The Muscle-Bone Relationship in X-Linked Hypophosphatemic Rickets

Louis-Nicolas Veilleux, Moira S. Cheung, Francis H. Glorieux, and Frank Rauch
Shriners Hospital for Children and Department of Pediatrics, McGill University, Montréal, Québec, Canada

Context: We recently found that patients with X-linked hypophosphatemic rickets (XLH) have a muscle function deficit in the lower extremities. As muscle force and bone mass are usually closely related, we hypothesized that patients with XLH could also have a bone mass deficit in the lower extremities.

Objective: The study objective was to assess the muscle-bone relationship in the lower extremities of patients with XLH.

Setting: The study was carried out in the outpatients department of a pediatric orthopedic hospital.

Patients and Other Participants: Thirty individuals with XLH (6 to 60 y; 9 male patients) and 30 age- and gender-matched controls participated.

Main Outcome Measures: Calf muscle size and density as well as tibia bone mass and geometry were assessed by peripheral quantitative computed tomography. Muscle function was evaluated as peak force in the multiple 2-legged hopping test.

Results: Muscle force was significantly lower in XLH patients than in controls but muscle cross-sectional area did not differ (after adjustment for tibia length). External bone size, expressed as total bone cross-sectional area, was higher in the XLH group than in controls. The XLH cohort also had statistically significantly higher bone mineral content.

Conclusions: Patients with XLH have increased bone mass and size at the distal tibia despite muscle function deficits. (J Clin Endocrinol Metab 98: 0000–0000, 2013)

Hereditary hypophosphatemic rickets is characterized by hypophosphatemia due to renal phosphate wasting, resulting in rickets, deformities of the lower extremities, and short stature (1, 2). The condition is most commonly caused by mutations in the phosphate-regulating endopeptidase gene (PHEX), which leads to X-linked hypophosphatemic rickets (XLH; OMIM 307800) (1, 2). Autosomal-dominant (OMIM 193100) and autosomal-recessive (OMIM 241520) forms of the disease have also been reported and are caused by mutations in fibroblast growth factor 23 and dentin matrix protein 1, respectively (1, 2). However, these latter forms are much rarer than XLH (3).

Current standard therapy of XLH consists of oral phosphate supplementation and calcitriol; the latter aims at preventing secondary hyperparathyroidism that otherwise would develop with high-dose phosphate supplementation (2). This regimen corrects the mineralization defect at the level of the growth plates and thus heals the rickets, but some degree of mineralization defect persists in the bone tissue despite treatment (4).

Muscle force is strongly correlated with measures of bone strength in healthy subjects (5). For example, it has been shown that the maximal ground reaction force during hopping on the forefoot predicts as much as 84% of bone mineral content (BMC) of the tibia in healthy chil-
dren, adolescents, and adults (5). We recently found that XLH patients have muscle function deficits as compared to age- and gender-matched controls (6). If the muscle-bone relationship follows the same pattern in patients as it does in healthy subjects, one would expect that the muscle function deficit in XLH will lead to weaker bones in patients.

To test this hypothesis, we used a recently established approach to assess specifically muscle-bone interaction in the lower leg (5). Muscle size, bone mass, and bone geometry at the lower leg are assessed by peripheral quantitative computed tomography (pQCT), and muscle function is evaluated by jumping mechanography.

Subjects and Methods

Study population

The patient population comprised 30 individuals with a diagnosis of XLH who were at least 6 years of age, the lower age for reliably performing pQCT and jumping mechanography assessments. The XLH population consisted of the following 2 subgroups: patients under 21 years of age who were actively followed at the Shriners Hospital for Children in Montreal, and participants aged 21 years or older, who were former patients invited to the clinic for the purpose of the present study. Exclusion criteria were fractures of the lower limbs in the past 6 months or lower limb surgery in the past 12 months. Thirty patients with XLH (age 6 to 60 y; 9 male patients) agreed to participate in this study.

The diagnosis was based on the presence of low serum phosphorus and normal results for serum calcium and parathyroid hormone levels, plus radiological evidence of rickets or a family history of XLH. In 25 of the 30 study patients, PHEX mutation analysis had been performed and revealed disease-causing mutations in 19 patients. Regarding treatment status at the time of this study, 14 patients were actively being treated with calcitriol and phosphate supplementation and had been receiving this treatment for 1.1 to 15.9 years (mean ± SD: 8.5 ± 4.0 y). Ten patients had received the same therapy in the past and had discontinued treatment when they had reached final height (ie, 2.9 to 18.6 y) prior to the present testing (mean ± SD: 8.9 ± 5.7 y). Six patients had never received phosphate supplementation. Results in this population were compared with those of 30 healthy age- and gender-matched control subjects (age range: 6 to 55 y) who were recruited among hospital staff and their children as well as among healthy siblings of patients. The study was approved by the Institutional Review Board of McGill University. Informed consent was provided by participants or, for minors, their parents. Assent was provided by participants aged 7 to 17 years.

The patient and control populations included in the current study were drawn from the same cohort as that of our previous study (6), with the exception of 4 patients and their respective matched controls who were excluded from the present study either because they could not perform the multiple 2-legged hopping (n = 2) or because they did not have valid pQCT measurements due to movements artifacts (n = 2).

pQCT

pQCT was performed on the left tibia using the Stratec XCT2000 (Stratec Inc, Pforzheim, Germany). Tibia length was measured using a ruler as the distance between the medial border of the tibial plateau and the medial malleolus. The angle between the foot and lower leg was set at 120°. The scanner was positioned on the distal lower leg and a scout view was carried out to determine the position of the “reference line.” In patients with an open growth plate, the reference line was drawn through the most distal portion of the growth plate. When the growth plate was no longer visible, the reference line was drawn through the middle of the medial border of the articular cartilage. The lower leg was scanned at 4, 14, and 66% of tibia length, measured as the distance from the reference line. At each of the 3 measurement sites, a tomographic slice of 2.0 mm thickness was obtained at a voxel size of 0.4 mm × 0.4 mm × 2 mm. The speed of the translational scan movement was set at 20 mm/s.

Image acquisition, processing, and the calculation of numerical values were performed using the manufacturer’s software package (version XCT 6.00B). The tibia was analyzed at the 4% site (metaphysis, trabecular bone) and 14% site (metaphyseal-diaphyseal transition site, cortical bone), whereas muscle size was determined at the 66% site. The 14% site was selected because this is the site where previous results in healthy subjects had shown the strongest correlation between BMC and maximal muscle force during multiple 1-legged hopping (5). This is also the location where BMC of the tibial cross-section is at its minimum (7). The 66% site was selected to obtain calf muscle physiological parameters because this is the region of largest outer calf diameter (8).

At the 4% site, the outer bone contour was detected at the default threshold of 280 mg/cm³ and parameters were calculated using the software’s CALCBD routine. The cortical bone at the 14% site was analyzed at a threshold of 710 mg/cm³ using the software’s CORTBD routine. At the 66% site, the combined cross-sectional area (CSA) of muscle and the 2 bones (fibula and tibia) was determined at a threshold of 40 mg/cm³, and the CSA of the 2 bones was determined with the threshold set at 280 mg/cm³. Muscle CSA was calculated by subtracting the bone CSA from the combined muscle and bone CSA. Muscle density was calculated as the mean density of the tissue that was included in the measurement of muscle CSA.

The main parameters of pQCT analysis at the tibia are as follows: BMC, corresponding to the amount of mineral per millimeter of cross-sectional slice thickness (unit: mg/mm; measured at the 4 and 14% sites); total bone CSA, the surface area of the entire bone cross-section, including cortex and marrow space (unit: mm²; 4 and 14% sites); cortical CSA, the surface area of the cortical bone cross-section excluding marrow space (unit: mm²; 14% site); total volumetric bone mineral density (vBMD; bone mineral density averaged across the entire bone cross-section; unit: mg/cm³; 4 and 14% sites); trabecular vBMD, the average mineral density in the trabecular compartment, determined in the central 45% of the tibial cross-section (unit: mg/cm³; 4% site); cortical vBMD, the average mineral density of the cortical compartment (unit: mg/cm³; 4% site); cortical thickness, determined from total and cortical CSA using the ring model (unit: mm; 14% site).
tests (9). A disorder that might interfere with their ability to perform the test was excluded from the hopping test if they reported any symptoms or discomfort.

The main parameter to investigate the muscle-bone interaction is peak force (5). Because the subject’s test result is the best result is defined as the hop with the highest peak force (FM2LH) (9). The main parameter to investigate is peak force (5). Because mechanography assesses maximal muscle performance, participants were excluded from the hopping test if they reported any disorder that might interfere with their ability to perform the test (9).

Results are given as mean (SD). All values are corrected for tibia length.

### Table 2. Bone Characteristics at the Tibia as Measured by pQCT

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 30)</th>
<th>XLH (n = 30)</th>
<th>%Δ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaphysis (“4% site”)a</td>
<td>Total CSA, mm²</td>
<td>790 (240)</td>
<td>1078 (252)</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Total BMC, mg/mm</td>
<td>250 (76)</td>
<td>313 (84)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Total vBMD, mg/cm³</td>
<td>317 (35)</td>
<td>291 (51)</td>
<td>-8</td>
</tr>
<tr>
<td></td>
<td>Trabecular vBMD, mg/cm³</td>
<td>214 (36)</td>
<td>217 (54)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Transition site (“14% site”)</td>
<td>Total CSA, mm²</td>
<td>334 (88)</td>
<td>448 (113)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total BMC, mg/mm</td>
<td>203 (55)</td>
<td>236 (63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortical vBMD, mg/cm³</td>
<td>1049 (66)</td>
<td>1012 (83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortical thickness, mm</td>
<td>2.6 (0.6)</td>
<td>2.4 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortical CSA, mm²</td>
<td>146 (39)</td>
<td>164 (47)</td>
</tr>
</tbody>
</table>

Results are given as mean (SD). All values are corrected for tibia length.

a Data of one participant removed because of movement artifacts.

### Anthropometric measurements

Height was measured using a Harpenden stadiometer (Holtain, Crymych, United Kingdom). Weight was determined using the Leonardo Mechanograph GRFP (Novotec Medical GmbH). Height and weight measurements were converted to age- and gender-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (12). Lower extremities were classified as straight legs, genu varum, or genu valgum. Legs were considered straight if the intercondylar and the intermalleolar distances were less than 4 cm. Genu varum was classified as mild when the intercondylar distance was between 4 and 8 cm and classified as severe when the intercondylar distance was more than 8 cm. Genu valgum was classified as mild when the intermalleolar distance was between 4 and 8 cm and as severe when the intermalleolar distance was more than 8 cm (6).

### Statistical analyses

Descriptive statistics are presented as means and SDs. All tests were 2-tailed and throughout the study P < .05 was considered significant. Paired t tests were used to detect significant differences between XLH patients and controls for anthropometric data. Data on muscle characteristics (force, cross-section, and density) represent a subset of previously reported results (6). To avoid redundancy, muscle characteristics are shown in the Supplemental data section, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org.

To compare bone parameters between the XLH and control groups, we first computed the percentage difference between each XLH patient and the matched control for each bone parameter. This new variable was used for ANOVAs, with treatment status (currently treated, previously treated, never treated) and leg deformities as the between-subjects factors. Additional covariates were age, tibia length, and height z-scores. It was found that tibia length and leg deformities were the only covariates significantly impacting bone parameters. Therefore, simpler univariate analyses of variance were computed for each bone parameter, in which the disease status (XLH vs control group) was set as the between-subjects factor and tibia length and leg deformities were used as covariates (ie, data adjusted for tibia length and presence of deformities).

Independent stepwise regression analyses were used to assess predictors of BMC and total bone CSA. Independent predictors...
were muscle force ($F_{M2LH}$, muscle function model) or its physiological surrogates (muscle CSA and muscle density that make up the muscle anatomy model), disease status (Control = 0; XLH = 1), leg deformity (straight legs = 0; mild deformities = 1; severe deformities = 2), age, and tibia length. These calculations were performed using the PASW Statistics software version 18.0 (SPSS Inc, Chicago, Illinois).

**Results**

Compared with age- and gender-matched controls, patients with XLH were shorter and had shorter tibias but were of similar weight (Table 1). Eight patients had severe leg deformities (genu varum or valgum); 9 had mild leg deformities and 13 had straight legs.

External bone size, expressed as total bone CSA, and BMC were higher in the XLH group than in controls at both sites (Table 2). Trabecular vBMD was similar between groups, but cortical vBMD was lower in XLH patients than in controls. The patient group had higher cortical CSA, whereas cortical thickness was similar between groups.

Stepwise regression analyses were performed to determine predictors of BMC at the 4 and 14% sites (Table 3). Apart from tibia length and disease status, we evaluated parameters of either muscle function ($F_{M2LH}$ Supplemental data) or of muscle anatomy (muscle CSA, muscle density) as predictors. In the muscle function model (Figure 1A), $F_{M2LH}$, leg deformity, and tibia length were significant predictors of BMC at both bone sites, whereas disease status was a significant predictor at the 4% site only. In the muscle anatomy model (Figure 1B), muscle CSA but not muscle density, leg deformities, and tibia length were significant predictors of BMC at the 2 measurement locations (Table 3).

Stepwise regression analyses were also computed to determine predictors of total bone CSA (Table 4). Muscle force (Figure 1C), muscle CSA (Figure 1D), and tibia length were predictors of total bone CSA at both sites. Disease status was a significant predictor of total bone CSA at both the 4% and the 14% sites with greater CSA in XLH. Leg deformity was not a significant predictor of total bone CSA.

### Table 3. Predictors of BMC (mg/mm) at the 4 and 14% Tibia Sites

<table>
<thead>
<tr>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle function model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$-18 + 50 \times (F_{M2LH}; \text{kN}) + 50 \times (\text{Leg deformity}; 0, 1, 2)$</td>
<td>0.71</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>$+ 34 \times (\text{Disease status}; 0, 1) + 0.52 \times (\text{Tibia length}; \text{mm})$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14% Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$-21 + 36 \times (F_{M2LH}; \text{kN}) + 34 \times (\text{Leg deformity}; 0, 1, 2)$</td>
<td>0.69</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>$+ 0.41 \times (\text{Tibia length}; \text{mm})$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Muscle anatomy model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$-99 + 0.020 \times (\text{Muscle CSA; mm}^2) + 0.70 \times (\text{Tibia length}; \text{mm}) + 53 \times (\text{Leg deformity}; 0, 1, 2)$</td>
<td>0.70</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>14% Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$-58 + 0.022 \times (\text{Muscle CSA; mm}^2) + 0.41 \times (\text{Tibia length}; \text{mm}) + 25 \times (\text{Leg deformity}; 0, 1, 2)$</td>
<td>0.78</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

The muscle function model tested the following predictors: muscle force, disease status, leg deformity, tibia length, and age. The muscle anatomy model tested the following predictors: muscle CSA, muscle density, disease status, leg deformity, tibia length, and age.
The results also showed that disease status (rather than leg deformation) had a significant independent influence on the relationship between muscle size or force and bone size. As the age range of the study participants ranged from preteens to adulthood, there was no indication that the study results depend on developmental stages. In contrast, leg deformation had a significant independent influence on the relationship between muscle size or force on the one hand and BMC on the other hand. The results also showed that disease status (rather than leg deformation) had a significant independent influence on the relationship between muscle size or force and bone size. The “muscle anatomy model” indicated a leg deformation effect of 2.5 mg/mm for BMC (Table 3) and a disease status effect of 107 mm² for total bone CSA (Table 4). Assuming that patients and controls had the same tibia length and muscle CSA, this means that relative to the average control, patients and controls had the same tibia length and muscle CSA.

This was a clinical observational study that was not designed to provide mechanistic data. Nevertheless, it is possible to interpret these results in the framework of the mechanostat model. According to this model, bone adapts to muscle forces in a manner that maintains bone tissue deformation caused by mechanical muscle loads within safe limits (13). In XLH, the bone matrix is presumably softer than normal, due to the mineralization defect that may persist despite current standard therapy with phosphate supplementation and calcitriol (14). The observation that cortical vBMD was low in XLH patients suggests that a mineralization defect was indeed present in this population. At a given force level, undermineralized bone should deform more than normally mineralized bone, which, according to the mechanostat model, should lead to higher than normal bone mass. If this interpretation is correct, treatment approaches that normalize bone mineralization in XLH during bone development should lead to smaller bones and lower BMC in such patients. This hypothesis can be tested in ongoing treatment trials.

Other explanations for the increased bone mass and size in XLH patients are also possible. For example, PHEx is expressed in osteoblasts and mutations in that gene might therefore directly influence the activity of those cells (15). It has also been reported that mice with PHEX mutations have decreased expression of sclerostin (16), which would lead to increased production of bone tissue. Another possibility is that hypophosphatemia alters the secretion of muscle-derived hormones (myokines) that might have an effect on bone metabolism (17).

In the present study we used both muscle anatomy (muscle size and density) and muscle function (peak force in M2LH) to assess the muscle-bone relationship. Muscle force as measured by mechanography (kN) and muscle cross-sectional area (mm²) are known to be highly correlated (18) ($R^2 = 0.53, P < .001$; $R^2 = 0.75, P < .001$, for the XLH and control participants, respectively). Nevertheless, in healthy subjects tibia BMC is better predicted by muscle force in the M1LH than it is by muscle CSA (5). This means that the influence of muscle on bone strength should be analyzed through measurements of dynamic muscle force rather than through measurements of muscle force surrogates. At present it is unknown if this remains true in metabolic disorders such as XLH. The conclusions
reached with the two approaches were identical and the correlation coefficients between muscle and bone parameters tended to be higher when using muscle CSA than when using $F_{M2LH}$. This indicates that the muscle-bone interaction can simply be assessed through these 2 pQCT measurements (ie, at 14% [cortical bone] and 66% [muscle CSA]). However, the higher correlation coefficient observed in the muscle anatomy model might simply reflect that muscle force ($M2LH$) data vary within a more narrow range than muscle CSA data. Therefore, although functional testing using jumping mechanography does not seem to be essential for such studies, it nevertheless provides unique information with regard to muscle force.

In conclusion, this study found that patients with XLH have increased bone mass and size at the distal tibia even though they have some muscle function deficits. The mechanisms leading to higher bone mass in XLH warrant further investigation.

Acknowledgments

Address all correspondence and requests for reprints to: Louis-Nicolas Veilleux, Shriners Hospital for Children, 1529 Cedar Avenue, Montréal, Québec, Canada H3G 1A6. E-mail: lnveilleux@shriners.mcgill.ca.

This work was supported by the Shriners of North America, the Fonds de la Recherche en Santé du Québec (FRSQ), the MENTOR-Réseau de recherche en santé buccodentaire et osseuse (RSBO) program supported by the Canadian Institute for Health Research (CIHR), and the FRSQ.

Disclosure Summary: The authors have nothing to disclose.

References