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Original Article

Hylonome

Vitamin D status and muscle function in children with neurofibromatosis type 1 (NF1)

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Abstract

Objectives: The aim of this cross-sectional study was to assess the vitamin D status and muscle function in children with NF1 compared with their unaffected siblings. **Methods:** NF1 children between 5 and 18 years of age and who had at least one unaffected sibling were identified. Serum concentrations of 25-hydroxyvitamin D (25(OH)D), calcium, inorganic phosphate, alkaline phosphate, parathyroid hormone and 1,25-dihydroxyvitamin D were measured. The Leonardo Mechanography Ground Reaction Force Platform (GRFP) was used to measure EFI, jump power, force and height. **Results:** There was no significant difference in 25(OH)D between NF1 subjects and unaffected siblings. Relative jump power and force were found to be significantly different. The adjusted means (95% confidence limits) of non-NF1 and NF1 children for relative jump power (W/kg), controlling for body mass and age, were 37.31 (34.14, 40.49) and 32.51 (29.34, 35.68), respectively (P=0.054); and force (N/kg), controlling for body mass, age and gender, were 25.79 (24.28, 27.30) and 21.12 (19.61, 22.63), respectively (P<0.0001). Jumping parameters were not related to serum 25(OH)D. **Conclusions:** There was no significant relationship between vitamin D status and NF1 status in children. NF1 children had significantly impaired jumping power and force, when compared to their unaffected siblings.

Keywords: Jumping Mechanography, Parathyroid Hormone, Myopathy, Pediatrics, Neurofibromas

Introduction

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is the most common autosomal dominant disease affecting about 1 in 3,500 individuals[1,2]. The primary phenotypic features of NF1 are café-au-lait spots, axillary freckling and cutaneous neurofibromas. NF1 is classically characterized as a neurocutaneous disorder, but other systems throughout the body (vascular and skeletal) are also affected by NF1. Ten to thirty-five percent of patients are affected by skeletal manifestations, which include kyphoscoliosis, spondylolisthesis, congenital bone bowing, pseudoarthrosis, sphenoid wing lesions, subperiosteal bone proliferation and local bone dysplasia[3-6].

Several studies have reported significantly lower mean serum 25-hydroxyvitamin D (25(OH)D, a measurable parameter of an individual's vitamin D status) concentration in adults with NF1, compared with healthy controls[4,5,7]. Lammert et al. found that NF1 subjects had an inverse relationship between the number of dermal neurofibromas and serum 25(OH)D concentration[7]. Vitamin D deficiency primarily affects calcium and bone homeostasis[8,9]. Patients with extremely low vitamin D serum levels (25(OH)D) have manifestations of nonspecific limb pain, lower limb and pelvic deformities, rickets, tetany, and hypocalcaemic convulsions[10,11]. In addition to these manifestations, vitamin D deficiency leads to reduced muscle strength[10,12,13]. This myopathy is seen more in the proximal muscles and muscles play a key role in the sculpting of bone size and maintenance of bone integrity[10,14,15].

R. Rawer is an employee of Novotec Medical GmbH. All other authors have no conflict of interest.

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List of Abbreviations	
Neurofibromatosis Type 1	NF1
Leonardo Mechanography Ground Reaction Force Platform	GRFP
Bone Mineral Density	BMD
25-hydroxyvitamin D	25(OH)D
1,25-dihydroxyvitamin D	$1,25(OH)_2D$
Parathyroid hormone	PTH
Esslinger Fitness Index	EFI

Studies conducted in children by Stevenson et al., found significant negative associations between NF1 and both 25(OH)D concentrations (P=0.01) and muscle cross-sectional area (P<0.001)[16,17]. In another study conducted by Souza et al., NF1 patients aged 7-60 years were found to have reduced muscle force when compared to healthy controls[18]. In recent years, reduced muscle strength has been found in individuals with RASopathies, such as NF1 individuals[6,17-20]. This finding may be due to the underlying condition of NF1, as well as, secondary to the vitamin D deficiency that appears to be more common in NF1 patients. To our knowledge muscle function and its relationship to vitamin D status has not been formally studied in NF1 children.

The Leonardo Mechanography Ground Reaction Force Platform (GRFP), a method that has been validated in children, athletes, and frail elderly[21-23], is used to measure the peak jumping power (kW), jump height (m), vertical velocity (m/sec) and the ground reaction force (N) of an individual. Dynamic measurements, such as those obtained using the GRFP, might be more functionally relevant than static measurements collected with the isometric and dynamic isokinetic tests[22]. A single two-legged jump is performed on a GRFP, where the individual is asked to jump as high as possible; therefore obtaining the maximum elevation of the center of mass[21-23]. This type of jumping requires the use of the proximal muscles, such as the quadriceps, gastrocnemius and soleus, which are the first muscles affected in myopathy[10].

The aim of this cross-sectional study was to investigate the muscle function and vitamin D status, and their relationship to each other, in NF1 children compared to their non-affected sibling of a similar age. The single two-legged jump performed on the GRFP was used to calculate the anaerobic peak power to assess muscle function. We hypothesized that (a) children with NF1 would have lower serum 25(OH)D concentrations compared to their unaffected siblings and (b) serum 25(OH)D concentrations would be associated with decreased muscle function parameters, assessed by GRFP.

Participants and Methods

The NF1 Genetic Register obtained from the Genetic Department, St. Mary's Hospital, Manchester, UK, was used to identify children with NF1 who were between 5 and 18 years of age and who had at least one unaffected sibling. All families that fulfilled the inclusion criteria were sent child and parent information leaflets, and a reply letter containing contact information, along with a stamped return addressed envelope. The families who returned the reply letter were contacted and informed consent was obtained. Children with NF1 who did not have siblings without NF1, or had any leg deformity, such as pseudoarthrosis of the tibia, fibula, or plexiform neurofibroma in the lower limb that affects leg length, leg mobility, or the ability to jump were excluded from the study. There were a total of 30 children (15 children affected by NF1, 15 children non-affected by NF1) who completed the study. This study received ethical approval from the Liverpool Region Ethical Committee, Liverpool, UK (Ethics approval 10/H1002/17).

Anthropometric Measurements

Standing height was measured using a stadiometer and body mass was measured using a digital scale with shoes and outdoor clothing removed. Body mass index (BMI) was calculated by using the formula mass (kg)/height(m)^2.

Physical Examinations & Questionnaires

Questionnaires were completed by each child with the help of their parent and study personnel. Questionnaires provided information on muscle pain, sun exposure, physical activity recalls and food frequency intake. The muscle pain questions asked if the children had suffered from any of the following: aches and pains in the legs, pain or difficulty climbing stairs, pain or difficulty getting out of a chair and muscle cramps. A previously used questionnaire was used to assess sunlight exposure, and modified for children participating specifically for this study[24]. An NF1 assessment form was completed on each NF1 child. If the assessment had been completed within the past year, the information was obtained from the child's medical record. Dr Mughal, a Pediatric Consultant or Dr Huson, a Genetic Consultant completed all NF1 assessments.

A 7-day activity recall was obtained using a Seven Day Physical Activity Recall (SDPAR) form[25], which was completed by study personnel via individual interview with each child and parent. The SDPAR requires the child and mother and/or father to determine the average amount of time spent sleeping, sitting, or participating in moderate or vigorous activity. The remaining time was classified as light activity. Vigorous activity was defined as any activity leading to an increase in heart rate or heavy breathing, including activities such as running and brisk walking. Moderate activity was defined as any activity that required significant movement but not at the level of noticeable increased heart rate or heavy breathing. Sedentary time was defined as any time that involved minimal or no active movement such as sitting, watching TV, school work, or working/playing on the computer. Activity patterns for both weekdays and weekend days were included and the number of days per week considered. From this information the average percent of time spent in moderate plus vigorous activity and sedentary time per day were calculated. Information on weight-bearing activity also was obtained. Weight-bearing activity was defined as any activity that required force against gravity to be applied on the muscle-bone unit, such as weight lifting, gymnastics or karate. Although not validated in pediatric populations, the SDPAR has been used in numerous studies[26-28].

A food frequency questionnaire was completed to evaluate the subjects' average daily intake of vitamin D and calcium containing foods. The Composition of Foods Integrated Dataset (CoF IDS) (McCance & Widdowson, 2002) was used to obtain the amount of calcium and vitamin D for each food option, per 100 g and then the average weekly and daily intake of calcium (mg) and vitamin D (μg) for each participant was calculated.

Ground Reaction Force Platform (GRFP)

Peak jumping power, peak jumping force and vertical velocity were measured using the Leonardo Mechanography Ground Reaction Force Platform (GRFP), software version 4.2 (Novotec Medical GmbH, Pforzheim, Germany). Each participant performed three maximum countermovement jumps with arms moving freely, and at rest between each jump. Each jump was two-footed and participants were instructed to jump as high as possible, therefore obtaining the maximum elevation of the center of mass.

The GRFP measures ground reaction forces (N) over time. Together with the body mass it calculates the effective acceleration (m/sec^2) acting on the center of gravity and by integrating these, computes the vertical velocity (m/sec). Jump height (m) can be calculated by the integration of velocity (m/sec) over time taken (sec) to jump. The individual's body mass is assessed during a quiet stance immediately before the jump. Instantaneous power, the peak jumping power, is the product of force and velocity and is calculated following the jump. The Esslinger Fitness Index (EFI) is derived from the peak power output per kg of body mass and is expressed as a percentage of the mean value of the reference data normalized by age and gender[29]. As described by the manufacturer, efficiency is the relation of the peak power output to the peak force normalized by body mass, gender and age[29]. Z-scores for EFI and efficiency were calculated for each participant's jump using a reference population[29]. The jump with the greatest height was used for data analysis. The precision, measured as coefficient of variation, of muscle power and peak jumping force measurements in children were 3.7% and 8.9%[23].

Blood Biochemistry

Non-fasting blood samples (5-7 ml) were collected for biochemical measurements. All the blood samples were drawn over a two-month period during the spring. Serum was stored at -20ºC until measurement. Following extraction and purification, serum $25(OH)D_2$ and $25(OH)D_3$ were measured separately by normal phase HPLC (Waters Associates, Milford, MA) using a Hewlett-Packard Zorbax-Sil Column (Hicrom, Reading, Berkshire, UK) eluted with hexane: propanol (98:2) run at 2 ml/min and quantified by UV absorbance at 265 nm and corrected for recovery. Sensitivity was 2 ng/mL (5 nmol/L) and interassay variation was 6%. The assay laboratory is accredited to ISO 9001:2008 and ISO 13485:2003 and participates successfully in the Vitamin D quality assurance scheme (DEQAS). Following separation by HPLC, $1,25(OH)_2D$ was quantified by radioimmunoassay as described in detail elsewhere[30]. The adult reference range is 48-120 pmol/L, sensitivity 3 pmol/assay tube and intra-and-inter assay CV 7.8% and 10.5%, respectively[31]. Serum intact parathyroid hormone (PTH) was measured using the Roche Modular e170 analyser according to manufacturers instructions (Roche Diagnostics, Mannheim, Germany); the paediatric reference interval is 10 to 60 pg/mL (1.1 to 6.4 pmol/L: to convert pg/mL to pmol/L multiply by 0.105), Intra-&-inter assay CV 3% and 6%, respectively. Serum concentrations of calcium adjusted for albumin (Ca), inorganic phosphate (P) and alkaline phosphatase activity were measured using the Roche COBAS 6000 analyser according to manufacturers instructions (Roche Diagnostics, Mannheim, Germany). Assay performance data: PTH inter-assay coefficient of variation (CV) <3% at 22 and 123 pg/mL; ALP CV <2% at 57 and 203 IU/L, phosphate CV <3% at 0.67 and 2.05 mmol/L and calcium CV<2% at 1.55 and 3.22 mmol/L.

Data Analysis

Statistical analyses were performed using SAS 9.2 software (SAS Institute Inc. Cary, NC). Descriptive statistics were calculated for each study group (NF1 children, non-NF1 children) and compared using an unpaired student's t-test. Chi-squared analysis was used to evaluate differences between the two study groups for categorical dependent variables. Preliminary analysis was completed using correlation coefficients. Body mass, age and gender were found to be correlated with the jumping parameters, which are known covariates to be associated with jumping parameter outcomes[22,29]. Regression analysis was used to evaluate the association of the biochemistry and jumping parameters with the study groups and other possible covariates. As previous studies have suggested body mass has a quadratic relationship with jumping parameters[10], additional analyses were performed by adding a quadratic term for body mass; however this did not appreciably change the relationships between group (NF1 vs. non-NF1) and jumping parameters.

Results

A total of 30 children (15 NF1 children, 15 non-affected NF1 children) aged 5 to 18 years of age participated in the study (6-15 years of age for NF1; 6-18 years of age for Non-NF1). Among the study population, 60% (n=18) were female (10 NF1; 8 Non-NF1) and 40% (n=12) were male (5 NF1; 7 Non-NF1). Questionnaires and blood biochemistry data were obtained in all 30 children. A total of 26 children (13 NF1 children, 13 non-affected NF1 children) performed three single two-legged jumps on the GRFP. Four children (2 children affected by NF1, 2 children non-affected by NF1) did not participate in the GRFP portion of the study. One NF1 participant had a neurofibroma on her left foot that did not affect her mobility. Table 1 summarizes the demographic, blood biochemistry and dietary intake data. We calculated the height z-scores based on the CDC growth curves and found a significant difference in the z-scores between NF1 and controls (means of -1.21 + 0.77 and -0.06 + 0.90, $p<0.01$)[32]. Because only four of the 30 participants were classified as non-Caucasian race/eth-

	Non-NF1 Children	NF1 Children	P Value
Sex (M/F)	7/8	5/10	0.46
Age (yr)	11.5 (4.7)	11.8 (2.9)	0.84
Height (cm)*	140.5 (24.6)	140.1 (17.0)	0.96
Body mass (kg)*	42.8 (23.0)	40.0 (15.3)	0.71
Body Mass Index (kg/m^2)*	20.0 (5.0)	19.6 (3.4)	0.82
Serum 25(OH)D Total (ng/ml)#	16.6 (5.7)	15.6 (5.5)	0.65
Serum 25(OH)D_2 Total (ng/ml)@	1.1 (1.3)	1.6 (2.8)	0.57
Serum 25(OH)D_3 Total (ng/ml)@	14.9 (5.3)	13.3 (4.1)	0.42
Serum 1,25$(OH)_2$D Total (pmol/L)#	42.1 (10.7)	44.5 (13.5)	0.60
Adjusted Calcium (mmol/L)#	2.25 (0.09)	2.29 (0.06)	0.21
Calcium (mmol/L)#	2.26 (0.09)	2.30 (0.06)	0.24
Phosphate (mmol/L)^	1.3 (0.2)	1.27 (0.2)	0.74
Alkaline Phosphate (IU/L)#	185.5 (66.0)	151.7(56.8)	0.15
+Parathyroid Hormone (pg/mL)#	21.3 (10.1)	29.2 (8.9)	**0.05**
Dietary Calcium (mg)*	985.3 (357.3)	843.9 (454.8)	0.39
Dietary Vitamin D (μg)*	3.5 (2.1)	2.8 (1.7)	0.33
Sun Exposure Weekday			0.50
– >60 mins/day	12	11	
– 30-60 mins/day	1	3	
– 15-30 mins/day	2	1	
– <15 mins/day	0	0	
Sun Exposure Weekend			0.67
– >60 mins/day	12	11	
– 30-60 mins/day	3	4	
– 15-30 mins/day	0	0	
– <15 mins/day	0	0	

*non-NF1 (n=13); NF1 (n=13); ^non-NF1 (n=14); NF1 (n=13)
#non-NF1 (n=15); NF1 (n=14); @non-NF1 (n=12); NF1 (n=11)
+ to convert pg/mL to pmol/L multiply by 0.105

Table 1. Demographic, biochemical, sun exposure and diet data for NF1 and non-NF1 children. Data are presented as mean (SD).

	Non-NF1 Children	NF1 Children	P Value
EFI (%)	93 (15)	83 (13)	0.07
EFI Z-Score	-0.5 (1.0)	-1.4 (1.1)	**<0.04**
Force Efficiency	90 (6)	93 (13)	0.47
Force Efficiency Z-Score	-0.9 (0.5)	-0.7 (1.2)	0.58
Relative Jump Power (W/kg)*	37.31 (1.62)	32.51 (1.62)	**0.054**
Relative Jump Force (N/kg)@	25.79 (0.77)	21.12 (0.77)	**<0.0001**
Peak Jump Height (m)	0.34 (0.16)	0.32 (0.16)	0.67
Peak Velocity (m/sec)	1.96 (0.36)	1.97 (0.25)	0.95
Miles Walked (miles/day)	1.81 (0.72)	1.82 (0.87)	0.98
Flights (10 steps = 1 flight)	14(12)	11 (7)	0.98
% Moderate/Vigorous Activity (hours/day)	3.4 (1.0)	3.4 (1.6)	0.91
Sedentary time (hours/day)	6.5 (1.7)	7.0 (2.0)	0.54
Resistance Activity (minutes/day)	11 (15)	8 (12)	0.59

EFI = Esslinger Fitness Index
*Adjusted P-value for group differences after body mass and age were included in the analysis.
@Adjusted P-value for group differences after body mass, age and gender were included in the analysis.

Table 2. Ground reaction force platform and physical activity results for NF1 and non-NF1 children. Data are presented as mean (SD).

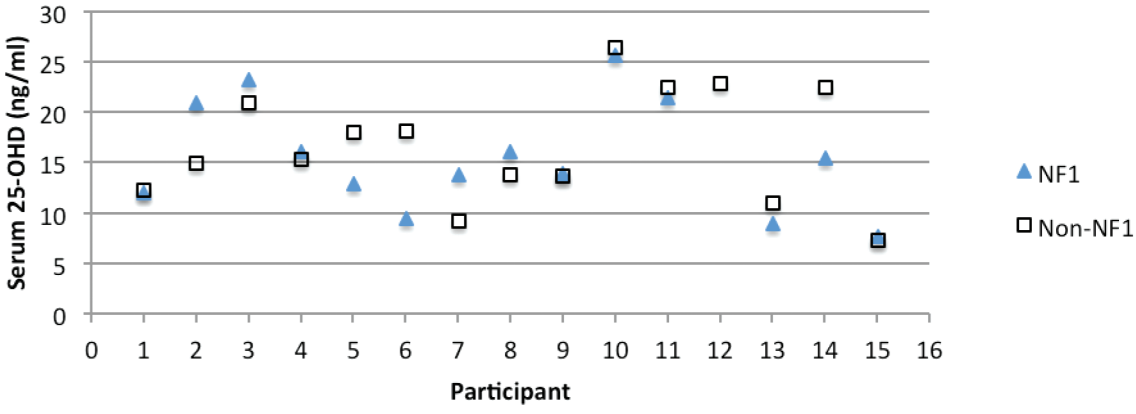

Figure 1. Serum 25-(OH)D concentrations (ng/ml) for NF1 children and their matched non affected sibling.

nicity, we were unable to include race/ethnicity as a covariate. There was no difference in sun exposure between the NF1 children and controls and there was no association between sun exposure and either serum 25-OHD concentrations or GRFP results. In addition, there were no significant differences in the demographic or dietary intake data between NF1 children and controls. The relationship between serum 25-OHD concentrations and GRFP results also was not significant.

Vitamin D deficiency is defined as a 25(OH)D<20 ng/mL and insufficiency as 21-29 ng/mL[33]. The mean total serum 25(OH)D concentrations were not significantly different between the two groups, but the overall mean of 16 ng/mL seen in the total population was within the deficient range (Table 1). There were 4/14 NF1 children and 5/15 non-NF1 children with 25(OH)D levels below 20 ng/mL. In addition to total serum 25(OH)D concentrations, serum concentrations of $25(OH)D_2$ and $25(OH)D_3$, total $1,25(OH)_2D$, corrected calcium, phosphate, alkaline phosphatase (AKP), and parathyroid hormone (PTH) were determined. Alkaline phosphatase concentrations showed a trend of lower concentrations in NF1 individuals compared to controls (p=0.15). PTH was the only blood biochemistry variable found to be significantly different between the NF1 and non-NF1 groups. The mean PTH was greater in children with NF1 compared to children without NF1 (p=0.05) (Table 1). There was no relationship between PTH and 25(OH)D for NF1 and non-NF1 children (Figure 1).

We could not demonstrate a relationship between total serum 25(OH)D concentrations and the jumping parameters as we had originally hypothesized. Total serum 25(OH)D concentrations were not found to be associated in the unadjusted or adjusted models for relative jump force (p=0.11 and p=0.65, respectively) and relative jump power (p=0.98 and p=0.57, respectively).

Jumping parameters are summarized in Table 2. Results from the physical activity questionnaire were not significantly different between groups and were not significantly associated with the jumping parameters (Table 2). However, the jumping parameters, relative jumping power and relative jumping force, were significantly different between non- NF1 and NF1 children. The unadjusted means (95% confidence limits) of non-NF1 and NF1 children for relative jump power (W/kg) were 36.11 (31.77, 40.44) and 33.72 (28.38, 38.06), respectively (*P*=0.45) (Figure 2a); and relative jump force (N/kg) 25.38 (23.61, 27.15) and 21.92 (20.15, 23.69), respectively (*P*=0.012)

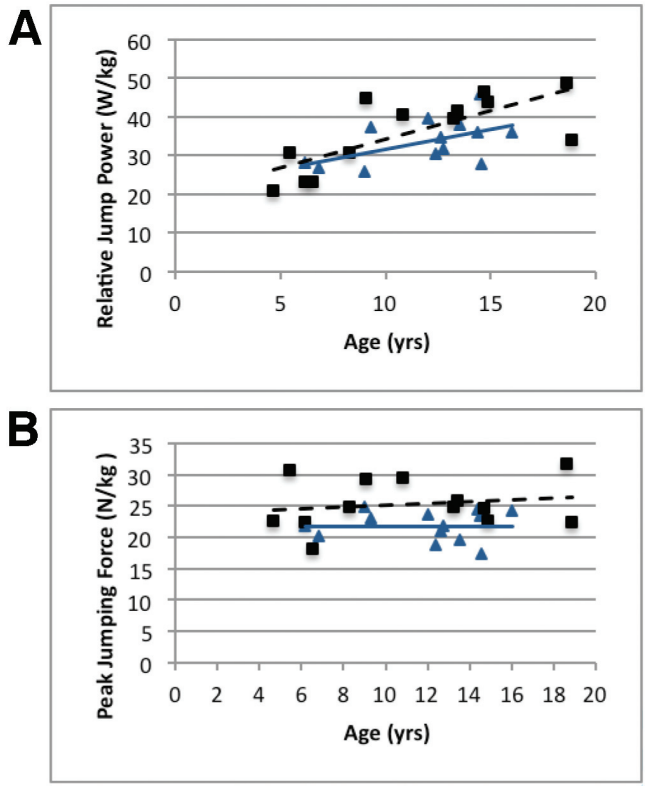

Figure 2. A. Relative jump power (W/kg) and age (yrs). Triangles and solid regression line are NF1 children and squares and dotted regression line are non-NF1 children. **B.** Relative jump force (N/kg) and age (yrs). Triangles and solid regression line are NF1 children and squares and dotted regression line are non-NF1 children.

(Figure 2b). The adjusted means (95% confidence limits) of non-NF1 and NF1 children for relative jump power (W/kg), controlling for body mass and age, were 37.31 (34.14, 40.49) and 32.51 (29.34, 35.68), respectively (P=0.054); and relative jump force (N/kg), controlling for body mass, age and gender, were 25.79 (24.28, 27.30) and 21.12 (19.61, 22.63), respectively (P<0.0001). Although sex was not a significant covariate in the model predicting jump power, if we include it the significance between NF1 and controls drops from p=0.054 to p=0.060. There also was a significant difference in the EFI Z-scores between non-NF1and NF1 children (p=0.04) (Figure 3). The mean EFI Z-score for NF1 children was less than zero (p<0.001), while the mean EFI Z-score for non-NF1 children was not significantly different than zero (p=0.09). The lower EFI Z-score among NF1 children occurred at all ages (age-by-group interaction not significant).

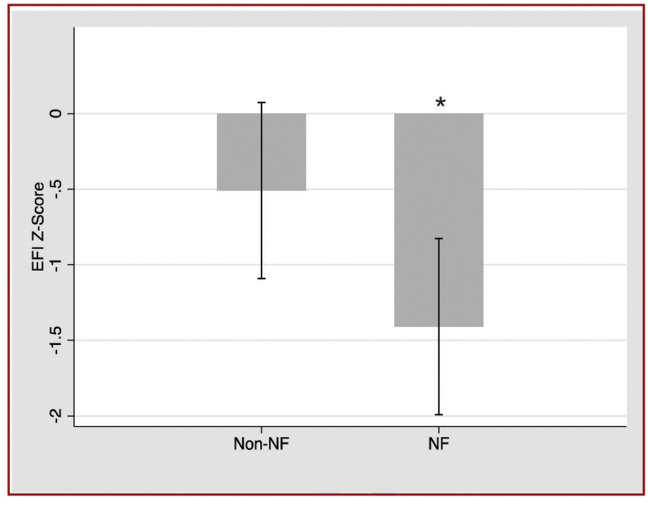

Figure 3. The EFI (Esslinger Fitness Score) Z-score for NF1 children is significantly lower (asterisk, p=0.04) than non-NF1 children. The error bars represent ±1 standard deviation (SD) away from the mean.

Discussion

The data presented here suggests there is no difference in total serum 25(OH)D and 1,25$(OH)_2$D concentrations between NF1 and non-NF1 children raised in the same environment. However, mean serum 25(OH)D concentration for the whole cohort was 16.1±5.5 ng/mL, which is below 20 ng/ml[33], a conservative level for vitamin D adequacy. To the best of our knowledge, five studies have specifically assessed vitamin D status in NF1 patients[1,4,5,7,16] and only two of those studies were focused on 25(OH)D concentrations in NF1 children[5,16]. Stevenson et al. measured 25(OH)D concentrations in 109 NF1 children (aged 2-17 years) and 218 healthy controls from the Intermountain West region of the USA and did not find a difference in mean 25(OH)D concentration (32±11 ng/mL and 34±12 ng/mL for NF1 and control, respectively). Only after dichotomization of the 25(OH)D concentrations did a significant association exist between NF1 children and lower 25(OH)D concentrations[16]. In a study conducted by Brunetti-Pierri et al., 25(OH)D concentrations were measured in a subset of 16 NF1 patients (aged 6-38 years) from Texas, USA, who had a mean lumbar BMD z-scores of -2.1. The mean 25(OH)D concentration was 21±5 ng/mL and approximately two-third of the patients had 25(OH)D concentrations <20.0 ng/mL; unfortunately, no controls were measured[5].

Vitamin D deficiency is common in England and could be explained by several factors, such as dietary intake, geographical location, or fortification regulations. Only a few foods, such as margarine, infant foods and breakfast cereals are fortified with vitamin D in the UK. This, together with paucity of vitamin D in natural foods might explain the low mean daily vitamin D intake for both NF1 and non-NF1 children in our study population (2.7±1.7 μg, 3.3±2.2 μg, respectively). The degree of 25(OH)D deficiency observed in this study population may also be due to environmental factors, such as geographical location or the amount of time spent in the sun. However, since unaffected siblings were used for the control group, many of the environmental effects that may have confounded this study and other studies were accounted for in the study design. Serum 25(OH)D concentration is the best indicator of vitamin D status, although the threshold levels indicating vitamin D deficiency are still highly debated. One reason we may not have observed a difference in serum 25(OH)D between NF1 and controls could be due to the overall low vitamin D status in both populations. It is possible that if we enrolled participants in the summer or fall, rather than the spring, we may have observed a difference. The differences in 25(OH)D concentrations seen in previous studies in NF1 adults may be a direct effect of increased Ras/MAPK signalling caused by NF1 mutations and/or secondary factors of the age-related manifestations of neurofibromas, such as lower sunlight exposure due to clothing choice and decreased outdoor activities because of increasing visibility of neurofibromas. In a study by Lammert et al., NF1 adults had significantly lower serum 25(OH)D concentrations when compared to controls[7]. An inverse correlation between the number of skin neurofibromas and low serum 25(OH)D concentrations was found, suggesting that low serum vitamin D concentrations were associated with the occurrence of neurofibromas.

High serum PTH concentrations are seen in vitamin D deficient individuals. We found NF1 children had higher serum PTH concentrations than non-NF1 children, while the mean 25(OH)D concentrations and dietary calcium intakes were not different. No differences were observed in serum concentrations of calcium, phosphate and alkaline phosphate concentrations between the two groups. Stevenson et al. observed one NF1 individual having PTH concentration above the assay reference interval of 15-75 pg/mL[16]. Brunetti-Pierri et al. also found high levels of PTH in 8 out of 16 NF1 patients, with a mean PTH of 44.1±24.8 pg/mL[5]. Collectively, these observations raise the possibility of an abnormality in secretion of PTH in NF1 patients. However, it is possible that our finding of higher mean PTH concentrations, especially with similar

25-OHD and 1,25-$(OH)_2$D concentrations, is spurious.

We used the GRFP to investigate muscle function in children affected with NF1. Although z-scores were obtained from the manufacturer's software, comparison of the NF1 and control data suggests that NF1 children have impaired jumping power and jumping force when compared to children not affected by NF1. Few studies have specifically assessed muscle function in NF1 patients[17,18]. Stevenson et al. used peripheral quantitative computed tomography (pQCT) to measure the total cross-sectional area of muscle and bone at the 66% site as well as the polar stress strain index, a measure of bone strength, in 40 NF1 children (aged 5-18 years) and 377 healthy control[17]. They found that NF1 children had decreased muscle and bone cross-sectional area compared to non-NF1 children. When the NF1 children were separated into two groups based on the presence of osseous abnormalities there were no significant differences between groups. In a study by Souza et al., muscular force per muscle area was assessed using a handgrip test instrument in 21 NF1 patients and 21 healthy controls (aged 7-60 years). They found the mean maximal voluntary muscular force (F_{area}) was greater in healthy males and females (9.8±3.2 $N.cm^{-2}$, 6.7±1.6 $N.cm^{-2}$, respectively) than NF1 males and females (5.7±2.6 $N.cm^2$, 5.7±1.9 $N.cm^2$, respectively)[18].

In this study, we did not use the pQCT to measure the muscle-bone relationship, but used the GRFP to measure muscle function. The GRFP assesses the maximal force developed in seconds, which is the type of force generated through anaerobic metabolism by type II fibers[34]. Recently, Kossler et al. suggested that neurofibromin is necessary for proper formation and maintenance of skeletal muscle[6]. The mechanostat theory postulates that increasing maximal muscle force during growth or in response to increased loading will have a positive effect on bone mass, size and strength. It has been shown that the development of muscle and bone during growth is influenced by gravitational force and physical activity, where the muscle forces create peak forces that act on bone[35]. Bone development is influenced by muscle forces acting on bones; since muscle forces are reduced in NF1, the bones are not experiencing full maximal loading.

Similar muscle function studies completed on individuals with cystic fibrosis (CF), found a decrease in peak jumping power and an increase in peak jumping force[36]. Some suggest this is explained by the lack of flexibility and the CF individuals compensate by increasing the force to generate tolerable power outputs[36]. Since we found peak jumping power and peak jumping force are decreased in NF1 children, compared to healthy controls, then this may suggest that the flexibility is there, but the overall ability to generate power is lower. If this is so, then the demands of everyday living on the NF1 body are lower and the body adjusts to a lower set point. This possible explanation fits the findings of Stevenson et al., where he found a decrease in the muscle and bone cross sectional area[17]; and Souza et al. where he found a decrease in F_{area}[18]. Therefore if there is less muscle, there is less bone; and the musculoskeletal system adapts to a lower set point, which produces 'weaker' muscles and the inability to produce the same force and power as healthy individuals.

The physical activity measurements between NF1 children and their unaffected siblings were not significantly different. Therefore, it is unlikely that decreased activity levels led to the decreased muscle function in our study population. However, an individual can participate in the same amount of hours of activity per day but generate different results. While the children do the same amount of activity, the typical peak force and peak power used during this activity may be different. This reasoning would help explain why we see a difference in the jumping parameters and not the physical activity levels.

One limitation of the current study is the use of a questionnaire to assess activity levels. Physical activity was not correlated with the jumping parameters, and it is possible that this questionnaire was not sensitive enough to accurately evaluate physical activity levels. The use of accelerometers may provide a better source of data on the type and duration of the physical activity conducted. It is known that physical activity is associated with muscle function and that physical activity can increase the mechanical loading placed on the muscle-bone unit. An exercise intervention study may be useful in determining the muscle-bone set-point, while increasing muscle strength in NF1 patients and possibly leading to improved bone health. This study investigated the role of 25(OH)D on the musculoskeletal system in NF1 children, which has not been previously studied. The decreased 25(OH)D concentrations seen in NF1 adults, but not in NF1 children, may be caused by a difference in 25(OH)D metabolism due to neurofibromas. We did not observe difference in 25(OH)D and we suggest that perhaps the *Nf1* gene may play an integral part in the metabolism of 25(OH)D, such as creating PTH resistance. Studies with larger sample sizes may be better suited to test the hypothesis that there is a 25(OH)D-by-*Nf1* interaction on serum PTH concentrations.

Another limitation is the relatively small sample size. As a result of the small sample size the statistical power is low. Based on our observed standard deviation for 25(OH)D and our available sample size, we would have been able to detect a difference between two means of 9 ng/ml at an alpha of 0.05 and 80% power. Given our results, we only had 11% power to detect a difference of 1.0 ng/ml between the two groups.

Our finding of no significant difference in 25(OH)D concentrations and lack of an association between muscle function and 25(OH)D indicate that low vitamin D status is not associated with the decreased muscle function observed in NF1 children. Since maintenance and function of the musculoskeletal system depends on both the skeleton and the musculature, the shown muscle weakness may contribute to some of the bone phenotypes seen NF1 patients. The relationship between the decreased muscle function and the known osseous abnormalities in NF1 patients is still unclear and further studies are needed to better understand the direct or indirect effect of the *Nf1* gene has on the muscle-bone unit.

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