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The muscle–bone interaction in Turner syndrome

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ABSTRACT

Objectives: Turner syndrome (TS) is associated with an increased fracture rate due to reduced bone strength, which is mainly determined by skeletal muscle force. This study aimed to assess the muscle force–bone strength relationship in TS and to compare it with that of healthy controls.
Methods: This study included 39 girls with TS and 67 healthy control girls. Maximum muscle force (F_{max}) was assessed through multiple one-legged hopping with jumping mechanography. Peripheral quantitative computerized tomography assessed the bone strength index at the tibial metaphysis (BSI 4) and the polar strength–strain index at the diaphysis (SSI polar 66). The effect of TS on the muscle–bone unit was tested using multiple linear regression.
Results: TS had no impact on F_{max} ($p = 0.14$); however, a negative effect on bone strength ($p < 0.001$ for BSI 4 and $p < 0.01$ for SSI polar 66) was observed compared with healthy controls. Bone strength was lower in the TS group (by 18%, $p < 0.01$, for BSI 4 and by 7%, $p = 0.027$, for SSI polar 66), even after correcting for F_{max}.
Conclusions: Similar muscle force induces lower bone strength in TS compared with healthy controls, which suggests altered bone-loading sensitivity in TS.

Introduction

Turner syndrome (TS) is caused by the complete or partial loss of one X chromosome. Its incidence is 1 in 2000 live female births [39]. Patients with TS are seen primarily by endocrinologists to address their short stature, ovarian failure or autoimmune thyroiditis. However, other specialists may be involved because TS is associated with an increased risk of congenital heart and urinary system malformations and celiac disease [13]. Additionally, an increased fracture rate [15] and decreased bone mineral density (BMD) [36] have been described in TS compared to healthy controls. Several other studies describe osteoporosis in TS [4,14,23], which suggests that skeletal complications are of concern in these patients.

The etiology and mechanism of bone fragility in TS have not been completely elucidated. While the results of some studies suggest inappropriate estrogen substitution [7,16,18], other studies claim that either a direct effect of the loss of the short stature homeobox-containing gene (*SHOX*) [28,37] or an indirect effect of the loss of Xp22.3, which leads to neuromotor impairment [31,44], causes bone fragility in TS.

Skeletal muscle force represents a major mechanical stimulus for bone development and determines bone strength [12,33,34]. According to Frost's mechanostat theory [11], every bone should be adapted to withstand the maximal voluntary muscle forces. Ground-reaction forces have been used to calculate that during the multiple one-legged hopping test, the plantarflexor muscle force amounts to approximately 9–10.5 times the body weight [3]. This value is 2.8–3.3 times higher than the dynamometry-derived peak plantarflexor force (approx. 4.8 times the body weight) [3]. Therefore, the mechanical forces at the tibial shaft are much greater during multiple one-legged hopping compared with isokinetic dynamometry, a method that was previously used to assess muscle torque in the pediatric population [19,29].

Moreover, a strong correlation has been observed between maximum force, as assessed using ground-reaction force plate (GRFP) measurements during multiple one-legged hopping, and tibial bone strength, measured using peripheral quantitative computed tomography (pQCT) [2]. This correlation, together with its good reliability [43] and the availability of reference data [6,24,40], make GRFP a suitable method for assessing muscle function in children.

We hypothesized that the muscle–bone interaction is impaired in TS patients compared with healthy controls, leading to decreased bone

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strength. Thus, the aims of the present study were 1) to assess the peak force and bone strength using GRFP and pQCT, respectively, in girls with TS and healthy controls; 2) describe the muscle–bone relationships in the participants with TS by implementing the GRFP and pQCT measures; 3) compare these relationships with those of healthy controls; and 4) test the influence of menarcheal status, course of puberty, karyotype, durations of growth hormone (GH) and estrogen (E) therapy and fracture history on muscle–bone relationships in the TS participants.

Participants and methods

Participants

This was a cross-sectional study involving 39 girls with TS from a single university hospital referral center. Eighteen TS participants in the present study were also included in the longitudinal BMD study, and the baseline values of that study have been published previously [36]. Several patients with TS were passed on to adult endocrinologists, while new young girls with TS were also included. However, the present cross-sectional study is based on measurements of the weight-bearing tibia bone, whereas the previous study was based on radius bone measurements. Muscle force data from TS girls reported in this study were published quite recently [35]. However, the novelty of the present study is the concurrent assessment of both peak muscle force and BMD, which allows direct observation of the muscle–bone interaction. Despite the fact that the radiation dose due to pQCT measurement is negligible, only 39 girls with TS (and/or their guardians) consented to be included in the present study (i.e., to have scans at the tibia in addition to the scans at the radius) compared with 60 girls with TS who agreed to the muscle function assessment [35].

The exclusion criteria were any chronic disease (except autoimmune thyroiditis) or medication (except recombinant human growth hormone [GH] and estrogen) with a known effect on the muscles or bones. Body height was measured with a stadiometer to the nearest 1 mm. Weight was measured with an electronic scale to the nearest 100 g. Height, weight and BMI Z-scores were calculated using the most recent national reference data [21]. The girls with TS presented with Tanner stage 1 (N = 13), 2 (N = 3), 3 (N = 2), 4 (N = 6) and 5 (N = 15) breast development. There were 21 pre-menarcheal girls and 18 post-menarcheal girls. Their karyotypes were either monosomy of X (N = 13), the classical mosaic form 45,X/46,XX (N = 4), other double- or triple-line mosaics with at least two aberrant lines (N = 18) or a structural defect of one X chromosome (3 patients with a deletion and 1 patient with an isochromosome).

All of the girls with TS were treated with GH at a starting dose of 50 μg/kg/d, which was then adjusted according to the clinical response [32]. The mean age at the start of GH treatment was 7.6 ± 3.9 years, and the mean duration of GH administration was 5.4 ± 3.8 years. On the day of the examination, 3 of the girls had just begun GH treatment, and another 14 girls had already stopped treatment because they had reached their final height.

Estrogen replacement was given to 14 patients to initiate their puberty and to 4 patients because of secondary ovarian failure; another 9 patients had a spontaneous pubertal development. The remaining 12 patients were pre-pubertal. Oral estrogen replacement began at the mean age of 13.6 ± 1.7 years, with a starting dose of 5 μg/kg/day of 17-β-estradiol given for 12–18 months. [30]. Afterwards, a dose of 10 μg/kg/day was administered for another 12–18 months, followed by an increase to 20 μg/kg/day. After a mean period of 3 months, the same dose of estradiol was continued from days 1 to 21, with 5 mg of medroxyprogesterone per day added from day 15 to day 21 of every cycle.

Thyroxine substitution was necessary in 8 patients with autoimmune thyroiditis. All of these patients had been euthyroid for at least 18 months prior to the study measurements. Altogether, there were 19 fracture events in the histories of 10 patients: 12 in the forearm, 3 finger fractures, 2 ankle fractures, 1 wrist fracture and 1 vertebral fracture.

As a control group, we selected 67 healthy girls with a wide physical activity range. The control girls were recruited from schools and different sports associations (volleyball, ice hockey, gymnastics and synchronized swimming). The exclusion criteria were any disease or medication affecting the musculoskeletal system. The muscle and the bone measures were obtained using the same methods that were used for the TS group (GRFP and pQCT) and by implementing the same technical settings and maneuvers. These data have been published in part as a subgroup of a larger study comprising 323 children and adults [2].

The present study was approved by the University Ethics Committee and conformed to the Declaration of Helsinki. All of the participants included in this study (or a parent if the patient was younger than 18 years) consented to the testing.

Muscle force assessment

Maximum ground reaction force (F_{max}; N), a surrogate for maximum plantarflexor muscle force, was assessed using the multiple one-legged hopping (M1LH) test performed on a Leonardo Mechanograph® GRFP (Novotec Medical, Pforzheim, Germany). To detect, store and calculate the data, we used the manufacturer's software (Leonardo Mechanography GRFP version 4.2; Novotec Medical). The test consisted of repeated hopping on the forefoot of one leg with a stiff knee and free movement of the arms. The subjects were lightly dressed and wore no shoes. The subject's task was to jump as quickly as possible at the beginning of the test and then, after achieving good balance, to jump higher with every subsequent jump to obtain F_{max}. The highest value for F_{max} was selected among the three consecutive tests performed with the non-dominant leg. F_{max} and its relation to body weight (F_{max}/BW; no unit) were recorded.

Bone strength assessment

An XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) was used for pQCT measurements at the non-dominant tibia. The leg dominancy was determined according to handedness, so that the leg on the same side of the body as the dominant hand was considered dominant. A single tomographic 2.0-mm-thick slice was taken at distances corresponding to the 4% and 66% bone lengths, as measured from the medial malleolus to the superior margin of the medial condyle. A voxel size of 0.4 × 0.4 × 2.0 mm was used. The images were processed and the numerical values were calculated using version 6.20 C of the integrated XCT software. At the distal tibia (4% site), the total bone mineral content (BMC 4), total volumetric bone mineral density (vBMD), total bone cross-sectional area (CSA) and trabecular vBMD were measured. The compressional bone strength index (BSI 4) was calculated as the product of the square of the total vBMD and the total bone CSA, as previously described [22]. At the proximal tibia (66% site), the total BMC (BMC 66) and the polar strength–strain index (SSI polar 66) were assessed. The SSI polar 66 was determined using a segmentation threshold of 480 mg/cm^3. The precision errors of the pQCT measures were not assessed at the tibia, but they have been found to be low at the radius [36]. The root mean square standard deviations gained from the 3 consecutive measurements with repositioning were as follows: at the distal radius: total BMC 0.009 g/cm, total bone CSA 9.617 mm^2, total vBMD 9.353 mg/cm^3 and trabecular vBMD 2.905 mg/cm^3; at the proximal radius: total BMC 0.007 g/cm and SSI polar 7.685 mm^3.

Biochemistry

Blood samples were obtained from the patients on the day of the muscle and bone assessment and were a part of their regular follow-up. Serum FSH levels were measured in our accredited hospital