Abnormalities in Muscle Density and Muscle Function in Hypophosphatemic Rickets

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Context: Animal studies suggest that hypophosphatemic rickets (HPR) is associated with muscle function deficits, but it is unknown whether humans with HPR have a muscle disorder.

Objective: Our objective was to assess calf muscle size and density (an indicator of muscle quality) and lower extremity muscle function in patients with HPR.

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

Patients and Other Participants: Participants included 34 individuals with HPR (6–60 yr; nine males) and 34 age- and gender-matched controls.

Main Outcome Measures: Calf muscle parameters (muscle cross-sectional area and density) were measured by peripheral quantitative computed tomography. Lower extremity muscle function (peak force per body weight and peak power per body mass) was measured by jumping mechanics through five tests with different levels of difficulty: multiple two-legged hopping, multiple one-legged hopping, single two-legged hopping, chair-rise test, and heel-rise test.

Results: Compared with age- and gender-matched controls, patients with HPR had normal muscle size (P = 0.58) but lower muscle density (P = 0.008) and lower peak muscle force and power (P < 0.001 in each test). Muscle function tests were also lower in the subgroup of patients with straight legs (n = 13) than in controls, even though patients with straight legs had higher muscle function test results than patients with severe leg deformities.

Conclusions: The present study suggests that muscle weakness is a clinical feature of HPR. Lower muscle quality and limb deformities contribute to this functional deficit. (J Clin Endocrinol Metab 97: E1492–E1498, 2012)

Hereditary hypophosphatemic rickets (HPR) is characterized by hypophosphatemia due to renal phosphate wasting, resulting in rickets, deformities of the lower extremities, and short stature (1, 2). HPR is most commonly caused by mutations in the phosphate-regulating endopeptidase gene (PHEX), which lead to X-linked hypophosphatemic rickets (OMIM 307800) (1, 2). Autosomal dominant (OMIM 193100) and autosomal recessive (OMIM 241520) forms of HPR 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 of HPR are much rarer than the X-linked disease (3).

The usual treatment for HPR consists of oral phosphate supplementation and calcitriol (1, 2). Early treatment and close monitoring aim at minimizing the skeletal complications of HPR and to optimize growth. Complications of the treatment can be nephrocalcinosis (calcium deposition in the kidneys) and secondary or tertiary hyperparathyroidism. Because the treatment schedule is quite burdensome and the main benefits are achieved during growth, therapy is often discontinued once final height is achieved.

Abbreviations: CSA, Cross-sectional area; GRFP, ground reaction force plate; HPR, hypophosphatemic rickets; pQCT, peripheral quantitative computed tomography.
It is well known that acute hypophosphatemia can affect muscles and give rise, for example, to rhabdomyolysis (4, 5). Animal experiments have shown that hypophosphatemia alters the transmembrane potential of muscle cells, changes the composition of skeletal muscle, and alters mitochondrial structure (6, 7). However, it is not clear whether HPR is also associated with muscle alterations. Some authors mention muscle weakness as being part of HPR (8), but others do not feel that patients with HPR have impaired muscle function (9, 10). Neither view can claim a strong evidence base, because muscle function in patients with HPR does not seem to have been studied in any detail. Nevertheless, a recent study demonstrated that Hyp mice, a murine homolog of X-linked hypophosphatemic rickets, had clearly decreased muscle function (11).

Muscle composition and muscle function can be assessed in the clinical setting by using peripheral quantitative computed tomography (pQCT) and jumping mechanography, respectively. The pQCT allows us to determine muscle density, a parameter that is inversely related to fat content and thus is regarded as a marker of muscle quality (12–14). Jumping mechanography is a method to dynamically assess muscle function through ground reaction force measurements (15–17).

In the present study, we aimed at assessing muscle composition and function in HPR. We examined 34 patients using pQCT and jumping mechanography and compared results to age- and gender-matched controls.

**Subjects and Methods**

**Study population**

The patient population consisted of individuals with a diagnosis of HPR who were at least 6 yr of age. Because pQCT and jumping mechanography assessments require substantial cooperation, children younger than 6 yr can usually not be assessed. The HPR population consisted of two subgroups: Patients under 21 yr of age were actively being followed at the Shriners Hospital for Children in Montreal, whereas participants aged 21 yr or older were former patients of the Shriners Hospital for Children who were 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-four patients with HPR (age 6–60 yr; nine males) agreed to participate in this study.

The diagnosis of HPR was based on the presence of low serum phosphorus and normal results for serum calcium and PTH levels, plus either radiological evidence of rickets or a family history of HPR. In 26 of the 34 study patients, PHEX mutation analysis had been performed and revealed disease-causing mutations in 22 patients. PHEX mutation analyses were not performed for the remaining eight study patients.

Regarding treatment status at the time of this study, 15 patients were actively being treated with calcitriol and phosphorus supplementation and had been receiving this treatment for 1.1–15.9 yr (mean ± SD = 8.5 ± 3.8 yr). Eleven patients had received the same therapy before but had discontinued the treatment 2.9–18.6 yr before the present testing (mean ± SD = 8.8 ± 5.8 yr), when they had reached final height. Eight patients had never received phosphorus supplementation.

Results in the HPR population were compared with those of 34 healthy age- and gender-matched control subjects (age range, 6–55 yr) 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 7–17 yr.

**Anthropometric measurements**

Height was measured using a Harpenden stadiometer (Holtain, Crymych, UK). Weight was determined using the Leonardo Mechanograph ground reaction force plate (GRFP) (Novotec Medical GmbH, Pforzheim, Germany). 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 (18). Lower extremities were classified as straight legs, genu varum, or as 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 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 severe when the intermalleolar distance was more than 8 cm.

**Biochemical analyses**

Serum total calcium and phosphorus were measured using standard methods. Serum levels of intact PTH were analyzed using a chemiluminescent immunoassay (Access Immunoassay Systems; Beckman Coulter Canada Inc., Mississauga, Canada). Reference data for serum calcium and phosphorus (20 yr and younger) were taken from a Canadian pediatric reference data study (19). Reference data for intact PTH (16 yr and younger) were obtained from a study published by Cioffi et al. (20). Reference data for adults were used as established by the reference laboratory.

**Peripheral quantitative computed tomography**

The pQCT was performed at the left lower leg (XCT2000; Stratec Inc., Pforzheim, Germany). The angle between the foot and lower leg was set at 120°. Tibia length was measured using a ruler as the total distance between the medial condyle and the medial malleolus of the tibia. A pQCT scan was performed at the site where the distance to the distal tibial articular surface corresponded to 66% of tibia length. This site was selected because this is the region of the largest outer calf diameter (21). From this scan image, the combined cross-sectional area (CSA) of muscle and bone (fibula and tibia) was determined at a threshold of 40 mg/cm², and the bone CSA was determined with the threshold set at 280 mg/cm². Muscle CSA (square centimeters) 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 pQCT scan could not be performed in two patients because they had intramedullary rods in their tibias, which makes pQCT analysis impossible. In one other patient, the pQCT scan had to be excluded from analysis because of significant movement artifacts. Thus, pQCT analyses were performed on 31 HPR patients.

**Jumping mechanography**

A force plate (Leonardo Mechanograph GRFP; Novotec Medical) was used to measure vertical ground reaction forces. The force plate was connected to a laptop computer, and force measurements were sampled at a frequency of 800 Hz. As described in detail elsewhere, all muscle function parameters reported here were derived from these force-time data using proprietary software (Leonardo Mechanography GRFP Research Edition software, version 4.2-b05.53-RES; Novotec Medical) (17).

We used the same procedures as described in detail elsewhere (17). Five different tests were performed as follows: 1) multiple two-legged hopping and 2) multiple one-legged hopping representing vertical hopping on one or on both feet (similar to rope-skipping), respectively, the aim being to achieve maximal vertical ground reaction forces during an eccentric contraction; 3) single two-legged jump, a vertical countermovement jump to achieve maximum jump height during a stretch-shortening cycle movement; 4) heel-rise test, consisting of five bilateral heel rises with the aim to achieve maximal speed during the upward movement; and 5) chair-rise test, a sit-to-stand test with five repetitions, with the aim to achieve maximal speed during the upward movement. The chair-rise test made use of a bench that was anchored to the force plate.

Each test was repeated three times, and the best result was recorded as the subject’s test result. The definition of best result was highest peak force for a given hop in the multiple one- and two-legged hopping (the force tests); highest peak power during the take-off phase during a single two-legged jump, during the first rise of the heel-rise test and for the second rise of the chair-rise test (the power tests) (17). For the multiple one- and two-legged hopping, the main outcome parameter was peak force relative to body weight, whereas for the single two-legged jump, the heel-rise test and the chair-rise test, the main outcome parameter was peak power relative to body mass.

**Statistical analyses**

Descriptive statistics are presented as means and sds. All tests were two-tailed, and throughout the study, \( P < 0.05 \) was considered significant. Univariate analysis was used to detect significant differences between HPR patients and controls for muscle CSA and muscle density, after adjustment for tibial length.

To investigate the effects of age and gender on mechanographic outcome parameters, we computed the difference (in percent) between each patient and the matched control for each of the five outcome parameters. We then performed a stepwise regression analysis for each of the five tests, in which the percent difference was the dependent variable, and gender (male = 0; female = 1) and age were the predictors. Because age and gender were not predictors of muscle function, HPR patients were compared with their matched controls as a single group through independent paired t tests.

To test for the impact of limb deformities on muscle function, we computed random-blocks ANOVA on the main outcome parameter of each test. The block factor was the sample group (HPR vs. control), and the confounding factor was the degree of limb deformity (straight, mild, or severe). To test for the impact of muscle quantity and quality, we performed stepwise regression analyses for each of the five mechanographic tests. Disease status (control = 0; HPR = 1), muscle density, and muscle volume index (muscle CSA multiplied by tibia length) were the predictors.

These calculations were performed using the PASW Statistics software version 18.0 (SPSS Inc., Chicago, IL).

**Results**

The study included 34 HPR patients between 6 and 60 yr of age. Thirteen patients had straight legs, nine had genu varum (four mild, five severe), and 12 had genu valgum (seven mild, five severe). Two patients had undergone surgery for limb deformities before testing. Compared with controls, HPR patients were shorter and had shorter tibias but were of similar weight (Table 1). At the time of testing, 29 patients had low phosphorus serum levels, but serum calcium was normal in all participants. Fifteen patients had serum PTH levels above the reference range (up to 19.7 pmol/liter).

The pQCT analyses yielded similar results for calf muscle CSA between HPR patients and controls, but muscle density and muscle volume index were lower in the HPR group (Table 2).

As to jumping mechanography, all tests could be performed by all controls. However, 12 HPR patients were unable to perform the multiple one-legged hopping, because they could not lift off the ground. For the same reason, two patients could not do the multiple two-legged hopping. All patients were able to perform the single two-legged jump, the chair-rise test, and the heel-rise test. Stepwise multiple regression analysis showed that the differences in jumping mechanography results between the HPR and control groups were not influenced by gender and age, with the exception of the multiple two-legged hopping test, where the group difference in force per body weight increased with age (\( r^2 = 0.18; P = 0.01 \)).

Compared with controls, the HPR group had significantly lower body weight-related peak force in the hopping tests, where the aim is to generate maximal force (Table 2 and Fig. 1). In the other three tests, where the aim is to achieve maximal power, HPR patients had between

<table>
<thead>
<tr>
<th>TABLE 1. Anthropometric data</th>
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<tr>
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<td>Gender (male/female)</td>
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<tr>
<td>Age (yr)</td>
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<td>Weight (z-scores)</td>
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<tr>
<td>Height (z-scores)</td>
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<tr>
<td>Tibia length (mm)</td>
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Results are given as mean (sd).
8.5% (for the heel-rise test) and 32% (for the chair-rise test) lower peak power per kilogram body mass than controls (Table 2 and Fig. 1).

To test the influence of limb deformities on mechanography tests, we analyzed results separately for patients with straight legs and those with mild and moderate leg deformities (Table 3). In each group and each test, HPR patients had significantly lower results than controls, with the exception of the heel-rise test in patients with straight legs. The muscle function deficit of patients with straight legs was significantly less than that of patients with severe deformities, with regard to the single two-legged jump (*P* < 0.001) and the heel-rise test (*P* < 0.001). More specifically, power per body mass was 26% (95% confidence interval = 5–47%) and 15% (95% confidence interval = 4–26%) lower in the subgroup with severe leg deformities than in the subgroup with straight legs for the single two-legged jump and heel-rise test, respectively. This difference in mean values did not reach significance for the multiple one-legged hopping test. However, the multiple one-legged hopping could be performed by only three of the 10 patients with severe lower limb deformity but by nine of the 13 patients with straight legs.

Multiple regression analysis was used to assess the impact of muscle mass (as reflected by the muscle volume index), muscle quality (represented by muscle density), and disease status on muscle function results (Table 4). Apart from the heel-rise test, disease status was a significant determinant of muscle performance in all tests. Muscle density was positively associated with outcome parameters in all five tests. Muscle volume index was a significant predictor in power tests (single two-legged jump, heel-rise test, and chair-rise test) but not in the force tests (multiple one-legged and two-legged hopping).

Within the HPR group, we investigated the potential influence of treatment status on muscle function as well as other confounding factors (serum intact PTH levels, serum phosphorus levels, and age). None of these factors was significantly associated with any of the muscle function outcomes.

**Discussion**

In the present study, we found that patients with HPR have muscle function deficits as well as low muscle density.
TABLE 4. Predictors of mechanographic tests results

<table>
<thead>
<tr>
<th>Regression equation</th>
<th>$r^2$</th>
<th>$P$</th>
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<tr>
<td>Multiple two-legged hopping (peak force per body weight)</td>
<td>$-2.10$ + $0.093 \times$ muscle density (mg/cm³) + $0.57 \times$ disease status</td>
<td>0.43</td>
</tr>
<tr>
<td>Multiple one-legged hopping (peak force per body weight)</td>
<td>$-1.65$ + $0.066 \times$ muscle density (mg/cm³) + $0.37 \times$ disease status</td>
<td>0.41</td>
</tr>
<tr>
<td>Single two-legged jump (peak power per body mass, W/kg)</td>
<td>$-72.9$ - $7.44 \times$ disease status + $1.43 \times$ muscle density (mg/cm³) + 0.006 muscle volume index (cm³)</td>
<td>0.60</td>
</tr>
<tr>
<td>Heel-rise test (peak power per body mass, W/kg)</td>
<td>$-13.0$ + $0.002$ muscle volume index (cm³) + $0.23 \times$ muscle density (mg/cm³)</td>
<td>0.34</td>
</tr>
<tr>
<td>Chair-rise test (peak power per body mass, W/kg)</td>
<td>$-17.4$ - $3.37 \times$ disease status + $0.39 \times$ muscle density (mg/cm³) + 0.002 muscle volume index (cm³)</td>
<td>0.52</td>
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These alterations were similar between genders and between younger and older patients. Thus, it appears that HPR affects muscles not only in the Hyp mouse (11) but also in humans.

The observation that the HPR group had significantly lower results than controls in each of the muscle function tests indicates that HPR affects various aspects of muscle function. Both eccentric (multiple one- and two-legged hopping) and concentric (heel-rise test, chair-rise test) contractions as well as the stretch-shortening cycle (22) (single-two legged jump) were affected in the HPR group. The eccentric contractions in the hopping tests achieve maximal forces and thus provide an indication of the maximal load that muscles exert on the skeletal system (15). The other tests assess power, which is determined as the ground reaction force multiplied by the speed of the body’s movement in a vertical direction. Power thus depends on the speed of muscle contraction in addition to force. Low muscle power can lead to functional deficits such as increased fall risks and limited mobility (23).

Many factors conceivably contribute to muscle function deficits in HPR patients, including poor muscle quality and quantity as well as lower limb deformities. Our data provide some indication of poor calf muscle quality and quantity in HPR, because pQCT demonstrated low muscle density and reduced estimated muscle volume. The lower muscle density suggests that HPR patients have higher muscle fat infiltration (12, 24) that is associated with decreased muscle force (25). Whether lower muscle density is a direct result of hypophosphatemia or instead mediated by indirect disease effect, such as possibly decreased physical activity, cannot be decided on the basis of the present data. Muscle volume was lower in the HPR group than in controls, because these patients had shorter distal lower extremities, whereas the other determinant of muscle volume, muscle CSA, was similar between groups. Muscle volume is known to be related to muscle power (26). This was also evident in the present study where muscle volume index was a determinant of outcomes in the power tests but not in the force tests.
Apart from muscle quality and quantity, our data also suggest that limb deformities play a role in reduced muscle function in HPR patients, because patients without lower limb deformities had better muscle function than those with severe deformities. However, muscle function was also reduced in patients with straight legs, suggesting that limb deformities are only one of several contributing factors to reduce dynamic muscle function in HPR.

In the present study, we did not find a significant influence of serum phosphorus levels, serum PTH levels, and treatment status on muscle function. Phosphorus serum levels undergo significant intraday fluctuations in X-linked hypophosphatemia patients (27), and therefore a single test result may not be representative. Elevated PTH levels were associated with decreased muscle function in previous studies on primary hyperparathyroidism (28) and vitamin D deficiency (29), but the clinical context in those studies differs greatly from our patient group, and therefore, results are difficult to compare. We did not find a significant influence of phosphorus supplementation on muscle function tests, but it must be acknowledged that the study design made it difficult to detect such treatment effects. Study participants receiving phosphorus and calcitriol were children and adolescents, whereas patients not receiving this treatment were mostly adults. To assess the effect of phosphorus and calcitriol therapy on muscle function, a randomized controlled study design would be more informative.

Although patients with HPR had measurable muscle function deficits, these deficits were not severe enough to impair everyday functional ability. In fact, all patients were able to perform the chair-rise test and the single two-legged jump, which are considered as high-function abilities (30, 31). Nevertheless, our study population was quite young, and only one participant was over 50 yr of age. It is possible that such muscle function deficits may impact on mobility function with aging. It is known that muscle force and in particular muscle power decrease with age (15, 32). Because HPR patients seem to embark on this age-related decline from a lower starting point, they may be more likely to experience functional deficits prematurely.

Conclusions

The present study suggests that muscle weakness is a clinical feature of HPR. It is therefore warranted to explore the etiology, long-term consequences, and possible treatments of muscle function deficits in HPR.

Acknowledgments

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