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Original Article

Hylonome

Case-control study of the muscular compartments and osseous strength in neurofibromatosis type 1 using peripheral quantitative computed tomography

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Abstract

Skeletal anomalies are observed in neurofibromatosis type 1 (NF1), but the pathogenesis is unknown. Given that muscle mass is important in the development of the strength of bone, peripheral quantitative computed tomography (pQCT) was utilized to compare measurements of muscle compartments between NF1 individuals and controls. Forty individuals with NF1 (age 5-18 years) were evaluated. Cross-sectional measurements, at the 66% tibial site, were obtained using pQCT (XCT-2000, Stratec) and variables were compared to controls without NF1 (age 5-18 years, N=380) using analysis-of-covariance controlling for age, height, Tanner stage, and gender. The NF1 cohort showed decreased total cross-sectional area [$p<0.001$], decreased muscle plus bone cross-sectional area [$p<0.001$], decreased muscle cross-sectional area [$p<0.001$], and decreased Stress Strain Index [$p=0.010$]. These data indicate that NF1 individuals have decreased muscle cross-sectional area and decreased bone strength than individuals without NF1.

Keywords: Neurofibromatosis Type 1, pQCT, Muscle

Introduction

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder affecting ≈1/3500 individuals worldwide. Clinical manifestations include café-au-lait macules, intertriginous freckling, distinctive osseous lesions, educational difficulties, seizures, Lisch nodules, neurofibromas, and other neoplasms (including optic pathway tumors). The criteria for the clinical diagnosis of NF1 have been set and are well accepted[1,2]. The *NF1* gene was mapped to the long arm of chromosome 17, cloned and characterized as a ras-GAP protein[3]. The *NF1* gene product, neurofibromin, interacts with the ras signal transduction pathway and has "tumor suppressor" aspects, but this does not easily explain the mesodermally-derived manifestations observed in NF1.

NF1 is classically characterized as a neurocutaneous disorder, but skeletal abnormalities are clearly associated with NF1. The common skeletal abnormalities include long bone dysplasia, sphenoid wing dysplasia and scoliosis. Rare manifestations include bone cysts, spinal canal widening, vertebral body narrowing, rib-penciling, and vertebral scalloping. Long bone dysplasia (seen in 5% of patients with NF1) typically presents with anterolateral bowing of the tibia often leading to fracture and non-union[4,5]. Between 10-33% of children with NF1 will have some vertebral deformity[6]. Sphenoid wing dysplasia is seen in 7-11% of NF1 individuals[7] and it is often associated with ipsilateral temporal-orbital plexiform neurofibromas. One study of NF1 patients followed at an NF Clinic reported 38% of patients had one or more orthopaedic findings[8]. Some orthopaedic manifestations of NF1 are highly morbid and require significant intervention.

Muscle mass is important in the development of bone strength, as voluntary muscle forces (the largest physiological load) impact skeletal response[9]. Using dual energy X-ray absorptiometry (DXA), decreases in bone mineral density of the lumbar spine have been seen in a set of 12 NF1 individuals with scoliosis[10]. It is unknown if the decrease in lumbar

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Variable	Healthy Controls (N=377)		NF1 Total (N=40)			Group A: NF1 without osseous dysplasia (N=32)			Group B: NF1 with osseous dyslasia (N=8)			
	Adjusted Mean	Adjusted SE	Adjusted Mean	Adjusted SE	p-value (vs. controls)	Adjusted Mean	Adjusted SE	p-value (vs. controls)	Adjusted Mean	Adjusted SE	p-value (vs. controls)	p-value (vs. Group A)
Total Area of Cross-section (mm^2)	7313	79	6356	248	p<0.001	6419	276	p=0.006	6103	545	p=0.086	p=1.0
Muscle & Bone Cross-sectional Area (mm^2)	4986	47	4344	147	p<0.001	4422	164	p=0.003	4031	322	p=0.011	p=0.828
Muscle Cross-sectional Area (mm^2)	4442	44	3886	137	p<0.001	3958	153	p=0.008	3600	301	p=0.018	p=0.859
Stress Strain Index (mm^3)	1314	16	1176	50	p=0.01	1186	56	p=0.088	1134	110	p=0.325	p=1.0

Table 1. pQCT statistical analysis at the 66% tibial site.
Comparison of the pQCT variables between NF1 and controls, NF1 individuals without (Group A) and with (Group B) osseous abnormalities and controls, and between Group A and Group B adjusted for gender, Tanner stage, height, and age using analysis-of-covariance with a fixed set of covariates. (pQCT= peripheral quantitative computed tomography; NF1= neurofibromatosis type 1; SE= Standard Error).

bone mineral density observed in these NF1 patients with scoliosis[10] is specific for scoliosis or a more generalized "dysplasia" of NF1 as no comparisons were made to NF1 individuals without scoliosis. DXA imaging, however, cannot assess bone shape or associated muscle geometry. The geometric properties of bone are important in determining the strength of bone[11], therefore, peripheral quantitative computed tomography (pQCT) has emerged as a useful technology to measure bone size and geometry with separation of the bone and muscle compartments.

Few syndromes have been evaluated using pQCT looking specifically at the muscle compartments. Musculoskeletal analyses using pQCT have been used in Turner syndrome[12], and may be of use in other syndromes with musculoskeletal involvement. This report uses pQCT to evaluate bone strength and mass of the muscular compartments of individuals with NF1.

Methods

NF1 individuals were recruited from an NF1 clinic at the University of Utah and all fulfilled the diagnostic criteria for NF1[1,2]. Forty individuals with NF1 (ages 5-18) were included in the study. Those with other chronic illnesses known to influence bone health, e.g., illnesses requiring systemic steroids, anorexia, pregnancy, lactation, oral contraception or hormone replacement were excluded. Controls consisted of a cohort of healthy individuals without NF1 (N=377) collected by the Center for Paediatric Nutrition Research at the University of Utah.

Physical examinations and medical histories were obtained on all NF1 individuals. Cross-sectional measurements at the 66% tibial site were obtained using peripheral quantitative computed tomography (pQCT) (XCT-2000, Stratec, White Plains, New York) with a resolution of 0.6mm, and compared to data from local controls. NF1 individuals were subsequently separated into 2 groups (Group A=NF1 individuals without osseous abnormalities; Group B=NF1 individuals with osseous abnormalites). For this study, an osseous abnormality was defined as long bone dysplasia, scoliosis, or sphenoid wing dysplasia. For statistical analysis, an analysis-of-covariance with a fixed set of covariates (age, height, Tanner stage, and gender) was used. The NF1 group and subgroups (Group A and B) were compared to controls. Subsequently, measurements between Group A and Group B were compared. To determine whether there might exist trends across the three study groups (controls, Group A, and Group B), a trend ANOVA was performed.

Institutional Review Board approval at the University of Utah and informed consent from participants were obtained.

Results

The NF1 individuals consisted of 18 girls and 22 boys [age (mean 10.5 years, SD±3.7); height (mean 133 cm, SD±19); weight (mean 32kg, SD±12); Tanner Stage (mean 2.1, SD±1.3)]. Controls consisted of 377 individuals without NF1 [age (mean 11.7 years, SD±3.8); height (mean 147cm, SD±21); weight (mean 45kg, SD±20); Tanner Stage (mean 2.4, SD±1.5)]. Osseous abnormalities were not seen in 32/40 NF1 individuals (Group A). In Group A there were 14 girls and 18 boys, the average age was 10.1 years (range 5.3-18.8

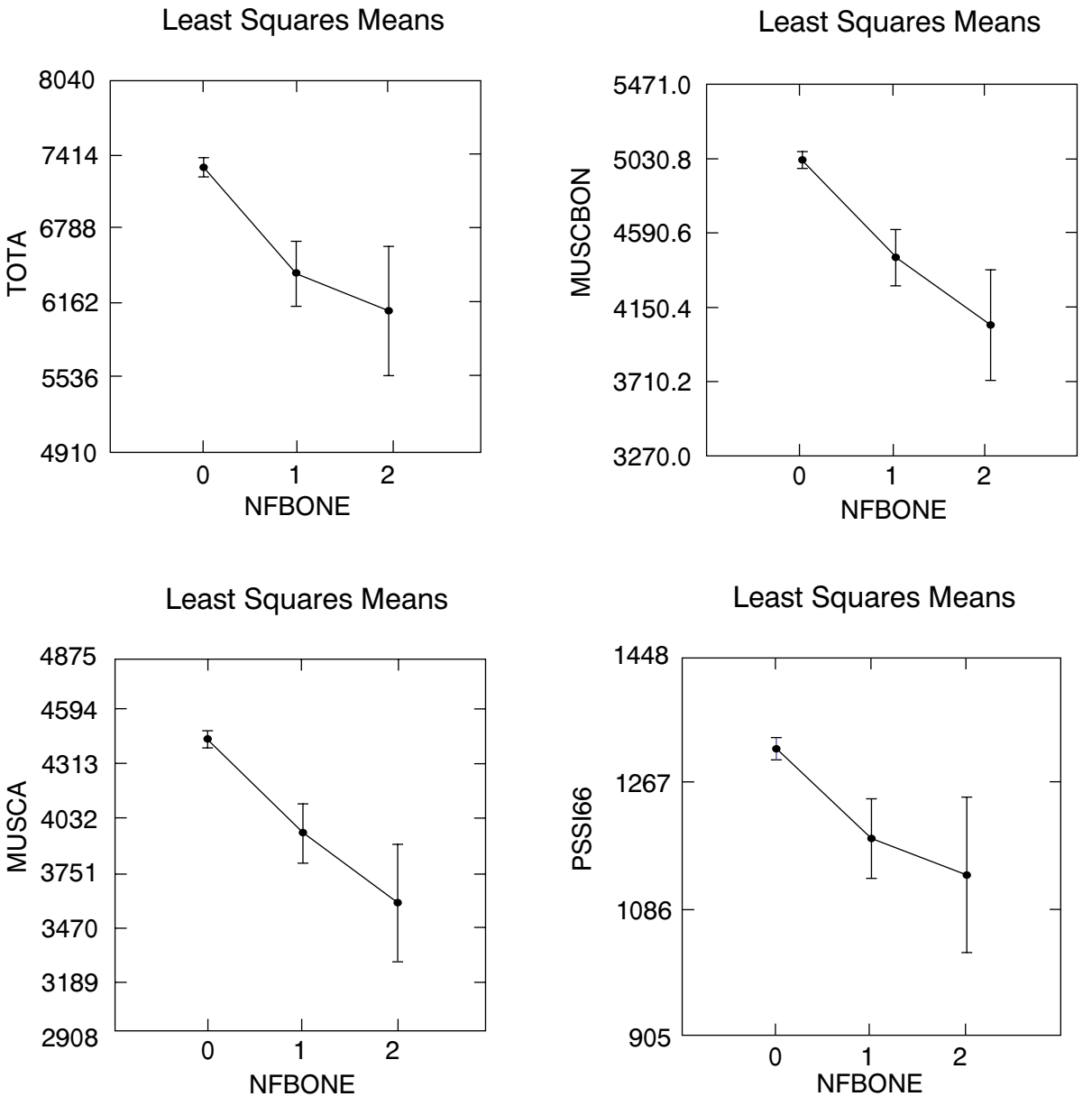

Figure 1. Graph of trend ANOVA in 4 variables from pQCT.
Graphical illustration of the comparison of controls (N=377), NF1 individuals without osseous abnormalities (N=32), and NF1 individuals with osseous abnormalities (N=8) showing a downward trend for 4 measurements from peripheral quantitative computed tomography (pQCT) images at the 66% tibial site. NFBONE describes the category of groups compared (0= controls; 1= NF1 individuals without osseous abnormalities; 2=NF1 individuals with osseous abnormalities). TOTA=Total Area of Cross-section (mm^2) [p=0.029], MUSCBON =Muscle plus Bone Cross-sectional Area (mm^2) [p=0.004], MUSCA= Muscle Cross-sectional Area (mm^2) [p=0.006], PSSI66= Stress Strain Index (mm^3) [p=0.108].

years), and 72% had a Tanner Stage ≤2 (range 1-5; median 2). Upon clinical evaluation 8/40 NF1 individuals had classical osseous dysplasias (6 with scoliosis, 1 with sphenoid wing dysplasia, and 1 with tibial dysplasia) and placed in Group B. In Group B there were 4 girls and 4 boys, the average age was 11.7 years (range 5.8-15.8 years), and 63% had a Tanner Stage ≤2 (range 1-5; median 2).

Cross-sectional measurements at the 66% tibial site were compared between NF1 individuals and controls adjusting for age, height, Tanner stage and gender. These comparisons are summarized in Table I. Statistically significant decreases were seen in the total cross-sectional area [p<0.001], muscle and bone cross-sectional area [p<0.001], muscle cross-sectional area [p<0.001], and the Stress Strain Index [p=0.010].

When NF1 individuals were separated into those who have osseous abnormalities and those who do not (Groups A and B), there were statistically significant differences for each group when individually compared to controls (see Table I). However, there were no differences between the NF1 groups. A trend ANOVA was performed to explore the possibility of trends across study groups (controls, Group A, and Group B). Since Group B had only 8 individuals and it is not yet established *a priori* that these groups reflect a pathophysiologic continuum, the trend ANOVA results should be considered preliminary and exploratory in nature. The trend ANOVA showed a downward trend in total area of cross-section (mm^2) [p=0.029], muscle plus bone cross-sectional area (mm^2) [p=0.004], and muscle cross-sectional area (mm^2) [p=0.006]. There also appears to be a downward trend in Stress Strain Index (mm^3), but this did not reach statistical significance (p=0.108). Figure 1 illustrates the downward trend in these variables. Comparison of regression of cortical bone area (mm^2) by muscle cross-sectional area (mm^2) at the 66% tibial site in all 3 groups (controls, Group A, and Group B) was performed, but ANCOVA testing of the slopes of the curves did not reach statistical significance.

Discussion

These data suggest that NF1 individuals have a different muscular geometry compared to the general population and documents that pQCT is an effective modality to assess musculoskeletal geometry in NF1. We hypothesize that sarcopenia contributes to the progression of bone dysplasia in NF1 individuals, but our data alone do not confirm this hypothesis, as prospective longitudinal measurements were not obtained during the development of osseous abnormalities. If future prospective studies determine that sarcopenia contributes to osseous dysplasias in NF1, then intervention to increase muscle mass could be of benefit.

Bone architecture has been shown to depend critically on muscle cross-section and tension development[13], and muscle strength is generally considered to play a role in fracture risk[14]. Physical activity is associated with bone geometry and lean tissue mass (predominantly muscle) explains some of this association[15]. Modeling increases bone strength when muscle forces exceed a threshold range, but decreased mechanical loads can result in removal of bone neighboring the marrow, which results in decreased bone strength[16]. Muscle size is also an important determinant of cortical area[17], and the cortical shell of the tibia in some NF1 individuals is dysplastic. Given that the NF1 individuals in this study had statistically significant decreases in muscle cross-sectional area, the muscle mass may contribute to the abnormalities seen clinically in the long bones of some NF1 patients.

Although it is well established that NF1 individuals have osseous abnormalities, the biological basis is not well characterized. Inactivation of the *NF1* gene likely contributes to the geometry of osseous structures and muscle compartments in NF1 patients, but modifier genes, extrinsic non-osseous forces, and environmental factors may play a role in the clinical variability of the musculoskeletal features observed in this condition. One such example is a spinal neurofibroma that exerts pressure on vertebral elements.

The treatment of the osseous dysplasias in NF1, including long bone bowing leading to fracture and non-union, is difficult and not uniformly agreed upon among health care providers. It is unknown how diet or exercise changes will improve bone strength and muscle mass in NF1 individuals prospectively. Physical activity can increase the mechanical load placed on the body as a whole[18], and high-impact exercise has been shown to be an effective strategy for site-specific gains in bone strength in pre-pubertal boys[19]. Improving muscle mass through exercise regimens may be of benefit to NF1 individuals to subsequently improve bone mineral acquisition and bone strength. Future longitudinal studies on the effects of exercise and diet on the musculoskeletal system in NF1 should provide insights into preventive or therapeutic strategies, and the pathophysiology of the differences observed in this study.

We cannot rule out the possibility that skeletal dysplasias in NF influence muscle development. It is unclear whether the decreased muscle area contributes to osseous abnormalities in NF1 or if impaired mobility due to osseous dysplasias cause the observed decrease in muscle area. The NF1 patients without clinical osseous abnormalities, however, also had statistically significant decreases in muscle mass compared to controls. The osseous matrix may be abnormal without evidence of clinical findings. Combinations of extrinsic forces including decreased muscle mass could compromise this potentially abnormal osseous matrix. We theorize that a combination of genetic and biomechanical factors influence the skeletal defects in NF1. Prospective studies and a larger sample size will be needed to understand the pathophysiology of the osseous abnormalities in NF1.

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