

Duchenne Muscular Dystrophy and Bone Mineral Density

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Research Article

Keywords: Bone mineral density, bone health, Dual-energy X-ray absorptiometry, neuromuscular disorder, Pediatrics.

Posted Date: October 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-972105/v1>

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Abstract

Duchenne muscular dystrophy (DMD) patients are at risk of developing conditions that can compromise their bone health, such as fractures and walking impairment. We aimed to assess bone mineral density as a function of the age of DMD patients. The cross-sectional study included 48 patients distributed into four groups by age: G1 (5.1-9.2 years), G2 (9.3-10.7 years), G3 (11.2-15.9 years), and G4 (18-24.7 years). Lumbar spine and total body bone mineral density (BMD) measurements were performed with dual-energy X-ray absorptiometry. There was a moderate negative correlation between age and the lumbar spine BMD z-score ($p = 0.001$; $r = -0.45$) and a strong negative correlation between age and the total body BMD z-score ($p = 0.001$; $r = -0.79$). The lumbar spine BMD z-scores in G3 and G4 were significantly lower than those in G1 and G2 ($p < 0.05$). The total body BMD z-score in G3 was lower than those in G1 and G2 ($p < 0.05$), and in G4 was lower than those in the other groups ($p < 0.05$).

Conclusion: The older patients had lower lumbar spine and total body BMD z-score values than younger patients. Moreover, these values were negatively correlated with the age of the patients.

What Is Known:

- Patients with DMD may have impaired bone health.

What is New:

- A negative and moderate correlation between age and lumbar spine bone density of patients with DMD.
- A negative and strong correlation between age and total body bone density of patients with DMD.

Introduction

DMD is an autosomal-recessive disorder on the X chromosome that is defined by mutations in the dystrophin gene, leading to deficiency in dystrophin protein [1]. Dystrophin stabilizes skeletal and cardiac muscle by connecting actin in muscle fibers to the extracellular matrix; in the absence of dystrophin, recurrent muscle fiber injury leads to chronic inflammation, changes in body composition and weakness [2]. Additionally, patients with this condition have cardiorespiratory complications and impaired bone health with disease progression [3].

Corticosteroids are the only proven effective pharmacological treatment for DMD. Corticosteroids improve muscle strength and function in boys with DMD. Although that, glucocorticoid therapy predisposes for osteoporosis by decreasing bone formation and increasing bone resorption, growth and pubertal delay. The proximal femur and spinal vertebrae in boys with DMD show markedly reduced bone mineral density (BMD) [4].

The low BMD results in a high incidence of fragility fractures, which greatly impairs function and quality of life in population with DMD [5]. Vertebral fractures have significant consequences of chronic back pain and spinal deformities, scoliosis, while lower long bone fractures precipitate permanent loss of ambulation in 20–70% of DMD patients [6].

Most of studies on bone density in DMD patients did not divide the study participants by age, a factor that may be associated with bone mineral density. Furthermore, most of studies investigating the relationship between age and BMD in patients with this dystrophy analyzed the spine region or the total body exclusively [7–11].

Thus, the importance of assessing bone mineral density in DMD patients is evident. This parameter needs to be assessed to establish adequate bone health management and prevent fractures, spinal deviations, and bone pain. Therefore, this study aims to assess bone mineral density as a function of the age of DMD patients.

Methods

Research Design

This study is an observational, cross-sectional survey and was approved by the appropriate research ethics committee of the Hospital Universitário Onofre Lopes (HUOL) (CAAE: 57345516.0.0000.5292). The study included male DMD patients treated in the neurology outpatient clinic of the HUOL from October 2016 to October 2019.

The inclusion criteria were a confirmed genetic diagnosis of DMD and an age equal to or greater than five years. The use of bisphosphonates was the exclusion criterion. The study population (n=48) was distributed into the following four subgroups by age. Distribution by age group was performed since DMD is a progressive disease and the population evaluated is heterogeneous. In essence, group 1 (G1) was 5.1 to 9.2 years, group 2 (G2) was 9.3 to 10.7 years old, group 3 (G3) was 11.2 to 15.9 years old, and group 4 (G4) was 18.0 to 24.7 years old.

Anthropometric Assessment

Bodyweight (kg) and height (cm) were measured using an electronic scale (BK50F, Balmak, Brazil) and a stadiometer (ES2030, Sanny, Brazil), respectively. For participants who could not ambulate, a digital platform scale with a maximum capacity of 500 kg was used to measure their body weight (KN P/R500/50, KN Waagen, Brazil), and height was estimated by measuring their recumbent height using an inelastic tape, measured from the top of the head to the base of the feet [12]. BMI was calculated as the participant's body weight (kg) divided by their height squared (m^2).

This study determined weight-for-age, height-for-age, and BMI-for-age z-scores by WHO AnthroPlus v1.0.4[®] software. The anthropometric evaluation was based on the weight-for-age (5 to 10 years old),

height-for-age (5 to 19 years old), and BMI-for-age z-scores (5 to 19 years old) or BMI classification (>19 years old), as recommended by the World Health Organization [13–16]. In Table 1, some patients were not classified according to weight-for-age, as this parameter assesses individuals up to ten years of age. Therefore, the weight-for-age of the G3 and G4 were identified as not applicable.

Bone mineral density and patients' clinical history of bone fractures

Bone mineral density (BMD) was assessed in the lumbar spine (from vertebrae L1 to L4) and the total body. BMD was measured using a DXA (Lunar DPX NT, General Electric Company, USA) and pediatric evaluation software. A trained technician performed the examination, and the participants wore light clothing free of metal fasteners. The data are expressed as measurements in g/cm² and z-scores. For the analysis, a z-score of ≤ -2.0 indicated low bone mineral density, as suggested by the International Society for Clinical Densitometry (ISCD, 2013) for young, adolescent, and adult males [17].

Each participant or his guardian reported their clinical history of bone fractures during anamnesis. Information on the previous occurrence of bone fractures was collected.

Statistical analysis

The data obtained in the study were tabulated and analyzed with Intel SPSS v24[®] software. Additionally, the Shapiro-Wilk test was used to assess the normality of the data, and the data are presented as descriptive measures (mean \pm standard deviation, or median and interquartile range). Spearman correlation tests were also performed between the lumbar spine BMD z-score and age and between the total body BMD z-score and age. Then, the distribution of the study population in the four age groups was evaluated, and a one-way between-groups analysis of variance (ANOVA) was conducted to explore the effect of age on BMD values (z-score). Tukey's post-test differences were considered statistically significant when $p < 0.05$.

Results

In total, 48 participants aged between 5 to 24 years were recruited for this study. Regarding the weight-for-age z-score, most of G1 had an adequate index (91.7%), and 50% of the participants in G2 also had an adequate weight-for-age. For the height-for-age z-score, the percentages of participants with short stature were 25% (G1), 33.3% (G2), 58.3% (G3), and 66.7% (G4), indicating a percentage increase in the groups of older patients. Regarding BMI assessment, obesity/overweight was frequently diagnosed in all groups, with proportions of 25% in G1 and G3 and 33.4% in G2 and G4. Additionally, thinness/underweight was frequently diagnosed in all groups, except G1; 25% of G2 and 33.3% of G3 were thin, while 33.3% of G4 were underweight. The anthropometric characteristics of the study population are presented in Table 1.

In G1, as well as in G2, eight participants could walk independently (66.7%). In G3, only three were able to walk (25%), and in G4, none of the participants were able to walk. Younger participants frequently used glucocorticoids (prednisone): all in G1, 11 in G2, nine in G3, and only two in G4 used glucocorticoids.

Regarding the bone fractures clinical history, no participants in G1, one in G2, and one in G3 reported a history of fractures, both of which occurred in the femur. Four participants in G4 had bone fractures (33.3%) in different regions: the humerus, the femur, the fibula, and both the ankle and knee. No patient reported a clinical history of vertebral fractures. The clinical characteristics of the study population are presented in Table 1.

The moderate negative correlation between age and the BMD z-score of the lumbar spine was significant ($\rho = -0.50$; $p < 0.001$). The strong negative correlation between age and the BMD z-score of the total body BMD was significant ($\rho = -0.82$; $p < 0.001$) (Figure 1).

Results for the lumbar spine (L1 - L4) and total body bone mineral density of the participants included in the study are shown in Table 2. Considering the mean BMD z-score values for each group, the BMD of the spine was low in G3 and G4, and the mean total body BMD was low in G4 (z-score ≤ -2.0).

Regarding the lumbar spine, there was a statistically significant difference ($p < 0.05$) in the BMD z-scores across the four age groups [$F(3, 44) = 6.4$, $p = 0.001$]. The effect size, calculated as eta squared, was $\eta^2 = 0.304$. The post hoc comparisons using the Tukey HSD test indicated that the mean BMD z-score from G1 (M=-1.15; SD=1.08) did not differ significantly from that in any other group. The z-score for G2 (M=-0.56; SD=1.10) was significantly different from those of G3 (M=-2.38; SD=1.31) and G4 (M=-2.29; SD=1.35). However, the z-scores of G3 and G4 were not significantly different (Figure 2).

When evaluating the total body BMD, there was a statistically significant difference ($p < 0.05$) in the z-scores across the four age groups [$F(3,44) = 25.6$, $p < 0.001$]. The effect size, calculated as eta squared, was $\eta^2 = 0.636$. The post hoc comparisons using the Tukey HSD test indicated that the mean BMD z-score for G1 (M=-0.21; SD=0.74) did not differ significantly from that of G2 (M=-1.02; SD=0.66), but those of G3 (M=-1.96; SD=0.68) and G4 (M=-2.83; SD=0.98) differed from each other and from those of groups 1 and 2 (Figure 2).

Discussion

One of the main findings of this study was that age was negatively correlated with both lumbar spine and total body BMD in the study population. This finding was corroborated by the significantly smaller mean BMD z-score values, both in the lumbar spine and in the total body, in the participants aged 9.3 to 10.7 years (G2) than in the others indicating that this range is a determinant age range for bone loss. Additionally, the older DMD patients had lower (z-score ≤ -2.0) lumbar spine and total body BMD z-score values than others (> 11.2 years and > 18 years, respectively).

The correlation between age and the lumbar spine BMD z-scores was negative and moderate, while the correlation between age and the total body BMD z-score was negative and strong. Nutritional complications get worse with age [18]. This interrelationship may be related to the mechanical load of skeletal muscles on the bones of the entire body has decreased due to the loss of muscle function over time. Larson et al. [19] compared the lumbar spine and the proximal femur BMD in DMD patients, and

there was a significantly larger reduction in the femur BMD than in the lumbar spine BMD. Spine BMD was affected when the patients lost the ability to walk, while that of the proximal femur was low even before they lost the ability to walk.

In this study, the BMI results corroborated the susceptibility of DMD patients to overweight and obesity (Table 1). Adipose tissue is associated with the production of inflammatory cytokines, which can negatively affect bone metabolism. On the other hand, adipose tissue also has an indirect positive effect on bone metabolism by producing hormones, cytokines, and adipokines, such as circulating leptin and insulin. These substances stimulate bone formation [20]. Given that this tissue affects bone metabolism in different ways, it is suggested that the prevalence of overweight and obesity is not the main determining factor for bone loss in the population studied.

Regarding height, larger percentages of participants with short stature were observed in the older groups, reaching 66.7% in G4. The etiology of short stature in populations with DMD is still unclear. However, previous studies suggest that low levels of growth hormones and the loss of muscle tone are partly responsible for short statures in DMD patients [21]. Moreover, height outcomes can be predicted by genetics. Distal deletions and central mutations of the DMD gene are associated with short stature [18].

The weaker correlation between age and the BMD z-score of the lumbar spine compared with that of the total body may be explained by the skeleton attempting to adapt to the gravitational load associated with the excess weight of participants [9]. DMD patients are susceptible to overweight and obesity due to the prolonged use of glucocorticoids, decreased mobility, and reduced energy expenditure, limiting their ability to perform physical activity [22].

The results of our study indicated that older DMD patients might have a more marked impairment in BMD than do younger patients, both in the lumbar spine and in the total body. Other studies have shown results that are like ours. In the study by Mayo et al. [10], there was low BMD in patients over 11 years of age. In the groups with average ages of 11 years and 14 years, the BMD z-scores of the lumbar spine were -2.4 and -3.6, which differed considerably. Regarding BMD of the total body, King et al. [7] concluded that DMD patients aged between 11 and 17 years had low BMD, with z-scores ranging between -3.0 and -4.0, which were lower than those in our study.

In addition, in our study, most of the participants in G4 (83.3%) had low BMD, which may be associated with disease progression. The damage to muscle fibers present in DMD patients is related to chronic inflammation of the muscles and the release of inflammatory cytokines, which impair the function of the cells involved in the formation of bone tissue. This damage can be a contributing factor to bone loss in DMD patients [11].

In addition, other factors are also associated with low BMD in DMD patients. The use of glucocorticoids has already been shown to produce oxidative stress in multiple tissues, including bone. There is an association between continued use of glucocorticoids and decreased bone formation and increased bone resorption, resulting in the increased renal excretion of calcium and reduced gastrointestinal absorption.

The continuous use of glucocorticoids is also related to pubertal delay and weight gain. These factors are associated with the incidence of fractures, osteoporosis, and the early loss of walking [8, 23–26]. The use of testosterone can lead to pubertal induction in DMD patients who use glucocorticoids in the long term and consequently improve bone mass accrual and bone healthy [26].

In G3, 25% of the participants used glucocorticoids. With the progression of the disease and muscle loss, the adverse effects on patient health caused by the chronic use of glucocorticoids may be more clinically relevant than are the potential benefits [27]. G3 and G4 showed significantly lower BMD z-score values for both, lumbar spine and total body, than other groups differing by age (G1 and G2). This finding may be related to adolescence from the G2 ages to the G3 ages, when peak bone mass occurs in healthy patients [28].

The use of glucocorticoids inhibits the production of factors that regulate the hypothalamic-pituitary axis during puberty, leading to a decrease in growth hormone production, resulting in pubertal delay. Most participants in the age groups G1 and G2 use glucocorticoids, and pubertal delay caused by these drugs is one of the main concerns reported by DMD patients, leading to psychosocial and bone health consequences [28]. In general, adolescents who do not have chronic diseases but have delayed sexual maturation have a reduced peak bone mass [29, 30].

Decreases in BMD in the lumbar spine and the total body are related to an increase in the risk of vertebral and long bone fractures, respectively, in DMD patients. Vertebral fractures cause spinal deformity, chronic pain, difficulty sitting, and decreased lung function, and long bone fractures may be associated with the permanent loss of ambulation [31].

According to a study by Ma et al. [32], approximately 20 to 60% of boys with DMD experienced low-trauma extremity fractures (usually of the femur or distal tibia), making it difficult or impossible for them to walk. Additionally, the risk of fractures increases as the DMD patients' survival increases [18]. In agreement with the results of previous studies, the older patients in this study (G4) had a higher prevalence of reported bone fractures (33.3%) than other groups (Table 1), which may be associated with the total body BMD z-score being lower in this group (Table 2). Among these fractures, there is a predominance of long bone fractures. No patient reported a clinical history of vertebral fractures. Vertebral fractures can be asymptomatic in their early phases and thus undetected in the absence of monitoring [33]. Among the four groups, G3 and G4 had the lowest percentages of participants who could walk independently and the lowest BMD z-scores, both in the lumbar spine and in the total body.

The early loss of ambulation caused by the progression of the disease gradually decreases the physical activity of these participants. The skeletal system has great modeling and remodeling dynamics, as it adjusts its properties in response to the load applied to it. Therefore, when there is a decrease in muscle use, bone loss occurs [9, 34]. Studies have shown that decreased muscle function and aging directly contribute to decreased bone density in patients with DMD [11], which is consistent with our results.

One of the strengths of our study is the sample size, which is larger than those of other studies that have also evaluated bone density in DMD patients [7, 8, 32]. Additionally, most studies have exclusively evaluated the lumbar spine or total body BMD, and in our study, BMD was evaluated in both areas.

The study has some limitations. As it is cross-sectional, it was impossible to analyze the cause-effects among glucocorticoids, walking ability, BMD, and disease progression. In addition, it is important to assess BMD associated with the assessment of bone fractures by imaging tests. Thus, longitudinal design studies should be conducted to assess the influence of these variables.

It is concluded that there is a moderate negative correlation between age and the BMD of the lumbar spine and a strong negative correlation between age and the BMD of the total body in the study population. Moreover, the older participants had a lower lumbar spine and total body BMD than younger ones. The results reinforce the relevance of assessing bone health in these patients at young ages to establish the most appropriate therapy and prevent bone fractures, as well as delay the early loss of walking and occurrence of fractures.

Abbreviations

BMD, bone mineral density; BMI, body mass index; cm, centimeters; DMD, Duchenne muscular dystrophy; DXA, dual-energy X-ray absorptiometry; G1; Group 1; G2, Group 2; G3, Group 3; G4, Group 4; HUOL, Hospital Universitário Onofre Lopes; UFRN, Universidade Federal do Rio Grande do Norte; GC, glucocorticoids; Kg, kilogram; m, meters.

Declarations

Acknowledgments: The authors thank Mrs. Ione Oliveira de Medeiros for her technical assistance regarding DXA.

Funding: The present study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) under process numbers 422667/2016-1 and 302298/2017-7. CNPq did not play a role in the design, analysis, or writing of this article.

Conflicts of interest/Competing interests: None of the authors has any conflict of interest to disclose.

Availability of data and material: Not applicable.

Code availability (software application or custom code): Not applicable.

Authors' contributions: All authors contributed to the execution of this study. L.C.L. wrote the manuscript. E.C.G. and S.H.L.V. contributed to the study design and data analysis. L.C.L., E.C.G., T.A.C., and B.G.M.A. contributed to the subject briefings and data collection. E.C.G, J.B.N, and S.H.L.V contributed to the interpretation of the data and the critical review of the manuscript. All authors approved the final version of the manuscript.

Ethics approval: The Research Ethics Committee of Hospital Universitário Onofre Lopes (CAAE: 57345516.0.0000.5292).

Consent to participate: Yes.

Consent for publication: Not applicable.

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Tables

Table 1. Characterization of Duchenne muscular dystrophy patients, according to age groups (n=48).

Variables	G1 (n=12)	G2 (n=12)	G3 (n=12)	G4 (n=12)
	5.1 to 9.2 years	9.3 to 10.7 years	11.2 to 15.9 years	18 to 24.7 years
Age (years)*	7.2 ± 1.3	10.0 ± 0.6	13.7 ± 1.7	20.5 ± 2.3
Weight (kg) [†]	21.9 (18.9, 26.7)	23.8 (21.9, 29.4)	34.9 (25.0, 50.0)	56.9 (42.1, 63.5)
Weight-for-age (Z-score) ^{*,‡}	-0.36 ± 1.28	-0.50 ± 2.70	NA	NA
Underweight (n (%))	1 (8.3)	2 (33.3)	NA	NA
Normal weight (n (%))	11 (91.7)	3 (50)	NA	NA
Overweight (n (%))	0 (0)	1 (16.7)	NA	NA
Height (cm)*	118 ± 14	130 ± 9	142 ± 8	153 ± 10
Height-for-age (Z-score) ^{*,§}	-0.95 ± 1.77	-1.31 ± 1.43	-2.37 ± 1.17	-3.15 ± 1.69
Short stature (n (%))	3 (25.0)	4 (33.3)	7 (58.3)	2 (66.7)
Adequate stature (n (%))	9 (75.0)	8 (66.7)	5 (41.7)	1 (33.3)
BMI (kg/m ²) ^{†,¶}	15.5 (15.1, 17.2)	15.1 (13.8, 17.8)	16.9 (14.1, 22.1)	22.7 (17.4, 27.0)
BMI-for-age (Z-score) ^{*,§}	0.39 ± 0.97	-0.47 ± 2.38	-1.27 ± 3.19	1.89 ± 1.41
Thinness / Underweight (n (%))	0 (0) 9 (75.0)	3 (25.0) 5 (41.6)	4 (33.3) 5 (41.7)	4 (33.3) 4 (33.3)
Eutrophy (n (%))	2 (16.7)	2 (16.7)	2 (16.7)	2 (16.7)
Overweight (n (%))	1 (8.3)	2 (16.7)	1 (8.3)	2 (16.7)
Obesity (n (%))				
Use of glucocorticoids (n (%))	12 (100)	11 (91.7)	9 (75)	2 (16.7)
Independent walking (n (%))	8 (66.7)	8 (66.7)	3 (25)	0 (0)
Bone fractures (n (%))	0 (0)	1 (8.3)	1 (8.3)	4 (33.3)

*Data presented as Mean \pm Standard Deviation; [†] Date presented as Median (Q1, Q3); [‡] classification for individuals aged 5 to 10 years (n=18); [§] classification for individuals aged 5 to 19 years (n=39); [¶] classification for individuals aged > 19 years (n=9); NA: Not applicable; BMI, body mass index.

Table 2. Comparison of bone mineral density (BMD) of the lumbar spine (L1 - L4) and total body of Duchenne muscular dystrophy patients, according to age groups (n=48).

BMD	G1	G2	G3	G4	p	η^2
	5.1 to 9.2 years	9.3 to 10.7 years	11.2 to 15.9 years	18 to 24.7 years		
Lumbar spine BMD	-1.15 \pm	-0.56 \pm	-2.38 \pm	-2.29 \pm	0.001	0.304
L1-L4 (z-score)	1.10 ^{a,b,c,d}	1.10 ^{a,b}	1.30 ^{a,c,d}	1.40 ^{a,c,d}		
Total body BMD (z-score)	-0.21 \pm 0.70 ^a	- 1.01 \pm 0.70 ^a	-1.96 \pm 0.70 ^b	-2.83 \pm 1.00 ^c	<0.001	0.636

Analysis of Variance Test (ANOVA) with Tukey's post-test. Different superscript letters indicate a significant difference between groups (p <0.05). η^2 = eta squared.

Figures

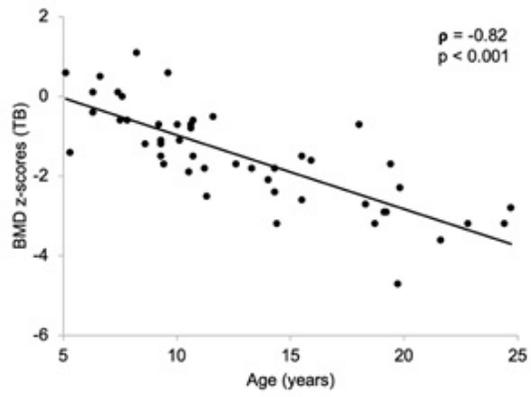
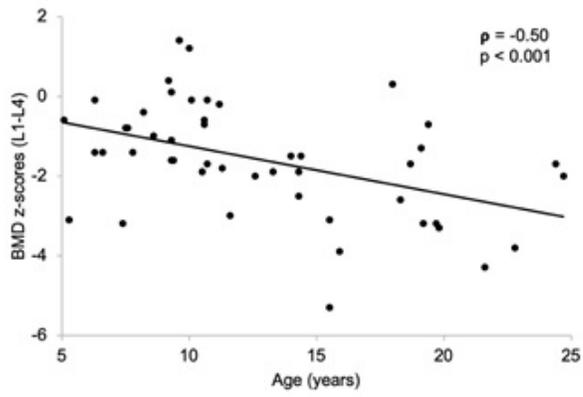


Figure 1

Correlations between age and the BMD z-score of the lumbar spine (L1-L4) and between age and the BMD z-score of the total body (TB).

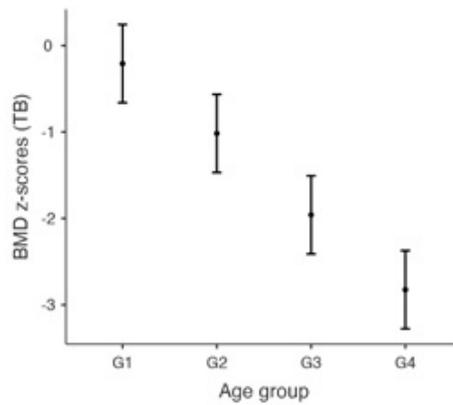
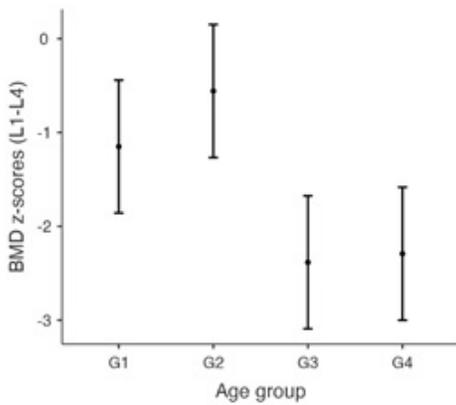


Figure 2

The mean and 95% confidence intervals of the BMD z-scores for the lumbar spine and total body of the patients with Duchenne muscular dystrophy, stratified by age group (G1 = 5.1 to 9.2 years; G2 = 9.3 to 10.7 years; G3 = 11.2 to 15.9 years; G4 = 18 to 24.7 years).