 (\pm) 0.08</td>
<td>1.15 (\pm) 0.11</td>
</tr>
<tr>
<td>Cortical ML</td>
<td>1.74 (\pm) 0.04</td>
<td>1.24 (\pm) 0.05**</td>
<td>2.01 (\pm) 0.10</td>
<td>1.72 (\pm) 0.12</td>
</tr>
</tbody>
</table>

Values shown are means \(\pm\) SE 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 GilKIH-GH-transgenic; AP, anterior-posterior diameter; ML, mediodiagonal diameter.

### Fig. 4. Cortical strength of femori from 10-wk-old male GHR/BP \(-/-\) and GHA mice and their respective W-T controls. 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 \(\pm\) SE \((n = 5–6)\); statistical comparisons made between the genetically modified and their respective W-T controls using Student’s \(t\)-test; *=\(P < 0.01\), **=\(P < 0.001\).
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±0.47</td>
<td>23.84±0.15</td>
<td>21.95±0.35</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, mg</td>
<td>102.4±3.2</td>
<td>81.0±5.5</td>
<td>97.8±2.8</td>
<td>76.2±4.0</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±1.8</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)].**
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, 53), the extent of the impairment in bone strength may be influenced by the degree of GH deficiency. This relationship may be nonlinear, as suggested by our current data in the murine models.
The removal of the powerful lipolytic influence of \( \text{GH} \) results in the accumulation of adipose tissue reserves and a consequent elevation in circulating leptin. However, the \( \text{dw/dw} \) rat is an exception, being unusually lean and remarkably hypoleptinemic (11). Although leptin has been shown to have differential effects on trabecular and cortical bone, we investigated whether elevating adiposity and circulating leptin may exacerbate the impairment of femoral strength in the \( \text{dw/dw} \) rat. This was achieved using two developmental manipulations known to elevate truncal adiposity and circulating leptin [neonatal MSG treatment (6) and maintenance on a high-fat diet (11)].

Neonatal MSG treatment, which elicited a profound increase in adiposity in \( \text{dw/dw} \) rats [circulating leptin increased 10-fold (11)], significantly exacerbated the compromised femoral strength in this model of \( \text{GH} \) deficiency. Although the geometry of the cortical bone was only minimally affected, this was clearly sufficient to account for the effect of MSG treatment since second moment of area was reduced without affecting UTc. Although these data appear to lend support to the hypothesis that the lean/hypoleptinemic phenotype of the \( \text{dw/dw} \) rat may partially ameliorate the impairment in bone strength in this model, we cannot exclude the possibility that the effects of neonatal MSG treatment are due to the suppression of residual \( \text{GH} \) secretion. We have shown previously that, although this manipulation does not alter the population of somatotrophs in the \( \text{dw/dw} \) pituitary (21), the activity of the \( \text{GH} \) axis, as indicated by the halving of circulating IGF-I and the reductions in femoral length, tibial length, and epiphyseal plate width (11), is clearly suppressed. Thus, since the further impairment of ultimate moment and second moment of area is abolished by correction for body weight and femoral length, the effect of MSG treatment may be due to the combined influence of increased adiposity and suppressed residual \( \text{GH} \) secretion.

To circumvent this problem, we investigated the effect of increasing adiposity in the \( \text{dw/dw} \) rat without reducing \( \text{GH} \) secretion. Maintenance on elevated dietary fat doubled truncal adiposity and circulating leptin, but circulating IGF-I, tibial epiphyseal plate width (11), and femoral length were unaffected. In this context, our observation that neither ultimate moment nor second moment of area was reduced by this diet is significant, implying that circulating leptin does not contribute to the impairment of bone strength in \( \text{GH} \) deficiency, at least over the 4-wk time course. Given the established relationship between increased load and increased bone formation (5, 29), we cannot exclude the potentially confounding influence of increased 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 \( \text{LH} \) 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 \( \text{GH} \) 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. 

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.

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