Received: 20 October 2021 | Revised: 19 April 2022 | Accepted: 23 April 2022

DOI: 10.1002/mus.27565

CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

Body composition and myokines in a cohort of patients with Becker muscular dystrophy

Andrea Barp MD[1] | Elena Carraro MD[1] | Giovanni Goggi MD[2,3] | Andrea Lizio MSc[1] | Alice Zanolini MD[1] | Carmelo Messina MD, PhD[4] | Silvia Perego MSc[5] | Chiara Verdelli MSc[6] | Giovanni Lombardi PhD[5,7] | Valeria Ada Sansone MD, PhD[1#] | Sabrina Corbetta MD, PhD[2,8#]

[1]Neurorehabilitation Unit, NeMO Clinical Center, University of Milan, Milan, Italy

[2]Endocrinology and Diabetology Service, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

[3]Department of Biotechnology and Translational Medicine, University of Milan, Milan, Italy

[4]IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

[5]Laboratory of Experimental Biochemistry & Molecular Biology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

[6]Laboratory of Experimental Endocrinology, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

[7]Department of Athletics, Strength and Conditioning, Poznań University of Physical Education, Poznań, Poland

[8]Department of Biomedical, Surgery and Dental Sciences, University of Milan, Milan, Italy

Correspondence
Andrea Barp, MD, Neurorehabilitation Unit, NeMO Clinical Center, University of Milan, Piazza Ospedale Maggiore 3, 20162 Milan, Italy.
Email: andrea.barp@centrocliniconemo.it

Abstract

Introduction/Aims: Becker muscular dystrophy (BMD) is an X-linked disease leading to muscle wasting and weakness. The decrease in lean body mass (LBM) in Duchenne muscular dystrophy, has shown correlation with loss of muscle function and bone density (BD). Myokines (including irisin) are hormones secreted by skeletal muscle that allow crosstalk between muscle and bone. The present study analyzed body composition and circulating myokine levels in a cohort of BMD patients; moreover, the association between dual energy X-ray absorptiometry (DXA) parameters, functional motor assessments, and myokine levels was investigated.

Methods: All patients underwent DXA, blood samples for myokine assays, and functional motor assessments. A group of healthy controls (HCs) was also included.

Results: Thirty BMD patients, median age at evaluation 36.0 y [26.0–41.0], were included. Twenty-nine patients underwent whole-body DXA. Median value of total body Z-score was −0.70. The prevalence of low skeletal muscle mass defined as appendicular skeletal muscle mass index (ASMMI) < 7.59 kg/m^2 was 83%. Irisin levels were significantly lower in BMD compared to HCs ($p = .03$). All DXA parameters showed significant correlation with the functional motor assessments, in particular the h^2-standardized lean mass lower limb index ($p = .0006$); h^2-standardized total fat mass showed negative correlations with North Star Ambulatory Assessment and 6 min walk test ($p = .03$).

Discussion: DXA is a useful tool to evaluate body composition in BMD patients; the decrease in BD and LBM is associated with a reduction of motor function in BMD.

Abbreviations: 10MRT, 10 meter run test; 10MWT, 10 meter walk test; 6MWT, 6 minute walk test; ALM, appendicular lean mass; ASMMI, appendicular skeletal muscle mass index; AUC, area under the curve; BD, bone density; BDNF, brain-derived neurotrophic factor; BMC, bone mineral content; BMD, Becker muscular dystrophy; BMI, body mass index; CTX-I, carboxy-terminal cross-linking telopeptide of type I collagen; DMD, Duchenne muscular dystrophy; DXA, dual energy X-ray absorptiometry; FDR, false discovery rate; FNDC5, fibronectin type III domain-containing 5; FSH, follicle-stimulating hormone; FSTL1, follistatin-like 1; FVC, forced vital capacity; HCs, healthy controls; HMGB1, high-mobility group box 1; LBM, lean body mass; LH, luteinizing hormone; mMRI, muscle magnetic resonance imaging; NSAA, North Star Ambulatory Assessment; PTH, parathormone; SHBG, sex hormone binding globulin; TFTs, Timed Function Tests.

# These authors contributed equally at this work.

KEYWORDS
Becker muscular dystrophy, bone mineral density, Duchenne muscular dystrophy, DXA, Irisin

1 | INTRODUCTION

Becker muscular dystrophy (BMD) is an X-linked disease caused, in most cases, by in-frame pathogenic variants in the sequence of the dystrophin gene (*DMD*), leading to lower but detectable dystrophin expression in muscle fibers.[1] The dystrophin deficiency leads to a progressive loss of skeletal muscle fibers that are replaced by adipose tissue, which may exert a negative impact on bone health.[2] A previous study has demonstrated that bone density (BD), assessed by *Z*-score, is reduced in patients with Duchenne muscular dystrophy (DMD) following ambulation loss,[3] resulting in the observation that older DMD patients have more significant bone health impairment than younger individuals who are still able to walk. In myopathies, fat body mass and lean body mass (LBM) (which mainly represents skeletal muscle,[4] measured by dual energy X-ray absorptiometry [DXA]) are increased and reduced, respectively.[5] Individuals with DMD have shown significantly altered whole-body composition compared to controls[4-6]; more specifically, the decrease in LBM in DMD correlates with loss of muscle function,[4] a decrease in regional lean mass, an increase in regional fat mass, and a decrease in strength.[7]

Myokines (such as irisin, brain-derived neurotrophic factor [BDNF], follistatin-like 1 [FSTL1], and high-mobility group box 1 [HMGB1]) are protein hormones secreted by skeletal muscle[8] that are involved in crosstalk with many different organs, such as bone, brain, and adipose tissue. Irisin is released by the cleavage of the extracellular domain of the transmembrane receptor fibronectin type III domain-containing 5 (FNDC5), mainly expressed in skeletal muscle. Previous studies demonstrated that irisin synthesis increases during myogenic differentiation of human myocites in vitro, supporting its myogenic potential.[9] In agreement with this hypothesis, irisin levels increase with physical activity and decrease in sedentary individuals, those with sarcopenia, and in those with a history of osteoporotic fractures.[10-13] These data suggest that irisin may represent a sensitive marker for muscle weakness and atrophy and, therefore, raise the interesting possibility that it could be a potential biomarker for muscle dysfunction.[14] In line with this hypothesis, a recent study[15] reported that patients affected by myotonic dystrophy types 1 and 2 have irisin levels significantly lower than healthy controls (HCs).

The primary aim of the study was to describe the body composition (by DXA) and the circulating myokine levels in a cohort of BMD patients, and to investigate the association between DXA parameters, functional motor assessments, and myokine levels.

2 | METHODS

2.1 | Study population

The present cross-sectional study involved a cohort of molecularly confirmed BMD patients from our neuromuscular center, according to the following inclusion criteria: age ≥ 18 y old and ≤65 y old; independent ambulation; baseline forced vital capacity (FVC) >80% (of predicted value); no evidence of dilated cardiomyopathy (left ventricular ejection fraction >50%); no significant cognitive impairment. Exclusion criteria were non-invasive ventilation or tracheostomy; daily use of wheelchair.

Twenty HCs, selected from the clinical staff, were also recruited. HCs included male subjects (age ≥ 18 y old and ≤ 65 y old) without any significant medical history, and taking no medication; HCs underwent blood tests for myokine levels only. All the participants (both BMD patients and HCs) provided written informed consent. The local ethical committee approved the study protocol (NM46-MaYBE_BMD). The study complied with the Declaration of Helsinki.

2.2 | Body composition assessment (DXA)

All patients underwent DXA using Hologic QDR-Discovery W densitometer (Hologic Inc., Bedford, MA, USA) in the same referral center (IRCCS Istituto Ortopedico Galeazzi in Milano, Italy).

BD and body composition of whole-body, total hip, distal femur (sites R1, R2, and R3), radius, and lumbar spine (L1–L4) were measured using DXA. BD was reported in grams per square centimeter (g/cm2). A Hologic software generated automatically age-matched Z-scores, that were used as normative data. The whole-body Z-scores were used to compare BD with age-matched normal ranges. A *Z*-score < −2.0 was used to identify subjects with BD below the expected range for age, whereas a *Z*-score ≥ −2.0 was indicative of BD within expected normal range for age.[16] The evaluations of body composition included the estimation of LBM, lean regional mass (in grams), and age-matched percentiles for LBM.[17] LBM was used as an estimation of muscle mass.[18] To obtain relative muscle mass, LBM percentage was calculated as LBM divided by total body mass in percentage,[19] and the appendicular lean mass (ALM) as the sum of lean mass of both upper limbs and lower limbs divided by total body mass in percentage.[20] To obtain absolute muscle mass, the appendicular skeletal muscle mass index (ASMMI) was calculated as ALM divided by height squared,[21] and the total LBM was directly derived from DXA in kilograms. In men, ASMMI drops gradually from the age group 20 to 29 y to the age group 60 to 69 y, and subsequently remains stable in older subjects.[22] Based on the 15th percentile (or 1 SD below the mean) of the ASMMI for the young adult reference Italian population, the cutoff for sarcopenia is 7.59 kg/m^2 in men.[22] Total fat mass was calculated automatically by DXA, which is able to discriminate different tissues and obtain an estimate of the fat mass in grams of all of the body compartments, using a double source of X-ray energy.

Anthropometric measures, including height, weight, and body mass index (BMI) were also collected for all subjects.

2.3 | Functional motor assessments

The clinical evaluation protocol included the following assessments: 6 min walk test (6MWT),[23] North Star Ambulatory Assessment (NSAA),[24] and Timed Function Tests (TFTs; [10 m walk test {10 MWT}, 10 m run test {10 MRT}, rise from floor {Gowers}, climb four steps]).[25] Trained evaluators performed the assessments as previously described.

2.4 | Laboratory assessment

Plasma irisin levels were measured by an irisin (FNDC5) (extracellular domain molecule: epitope 16–127) assay kit (Phoenix Pharmaceuticals, CA, USA; sensitivity 1.3 ng/mL, range 0.1–1000 ng/mL and linear range 1.29–27.5 ng/mL). The antibody used in this kit recognizes recombinant full length irisin, irisin (100%) and recombinant FNDC5, isoform 4 (9%), but not the irisin precursor C-terminal 48-mer FNDC5 and irisin. BDNF, FSTL1, and HMGB1were assessed at the same time in the same aliquot by multiplex fluorescent bead-based Luminex assay (Bio-Techne, Minneapolis, MN; USA) according to the manufacturer's guidelines.

Mineral and bone metabolisms were assessed in all BMD patients through the measurement of serum albumin-corrected calcium, serum phosphate, plasma parathormone (PTH), serum 25 hydroxyvitamin D (25OHD), total alkaline phosphatase, and serum carboxy-terminal cross-linking telopeptide of type I collagen (CTX-I) by routine assays. In addition, Leydig cell function was assessed measuring luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, sex hormone binding globulin (SHBG), and estradiol.

2.5 | Statistical analysis

For each variable, Shapiro–Wilk test was used to assess the normality of the distribution and Levene's test was used to assess the homogeneity of the variance. Data were summarized in text and tables as median and interquartile range for continuous variables, and number and percentage for categorical ones. For group comparison of myokine levels between BMD patients and HCs, the non-parametric Mann–Whitney U test was used to determine statistical significance. The discriminatory ability of the myokines between BMD and HCs was evaluated using the area under the curve (AUC) and considered as clinically relevant if the AUC resulted in equal to or greater than 0.70.[26]

The associations between the pre-selected DXA parameters and the performed motor evaluations were assessed using Spearman's rank correlation coefficient. Strength of correlations was assessed in accordance with Schober and colleagues guidelines.[27] The r^2 was used to assess the goodness-of-fit of each simple linear regression model considering the percentage of the variance in each motor evaluation explained by each DXA parameter. A p-value (two-tailed) <.05 was considered as statistically significant and was adjusted for multiple testing using the false discovery rate (FDR) approach as appropriate. All statistical analyses were performed using SAS 9.3 (SAS Institute, Inc, Cary, NC) software.

3 | RESULTS

3.1 | Patients and genetic profile

Thirty BMD patients with a median age at evaluation of 36.0 y [26.0–41.0] and 20 HCs with a median age of 34.0 y [33.0–38.0] (p-value: .7902) were included. The median BMI of the BMD cohort was 24.96 kg/m^2 [21.14–28.34]. None of the patients were currently taking steroids, but 15 were taking cholecalciferol or calcifediol.

Twenty-six (87%) patients harbored pathogenic variants in the *DMD* gene characterized by in-frame exon deletions (16 "del 45-x", 3 del 10–12, 1 del 11–24, 1 del 47, 2 del 48, 3 del "48-x"), one patient (3%) harbored exon duplications (dup 14–18), and three patients had nonsense variants (p.Gln1099*, p.Glu1343*, p.Tyr1274*). The patient carrying the nonsense variant p.Gln1099* underwent cardiac transplantation at 14 y old due to a severe dilated cardiomyopathy; at the time of enrolment, he was on an immunosuppressive therapy with tacrolimus and mycophenolate.

3.2 | DXA parameters

DXA parameters of the BMD cohort are shown in Table 1. Twenty-nine patients underwent whole-body DXA.

The median value of the total body Z-score of the present cohort was above the threshold for osteopenia diagnosis; 7 of 29 patients (24%) showed very low total Z-scores (<−2 SD): 3 patients harboring deletions of exons 10–12 in *DMD*, 3 harboring deletions of exons 45–48, and 1 with a duplication of exons 14–18. Of 29 patients, 7 carrying the "45-x" deletion in *DMD* gene (45–47, 45–48, 45–53 and 45–55) had mildly reduced Z-scores (−2 ≤ Z-score ≤ −1 SD); conversely, patients harboring del 48–49, del 48–51, single exon deletions (del 47 or del 48) and nonsense variants (p.Gln1099* and p.Glu1343*) were found to have Z-scores in normal range (>−1.0 SD).

The prevalence of low muscle mass, diagnosed as ASMMI <7.59 kg/m^2,[25] was 83% (24 of 29 patients).

BMD patients' ages correlated neither with total body BD (and total Z-score) nor with LBM. Mineral metabolism and Leydig cell function were investigated in all patients to exclude major hormonal diseases (hyperparathyroidism, hypovitaminosis D, osteomalacia, hypogonadism) that may cause a reduction of BD and skeletal muscle. Circulating bone and mineral markers were within the normal ranges in most patients, except for a mild hyperparathyroidism secondary to hypovitaminosis D in two. Leydig cell function was conserved in all patients (Supporting Information Table S1, which is available online).

3.3 | Circulating myokines levels

Table 2 summarizes the myokine levels, comparing BMD patients with a cohort of HCs. No significant differences in the levels of BDNF, FSTL1, and HMBG1 emerged between the two cohorts. Conversely BMD patients showed a significantly lower median irisin level than HCs. Despite this, the AUC index used for the discrimination of the two cohorts based on irisin levels was 0.6833, considered to be below the acceptable level.[26]

3.4 | Correlations of DXA parameters with functional motor assessments and myokine levels

All of the patients included in the study were able to perform the 6MWT and the NSAA protocol with median values of 382.0 m and 27.0, respectively. At the time of evaluation, 16 patients were not able to run, and 5 patients were not able to arise from the floor. In Table 1, median time values (seconds) are reported in detail.

Tables 3 and 4 show the association between the DXA parameters and the functional motor assessments. Most of the body composition parameters included in the analysis showed a significant moderate to strong correlation with the motor evaluations: in particular, the h^2-standardized lean mass lower limb index reported the highest r-squared value among all of the associations with each motor evaluation, emphasizing that the h^2-standardized lean mass lower limb index represents the DXA index that accounts for the highest percentage of the variability in each motor evaluation. Figure 1 shows the associations between the h^2-standardized lean mass lower limb index and each functional motor assessment. Conversely, parameters related to fat mass showed a less significant relationship with motor evaluations; in particular, total fat mass correlated negatively only with 6MWT, while h^2-standardized fat mass showed a weaker correlation with all of the functional motor scores except for the score "climb 4 steps."

Regarding the correlations between the total Z-scores and the functional motor assessments, BMD patients with total *Z*-score < −2 had significantly lower NSAA median values than patients with higher scores (≥ −2)(12.0 [10.0–13.0] vs. 31.5 [16.0–33.0], $p = .016$).

Analysis of association between DXA parameters and myokine levels showed a significant negative correlation between BD of the lower limbs and irisin levels; indeed patients with reduced BD values of the lower limbs had higher serum irisin levels compared to patients with higher BD values (Figure 2). No significant correlations were found between LBM and irisin, as well as between DXA parameters and the other myokines analyzed.

TABLE 1 DXA parameters and functional motor assessments of the BMD cohort

Body composition ($n = 29$)	
LBM (g)	**46 169.30 [39,500.50–51,120.50]**
LBM/height^2 (kg/m^2)	15.64 [13.07–16.69]
Total fat mass (g)	25 376.90 [16,581.30–32,210.80]
Total fat mass/height^2 (kg/m^2)	8.19 [5.80–11.26]
App. Lean mass/height^2 (kg/m^2)	6.77 [5.25–8.00]
Lean mass lower limbs (g)	13 458.10 [10,317.00–17,155.50]
Lean mass lower limbs/height^2 (g/m^2)	4594.94 [3550.12–5735.79]
LBM + BMC (g)	48,571.27 [41,467.74–53,741.05]
LBM + BMC/height^2 (g/m^2)	16,482.90 [13,801.34–17,548.10]
BD lower limbs (g/cm^2)	2.30 [19.19–24.40]
Z-score	−0.70 [−1.90 to −0.10]
Functional motor assessments	
6MWT (m) ($n = 30$)	382.00 [300.00–509.00]
NSAA total score ($n = 30$)	27.00 [12.00–33.00]
Climbing 4 steps (s) ($n = 28$)	3.51 [2.51–9.47]
10MWT (s) ($n = 28$)	6.49 [5.44–9.67]
10MRT (s) ($n = 14$)	3.88 [2.89–5.50]
Gowers (time) (s) ($n = 25$)	3.91 [3.11–8.50]

4 | DISCUSSION

The present study demonstrated that a reduction in LBM assessed by DXA correlates with poorer motor functional status, in agreement with previous data on DMD.[4-6,28,29] This correlation is particularly strong when we consider the lean mass of the lower limbs (the h^2-standardized lean mass lower limb index); probably because both the 6MWT and the NSAA explore mainly lower limb function. The significant correlation between BD of lower limbs and 6MWT/NSAA further strengthens the hypothesis that a loss of LBM (due to muscle weakness) results in a loss of muscle strength that consequently leads to a reduced bone load, that ultimately contributes to osteopenia/osteoporosis. A previous study by Jacques and colleagues did not show a significant reduction of LBM in BMD patients compared to a control group;

TABLE 2 Comparison of circulating irisin, BDNF, FSTL1, and HMGB1 levels between BMD patients and HCs

	BMD patients		HCs		
Myokine (ng/mL)	***n***	**Value**	***n***	**Value**	***p*-value**
Irisin	30	12.15 [10.60–13.10]	20	12.90 [12.10–15.55]	**0.0300**
BDNF	23	10.63 [8.44–12.35]	13	13.08 [10.85–13.90]	0.0605
FSTL1	26	8.69 [5.23–11.75]	18	7.09 [4.47–12.61]	0.5748
HMGB1	30	2.60 [1.33–4.05]	9	2.86 [2.08–4.63]	0.2340

TABLE 3 Associations between DXA parameters and functional motor assessments in the BMD cohort, part 1

	6MWT			NSAA total score			Climb 4 steps		
	Rho	*p*-value*	R^2	Rho	*p*-value*	R^2	Rho	*p*-value*	R^2
LBM	0.47	**0.0191**	0.1948	0.58	**0.0015**	0.2713	−0.51	**0.0101**	0.1112
LBM/height^2	0.46	**0.0191**	0.2138	0.62	**0.0006**	0.3062	−0.51	**0.0101**	0.0862
Total fat mass	−0.37	**0.0499**	0.1350	−0.35	0.0745	–	0.22	0.2599	–
Total fat mass/height^2	−0.40	**0.0363**	0.1608	−0.42	**0.0302**	0.1144	0.31	0.1508	–
App. Lean mass/height^2	0.60	**0.0018**	0.3658	0.76	**<0.0001**	0.4836	−0.68	**0.0004**	0.1791
Lean mass lower limbs	0.65	**0.0008**	0.4289	0.81	**<0.0001**	0.5291	−0.75	**<0.0001**	0.2401
Lean mass lower limbs/height^2	0.67	**0.0008**	**0.4429**	0.82	**<0.0001**	**0.5882**	−0.76	**<0.0001**	**0.2417**
LBM + BMC	0.47	**0.0191**	0.2231	0.58	**0.0015**	0.2699	−0.52	**0.0101**	0.1118
LBM + BMC/height^2	0.46	**0.0191**	0.2135	0.62	**0.0006**	0.3046	−0.50	**0.0101**	0.0865
BD lower limbs	0.62	**0.0017**	0.3798	0.68	**0.0002**	0.4446	−0.58	**0.0032**	0.1668
Z-score	0.45	**0.0191**	0.2049	0.65	**0.0003**	0.2773	−0.64	**0.0010**	0.1203

Note: Bold, statistically significant correlations; bold and underlined, highest r^2.
*The FDR adjusted *p*-values.

TABLE 4 Associations between DXA parameters and functional motor assessments in the BMD cohort, part 2

	10MWT (time)			10MRT (time)			Rise from floor (time)		
	Rho	*p*-value*	R^2	Rho	*p*-value*	R^2	Rho	*p*-value*	R^2
LBM	−0.52	**0.0078**	0.1988	−0.65	**0.0213**	0.2200	−0.62	**0.0013**	0.1226
LBM/height^2	−0.52	**0.0078**	0.1930	−0.59	**0.0364**	0.2225	−0.67	**0.0004**	0.1608
Total fat mass	0.36	0.0664	–	0.50	0.0694	–	0.35	0.0833	–
Total fat mass/height^2	0.44	**0.0270**	0.1462	0.57	**0.0412**	0.3465	0.43	0.0399	0.2092
App. Lean mass/height^2	−0.70	**0.0003**	0.3407	−0.88	**0.0008**	0.4806	−0.82	**<0.0001**	0.3179
Lean mass lower limbs	−0.77	**<0.0001**	0.3497	−0.89	**0.0003**	0.5288	−0.84	**<0.0001**	0.2992
Lean mass lower limbs/height^2	−0.75	**<0.0001**	**0.3739**	−0.89	**0.0002**	**0.5975**	−0.86	**<0.0001**	**0.3673**
LBM + BMC	−0.53	**0.0078**	0.1958	−0.65	**0.0213**	0.2170	−0.63	**0.0012**	0.1228
LBM + BMC/height^2	−0.52	**0.0078**	0.1895	−0.59	**0.0364**	0.2181	−0.67	**0.0004**	0.1605
BD lower limbs	−0.63	**0.0013**	0.2268	−0.83	**0.0008**	0.4582	−0.79	**<0.0001**	0.3462
Z–score	−0.62	**0.0013**	0.1190	−0.84	**0.0008**	0.2479	−0.78	**<0.0001**	0.1651

Note: Bold, statistically significant correlations; bold and underlined, highest r^2.
*The FDR adjusted *p*-values.

however, the technique used to determine body composition was different and less sensitive (bioelectrical-impedance) and the patients differed from the current study with respect to motor status (50% non-ambulatory), so that any comparisons must be interpreted cautiously.[30]

LBM also correlated with reduced BD.[31] There are several mechanisms involved in poor bone health: reduced muscle tension on bone due to loss of muscular strength, chronic inflammation in dystrophic muscle, where dystrophin deficiency leads to the activation of the pathway affecting osteoclastogenesis, and alteration of calcium homeostasis. Deficient vitamin D status and long-term steroid therapy (as observed in DMD[3]) can also impair bone mineral content (BMC), while this latter mechanism is less common in BMD. The good correlations between DXA parameters and functional motor assessments (6MWT and NSAA) are in line with what reported in other neuromuscular diseases: a greater lean mass and a good bone mineral health (particularly in the lower limbs) are associated with better muscular performance.[4-6] Conversely, parameters related to fat mass showed less significant correlations with functional motor assessments, probably due to the fact that the total fat mass does not include the intramuscular fat (fatty replacement of skeletal muscle) but only the body fat deposits, meaning that it is not necessarily related to the disease burden.

Reduced BD and LBM, and increased fat mass play a partial role in determining muscle weakness in BMD. The main determinant of muscle weakness in BMD remains the amount of dystrophin in skeletal muscle, which is related to specific pathogenic variants in the *DMD*

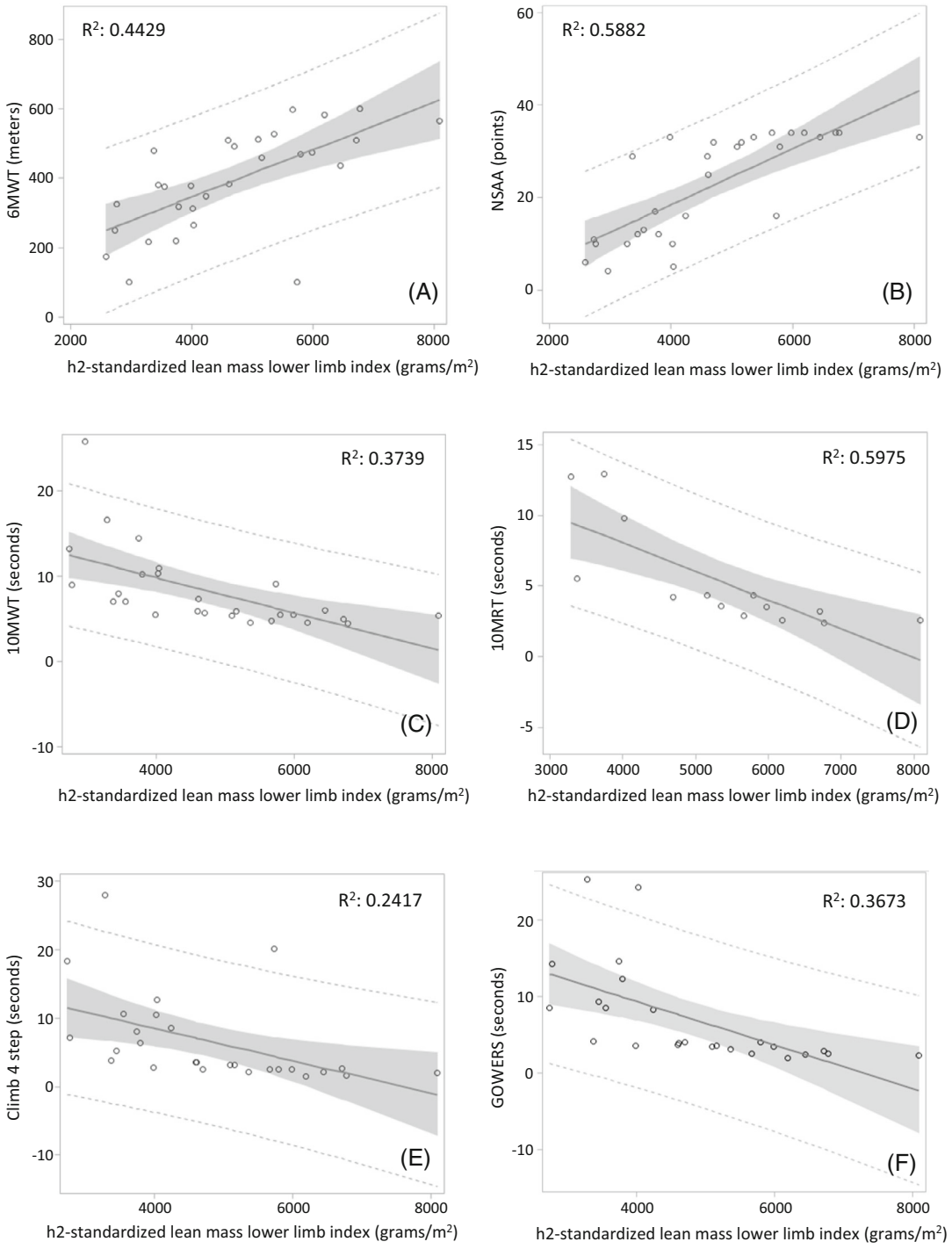

FIGURE 1 Correlation between h2-standardized lean mass lower limb index and functional motor assessments. The h2-standardized lean mass lower limb index values showed significant positive correlation with 6MWT (A) and NSAA (B), and negative correlation with 10MWT (C), 10MRT (D), climb 4 steps (E), and rise from floor (Gowers) (F).

gene. Different *DMD* in-frame exon deletions affect the properties of the resulting dystrophin protein: the loss of the crucial proximal N-terminal domain (including exons 10–12 and 14–18) of the dystrophin might result in a DMD-like phenotypes,[32] while the consequences of deletions in the dystrophin rod domain depend on the structural "phase" between spectrin repeats and hinge regions[33]; deletions including exons in the proximal rod domain,[34] or the hinge 3 domain encoded by exons 50–51[35,36] have been associated with mild phenotypes, whereas deletions situated in the exon 45–55 mutational hotspot,[37] but not including exons 50–51, usually cause "typical" BMD.[38]

The hypothesis that the incidence of osteoporosis is related to the loss of LBM, and that the latter is influenced by *DMD* pathogenic variants, finds support in our study, although we had no specific data on lumbar and femoral z-scores, only total Z-scores. BMD patients

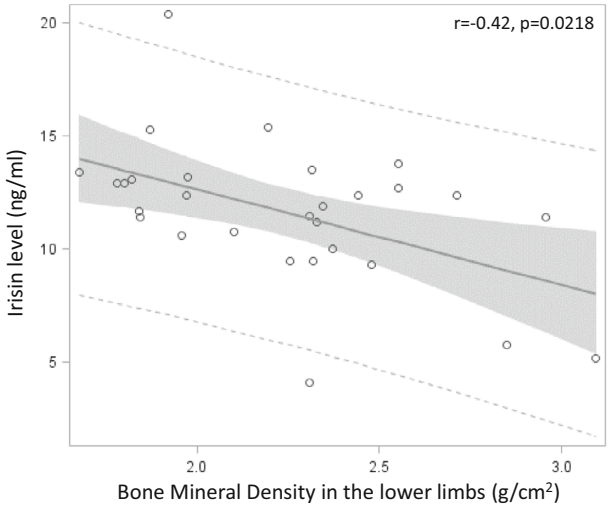

FIGURE 2 Correlation between bone mineral density of the lower limbs and irisin levels

with total z-scores < −2 in our cohort, who also showed poorer motor performance by NSAA compared to the median value of the whole cohort, harbored pathogenic variants in the crucial N-terminal domain (del 10–12 and dup 14–18) or in the 45–55 hotspot not including exons 50–51 (del 45–48).

At variance with a previous study that included male patients affected by myotonic dystrophies and reporting low circulating irisin levels,[15] circulating irisin levels were slightly reduced in BMD patients compared to healthy people, supporting the concept that low levels of irisin are associated with low mobility and sarcopenia.[10] Of note, it has been demonstrated that bone tissue is more sensitive than the adipose tissue to irisin action[39]; therefore, the small reduction of the circulating irisin concentrations detected in BMD patients is not expected to affect bone mineral density. Moreover, in the present study, circulating irisin levels correlated negatively with lower limb BD, suggesting resistance to irisin modulation at bone levels. This observation deserves further investigation to elucidate the underlying mechanisms.

This study has some limitations, such as the small sample size and the absence of a longitudinal follow-up, which did not allow us to speculate further on the association between *DMD* pathogenic variants, the body composition and the levels of myokines compared to DXA parameters.

5 | CONCLUSION

For several reasons, DXA application appears to be a useful tool to monitor the evolution of BMD: (i) moderate to strong correlations of LBM and functional motor assessments (6MWT and NSAA); (ii) the wide range of information that a single exam can provide on body composition and on BMC; (iii) the easy performance of DXA compared to muscle magnetic resonance imaging (mMRI), which is frequently more time demanding and expensive; and (iv) need for far less specialized expertise for post-processing DXA images compared to Dixon images of mMRI.[40] Further longitudinal studies are necessary to clarify the role of DXA and circulating myokines in predicting muscle deterioration, and to ascertain whether they can represent valid (surrogate) biomarkers in BMD.

ACKNOWLEDGMENT

Open Access Funding provided by Universita degli Studi di Milano within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL STATEMENT

The authors confirm that they have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Andrea Barp https://orcid.org/0000-0001-8015-2597

REFERENCES

1. Hoffman EP, Kunkel LM, Angelini C, Clarke A, Johnson M, Harris JB. Improved diagnosis of Becker muscular dystrophy by dystrophin testing. *Neurology*. 1989;39(8):1011-1017. doi:10.1212/wnl.39.8.1011
2. Joyce NC, Hache LP, Clemens PR. Bone health and associated metabolic complications in neuromuscular diseases. *Phys Med Rehabil Clin N Am*. 2012;23(4):773-799. doi:10.1016/j.pmr.2012.08.005
3. Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. *J Pediatr Orthop*. 2000;20(1):71-74.
4. Palmieri GM, Bertorini TE, Griffin JW, Igarashi M, Karas JG. Assessment of whole body composition with dual energy x-ray absorptiometry in Duchenne muscular dystrophy: correlation of lean body mass with muscle function. *Muscle Nerve*. 1996;19(6):777-779. doi:10.1002/(SICI)1097-4598(199606)19:63.0.CO;2-I
5. Kanda F, Fujii Y, Takahashi K, Fujita T. Dual-energy X-ray absorptiometry in neuromuscular diseases. *Muscle Nerve*. 1994;17(4):431-435. doi:10.1002/mus.880170411
6. Constantinides CD, Gillen JS, Boada FE, Pomper MG, Bottomley PA. Human skeletal muscle: sodium MR imaging and quantification-potential applications in exercise and disease. *Radiology*. 2000;216(2):559-568. doi:10.1148/radiology.216.2.r00jl46559
7. Skalsky AJ, Han JJ, Abresch RT, Shin CS, McDonald CM. Assessment of regional body composition with dual-energy X-ray absorptiometry in Duchenne muscular dystrophy: correlation of regional lean mass and quantitative strength. *Muscle Nerve*. 2009;39(5):647-651. doi:10.1002/mus.21212
8. He C, He W, Hou J, et al. Bone and muscle crosstalk in aging. *Front Cell Dev Biol*. 2020;8:585644. doi:10.3389/fcell.2020.585644
9. Huh JY, Dincer F, Mesfum E, Mantzoros CS. Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and metabolism in humans. *Int J Obes (Lond)*. 2014;38(12):1538-1544. doi:10.1038/ijo.2014.42
10. Jedrychowski MP, Wrann CD, Paulo JA, et al. Detection and quantitation of circulating human Irisin by tandem mass spectrometry. *Cell*

Metab. 2015;22(4):734-740. doi:10.1016/j.cmet.2015.08.001 Epub 2015 Aug 13.
11. Anastasilakis AD, Polyzos SA, Makras P, et al. Circulating irisin is associated with osteoporotic fractures in postmenopausal women with low bone mass but is not affected by either teriparatide or denosumab treatment for 3 months. *Osteoporos Int*. 2014;25(5):1633-1642. doi:10.1007/s00198-014-2673-x
12. Palermo A, Strollo R, Maddaloni E, et al. Irisin is associated with osteoporotic fractures independently of bone mineral density, body composition or daily physical activity. *Clin Endocrinol (Oxf)*. 2015;82(4):615-619. doi:10.1111/cen.12672
13. Singhal V, Lawson EA, Ackerman KE, et al. Irisin levels are lower in young amenorrheic athletes compared with eumenorrheic athletes and non-athletes and are associated with bone density and strength estimates. *PLoS One*. 2014;9(6):e100218. doi:10.1371/journal.pone.0100218
14. Chang JS, Kim TH, Nguyen TT, Park KS, Kim N, Kong ID. Circulating irisin levels as a predictive biomarker for sarcopenia: a cross-sectional community-based study. *Geriatr Gerontol Int*. 2017;17(11):2266-2273. doi:10.1111/ggi.13030
15. Dozio E, Passeri E, Cardani R, et al. Circulating Irisin is reduced in male patients with type 1 and type 2 myotonic dystrophies. *Front Endocrinol (Lausanne)*. 2017;8:320. doi:10.3389/fendo.2017.00320
16. McKiernan FE, Berg RL, Linneman JG. The utility of BMD Z-score diagnostic thresholds for secondary causes of osteoporosis. *Osteoporos Int*. 2011;22(4):1069-1077. doi:10.1007/s00198-010-1307-1
17. Weber D, Long J, Leonard MB, Zemel B, Baker JF. Development of novel Methods to define deficits in appendicular lean mass relative to fat mass. *PLoS One*. 2016;11(10):e0164385. doi:10.1371/journal.pone.0164385
18. Bijlsma AY, Meskers CG, van den Eshof N, et al. Diagnostic criteria for sarcopenia and physical performance. *Age (Dordr)*. 2014;36(1):275-285. doi:10.1007/s11357-013-9556-5
19. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc*. 2002;50(5):889-896. doi:10.1046/j.1532-5415.2002.50216.x
20. Estrada M, Kleppinger A, Judge JO, Walsh SJ, Kuchel GA. Functional impact of relative versus absolute sarcopenia in healthy older women. *J Am Geriatr Soc*. 2007;55(11):1712-1719. doi:10.1111/j.1532-5415.2007.01436.x
21. Baumgartner RN, Koehler KM, Gallagher D, et al. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol*. 1998;147(8):755-763. doi:10.1093/oxfordjournals.aje.a009520
22. Coin A, Sarti S, Ruggiero E, et al. Prevalence of sarcopenia based on different diagnostic criteria using DEXA and appendicular skeletal muscle mass reference values in an Italian population aged 20 to 80. *J Am Med Dir Assoc*. 2013;14(7):507-512. doi:10.1016/j.jamda.2013.02.010
23. McDonald CM, Henricson EK, Han JJ, et al. The 6-minute walk test in Duchenne/Becker muscular dystrophy: longitudinal observations. *Muscle Nerve*. 2010;42(6):966-974. doi:10.1002/mus.21808
24. Mazzone ES, Messina S, Vasco G, et al. Reliability of the north star ambulatory assessment in a multicentric setting. *Neuromuscul Disord*. 2009;19(7):458-461. doi:10.1016/j.nmd.2009.06.368
25. Scott E, Eagle M, Mayhew A, et al. Development of a functional assessment scale for ambulatory boys with Duchenne muscular dystrophy. *Physiother Res Int*. 2012;17(2):101-109. doi:10.1002/pri.520
26. Hosmer DW, Lemeshow S. Assessing the fit of the model. In: Shewart WA, Wilks SS, eds. *Applied Logistic Regression*. Vol 5. 2nd ed. New York, NY: John Wiley and Sons; 2000:160-164.
27. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesth Analg*. 2018;126(5):1763-1768.
28. Barzegar M, Niknam E, Habibi P, Shiva S, Tahmasebi S. Bone mineral density and bone metabolism in patients with Duchenne muscular dystrophy. Iran. *J Child Neurol*. 2018;12(1):77-83.
29. Del Rocío C-GO, Rodríguez-Cruz M, Almeida-Becerril T, Maldonado-Hernández J, Baeza CW. Muscle function and age are associated with loss of bone mineral density in Duchenne muscular dystrophy. *Muscle Nerve*. 2019;59(4):417-421. doi:10.1002/mus.26416
30. Jacques MF, Onambele-Pearson GL, Reeves ND, Stebbings GK, Smith J, Morse CI. Relationships between muscle size, strength, and physical activity in adults with muscular dystrophy. *J Cachexia Sarcopenia Muscle*. 2018;9(6):1042-1052. doi:10.1002/jcsm.12347
31. Akima H, Lott D, Senesac C, et al. Relationships of thigh muscle contractile and non-contractile tissue with function, strength, and age in boys with Duchenne muscular dystrophy. *Neuromuscul Disord*. 2012;22:16-25.
32. Kesari A, Pirra LN, Bremadesam L, et al. Integrated DNA, cDNA, and protein studies in Becker muscular dystrophy show high exception to the reading frame rule. *Hum Mutat*. 2008;29(5):728-737. doi:10.1002/humu.20722
33. Kaspar RW, Allen HD, Ray WC, et al. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in Becker muscular dystrophy. *Circ Cardiovasc Genet*. 2009;2(6):544-551. doi:10.1161/CIRCGENETICS.109.867242
34. Angelini C, Fanin M, Pegoraro E, Freda MP, Cadaldini M, Martinello F. Clinical-molecular correlation in 104 mild X-linked muscular dystrophy patients: characterization of sub-clinical phenotypes. *Neuromuscul Disord*. 1994;4(4):349-358. doi:10.1016/0960-8966(94)90071-x
35. Carsana A, Frisso G, Tremolaterra MR, et al. Analysis of dystrophin gene deletions indicates that the hinge III region of the protein correlates with disease severity. *Ann Hum Genet*. 2005;69(Pt 3):253-259. doi:10.1046/j.1529-8817.2005.00160.x
36. Anthony K, Cirak S, Torelli S, et al. Dystrophin quantification and clinical correlations in Becker muscular dystrophy: implications for clinical trials. *Brain*. 2011;134(Pt 12):3547-3559. doi:10.1093/brain/awr291 Epub 2011 Nov 18. Erratum in: Brain. 2016 Apr;139(Pt 4):e27.
37. White SJ, den Dunnen JT. Copy number variation in the genome; the human DMD gene as an example. *Cytogenet Genome Res*. 2006;115(3–4):240-246. doi:10.1159/000095920
38. Bushby KM, Gardner-Medwin D, Nicholson LV, et al. The clinical, genetic and dystrophin characteristics of Becker muscular dystrophy. II. Correlation of phenotype with genetic and protein abnormalities. *J Neurol*. 1993;240(2):105-112. doi:10.1007/BF00858726
39. Colaianni G, Grano M. Role of Irisin on the bone-muscle functional unit. *Bonekey Rep*. 2015;4:765. doi:10.1038/bonekey.2015.134
40. van de Velde NM, Hooijmans MT, Sardjoe Mishre ASD, et al. Selection approach to identify the optimal biomarker using quantitative muscle MRI and functional assessments in Becker muscular dystrophy. *Neurology*. 2021;97(5):e513-e522.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Barp A, Carraro E, Goggi G, et al. Body composition and myokines in a cohort of patients with Becker muscular dystrophy. *Muscle & Nerve*. 2022;66(1):63-70. doi:10.1002/mus.27565