Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 29 (2019) 671–677

www.elsevier.com/locate/nmd

Assessment of muscle involvement in patients with Duchenne muscular dystrophy via segmental multifrequency bioelectrical analysis

Satoshi Kuru[a,*], Tomoka Uchiyama[b], Ayako Hattori[c], Takatoshi Sato[d], Terumi Murakami[d], Keiko Ishigaki[d], Takahiro Nakayama[e]

[a] *Department of Neurology, National Hospital Organization Suzuka National Hospital, Suzuka, Japan*
[b] *TANITA Body Weight Scientific Institute, Tokyo, Japan*
[c] *Department of Pediatrics and Neonatology, Nagoya City University, Nagoya, Japan*
[d] *Department of Pediatrics, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan*
[e] *Department of Neurology, Yokohama Rosai Hospital, Yokohama, Japan*

Received 27 January 2019; received in revised form 7 August 2019; accepted 14 August 2019

Abstract

We investigated the usefulness of segmental multifrequency bioelectrical impedance analyses (MBIA) for assessing muscle involvement in Duchenne muscular dystrophy (DMD) patients. Bioelectrical impedance data of the upper arm, thigh, and lower leg were obtained from 29 boys with DMD (ages 2–17 years old; mean 10.8 ± 3.9 years) at three institutions along with 41 healthy controls (ages 3–16; mean 9.8 ± 3.5 years). Then the muscle density index (MDI: 1- Z_{250}/Z_5) was calculated using segmental MBIA and compared between groups. The MDI was lower in boys with DMD, relative to controls, with older DMD patients exhibiting a significant decrease in MDI. The MDI of patient thighs was significantly correlated with the percent muscle volume index (%MVI), as measured using computed tomography ($r = 0.79$). MDI values for the upper arm, thigh, and lower leg were all significantly correlated with the Brooke and the Vignos scales, respectively, with correlation coefficients of 0.56–0.77. Finally, MDI was significantly greater in the glucocorticoid-treated group, relative to the untreated group in all regions. Taken together, these data show that segmental MBIA is feasible for evaluating muscle involvement and might serve as an outcome measure in DMD.

Keywords: Duchenne muscular dystrophy; Multifrequency bioelectrical impedance analysis; Monitoring disease progression; Segmental muscle.

1. Introduction

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive degenerative disorder of the skeletal muscles that affects 1 in 3500 male births. It is caused by a mutation in the dystrophin gene, resulting in progressive muscle damage and its replacement by fat and connective tissue. No effective treatments are currently available; however, a number of promising genetic strategies have emerged as potential therapies [1,2], including stop-codon readthrough and exon-skipping with antisense oligonucleotides, with several treatments currently entering clinical trials. Assessment of clinical outcomes typically involves a combination of functional tests and muscle imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography [3–6]; however, these measures have limitations. Functional tests have the disadvantages of being dependent on motivation, attention, and coordination. CT remains less desirable due to the exposure to radiation. MRI remains a powerful, noninvasive tool for assessing muscle function; however, it is among the most expensive options, and is often unsuitable for young boys due to the need to remain motionless for long periods of time, requiring sedation to effectively perform the examination. There is therefore a pressing need for methods capable of frequently and easily assessing disease progression and treatment efficacy.

Bioelectrical impedance analysis (BIA) has been used to estimate skeletal muscle mass in healthy populations [7]. It is

* Corresponding author.
E-mail address: kuru.satoshi.rb@mail.hosp.go.jp (S. Kuru).

https://doi.org/10.1016/j.nmd.2019.08.006
0960-8966/

a safe, portable, and relatively inexpensive method, although its application has been limited in muscular dystrophy. Among BIA tests, Yamada et al. [8] showed that multifrequency BIA (MBIA) is superior to single-frequency BIA in elderly patients. Similarly, they found that MBIA is a better tool for evaluating the distribution between extracellular water (ECW) and intracellular water (ICW), relative to other methods.

Among other MBIA applications, McDonald et al. [9] reported high correlations between whole-body MBIA and DEXA in assessing the body composition of DMD patients. Further, electrical impedance myography (EIM), a type of MBIA instrument, has been utilised as an impedance measurement of localised muscle in clinical applications [10]. Here, we used segmental MBIA to evaluate impedance of certain segmental muscles, a method which has not been tested in DMD patients, and determine the usefulness of segmental MBIA for assessing muscle composition in DMD patients.

2. Materials and methods

2.1. Subjects

A total of 29 DMD male patients (mean age of 10.8 ± 3.9 years, ranging from 2 to 17) participated in this study. Patients were recruited among those followed in outpatient facilities from Suzuka National Hospital ($n = 6$), Nagoya City University ($n = 13$), and Tokyo Women's Medical University ($n = 10$). DMD was confirmed by genetic tests and/or muscle biopsies. Of the 29 boys with DMD, 10 were ambulatory and 19 were full-time wheelchair users. An additional 41 healthy boys served as a control group (mean age of 9.8 ± 3.5 years, ranging from 3 to 16).

Sixteen patients had been receiving glucocorticoid (GC); all other patients were GC-naïve. In Japan, GC-therapy for DMD patients had not been established until clinical practice guidelines for DMD were published in 2013 [11]. Of the 16 patients who had been receiving GC, only one patient stopped before the time of examination, who had been receiving GC treatment from age 8 to age 10 and entered this study at age 16. The average duration of GC therapy was 3.2 ± 1.7 years in 15 patients whose duration of GC treatment was available.

2.2. Ethical considerations and informed consent

Written informed consent was obtained from the participants' parents. All relevant study-related protocols were approved by the institutional review boards of their respective institutions prior to initiation of this study.

2.3. Bioelectrical impedance analysis and muscle density index

Recently, we proposed the muscle density index (MDI) as a novel index of BIA and demonstrated that it could accurately represent muscle development and muscle mass increases in healthy children [12]. This index is calculated as $MDI = 1 - Z_{250}/Z_5$, where Z is impedance in ohms and Z_5 and Z_{250} represent impedance at 5 kHz and 250 kHz, respectively.

2.3.1. Bioelectrical impedance measurement

Segmental impedance was measured in the middle part of the upper arm, thigh, and lower leg with an impedance analyzer (BCA-100 and MSd-100, Tanita Corp., Japan) using the 4-electrode method [13]. Measurements were taken with currents ≤100 μA at frequencies of 5 and 250 kHz. Disposable electrocardiogram electrodes (RedDot 2330, 3 M, Japan Ltd., Tokyo, Japan) were used to apply current, and were placed at the centre of the instep of each foot and at the back of each hand. The detecting electrode was a belt-type stainless steel plate (1 cm width), which was adhered to and then looped around the measurement location. For the upper arms, the reference electrodes were placed in the axillae, with the corresponding electrodes then placed distally to create a distance of 10 cm between the centres of the electrodes (or 8 cm when the child's height was ≤120 cm). For the thighs and lower legs, the corresponding electrodes were arranged in parallel and placed at 14 cm intervals between electrode centres (or 10 cm intervals when the child's height was ≤120 cm) on either side of either the femur or the central tibia. To minimise the effects of rapid shifts in body fluid caused by postural changes, the patients maintained a relaxed supine position on a bed for 20 min before and during the measurement. The bioelectrical impedance was measured three times consecutively at each location. After we acquired three impedance data sets, the relative error of each data at each frequency was automatically calculated within the device/equipment as below.

$$\text{relative error} = |(\text{average of data}) - (\text{data})| / (\text{average of data})$$

When the relative error of these datasets was ≤0.03, we calculated the average of the three datasets. Otherwise, we added the measurement one time, and when three out of four had a relative error of ≤0.03, we calculated the average of the three datasets. If there was not, we found no such three-dataset, we calculated the measurement no more than four times until we found three datasets whose relative error was ≤0.03among all datasets. If we were unable to find such three datasets, we adjusted conditions such as tightness of electrode and skin sweat and added the measurement not more than seven times until we found three datasets whose relative error was ≤0.03 among all datasets. When we found such three datasets, we calculated the average of the three datasets. When we were unable to find such datasets after we adjusted the measurement condition two times, we rejected the data as measurement error from the analysis (Fig. 1).

2.4. Muscle volume index (MVI) and %MVI from CT scan

Muscle volume index (MVI) and %MVI were obtained from CT scans at the mid-thigh. The CT scanning protocol was performed as previously described [3]. MVI is the estimated muscle volume within a given CT slice and is calculated using the CT histogram-based procedure after

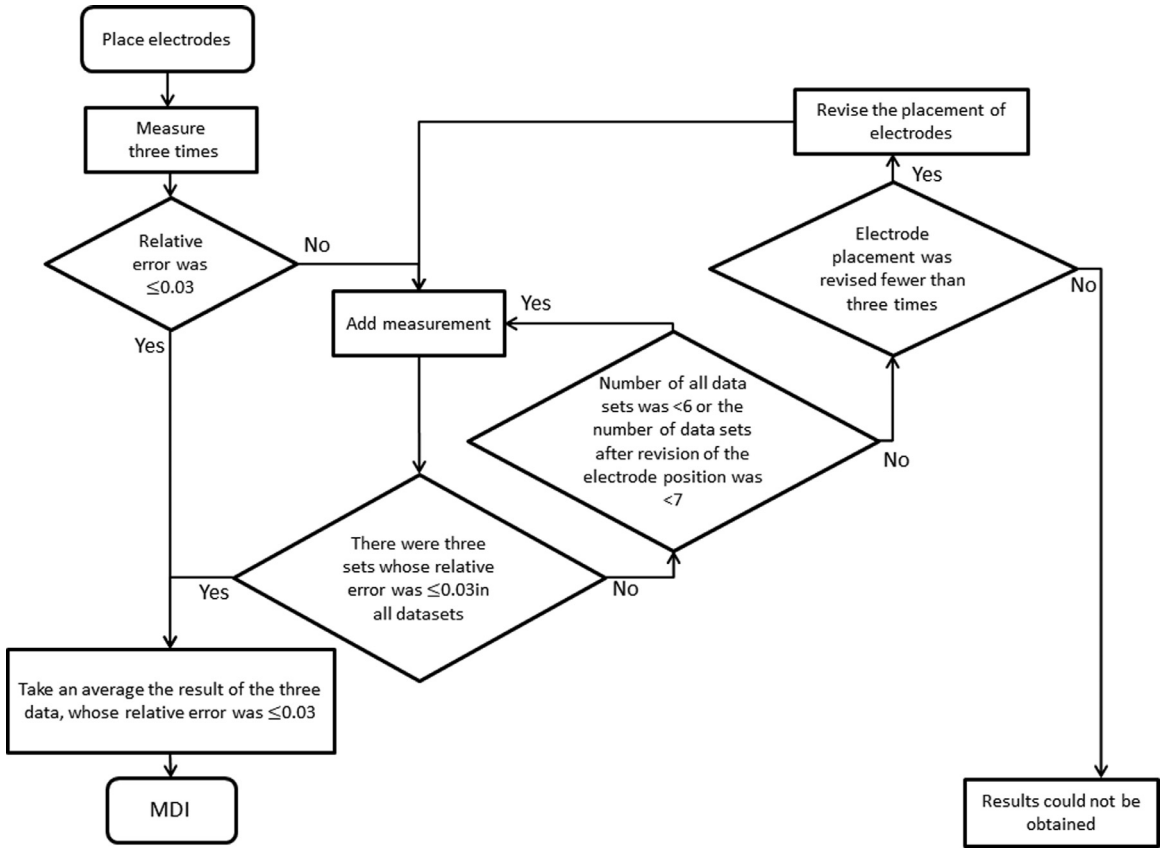

Fig. 1. The procedure of bioelectrical impedance measurement.

correcting for a partial volume effect. %MVI is calculated by dividing the MVI by the entire volume of the region of interest [3]. Each measurement is unilateral (right side).

2.5. Scaling of functional abilities

Functional activity was ranked using the Brooke Upper Extremity Functional Scale and the Vignos Leg Grading Scale [14,15]. The former is a 6-point scale that describes a variety of activities related to the upper limbs, and the latter is a 9-point scale that describes a variety of activities related to the lower limbs.

2.6. Statistical analyses

Statistical analyses were performed using Stat Mate III^{TM}. Data are expressed as means ± standard deviations. Pearson's correlation analyses were used to examine the relationship between CT indices (MVI and %MVI) and MDI. Differences between DMD patients and healthy controls were determined using a non-paired t-test. Multiple regression analyses in a stepwise manner were used to examine the contributions of age, height, weight, and body mass index to MBIA. Entry and reentry levels in a stepwise manner were set at 0.15. However, on the analysis for evaluation of steroid therapy the forced entry of above-mentioned selected variable was performed because the sample size is too small. The Spearman rank correlation coefficient was used to analyze the correlation between functional disability and MDI. Furthermore, we compared the GC-treated group to the naïve group. All subjects in the treated group were over 8 years of age. Therefore, four untreated subjects under 7 years of age were excluded for comparison. For all analyses, a p value < 0.05 was considered statistically significant.

3. Results

3.1. BI measurement

The average numbers of the BI measurement times of each limb were 5.00 in the upper arm, 4.93 in the thigh, and 4.44 in the lower legs. The adjustment of measurement condition was required in 8 limbs of 5 subjects. There was no measurement error in all 87 limbs.

3.2. Subject demographics

Patient demographics are shown in Table 1. The average (±standard deviation) age was 10.8 years (±3.9; $n=29$) in the DMD group and 9.8 years (±3.5; $n=41$) in the control group. Average heights and weights were 133.0 cm (±21.5) and 36.9 kg (±16.9) in the DMD group and 134.7 cm (±20.2) and 32.5 kg (±11.8) in controls. The differences between groups were not statistically significant ($p > 0.05$ by unpaired

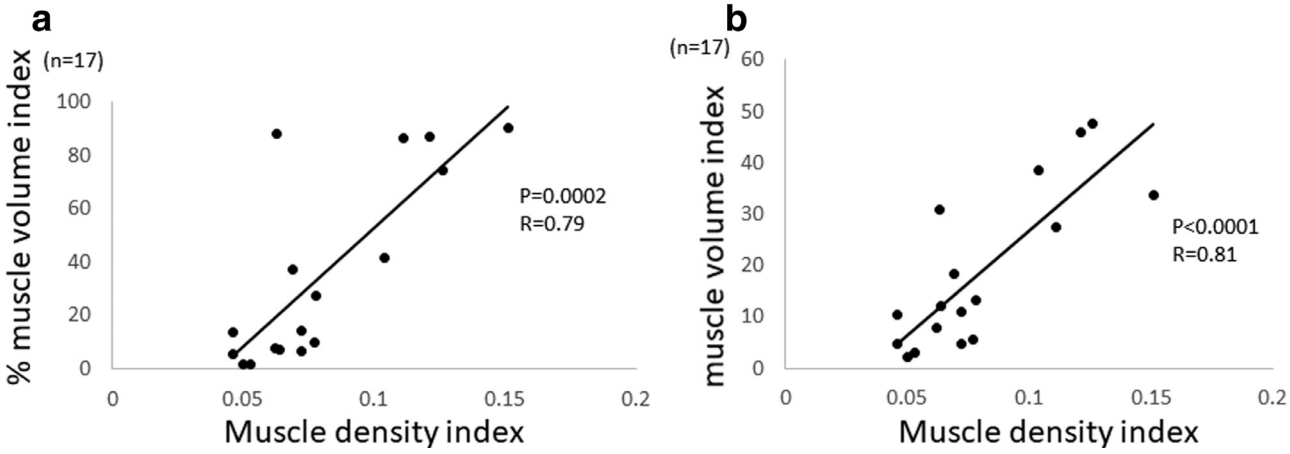

Fig. 2. the relationship between CT indices (MVI and %MVI) and MDI.

Table 1
Physical characteristics of subjects.

	DMD $n=29$ (min.- max.)	control $n=41$ (min.- max.)	non-paired t-test
Age (years)	10.8 ± 3.9 (2–17)	9.8 ± 3.5 (3–16)	N.S.
Height (cm)	133.0 ± 21.5 (88.4–164.0)	134.7 ± 20.2 (93.0–168.2)	N.S.
Weight (kg)	36.9 ± 16.9 (13.3–75.5)	32.5 ± 11.8 (13.2–62.7)	N.S.
BMI (kg/cm^2)	19.9 ± 5.2 (9.5–30.1)	17.2 ± 2.5 (13.9–25.7)	$p=0.004$

Values are mean ± standard deviation.
DMD: Duchenne muscular dystrophy group.
BMI: body mass index.

t-test). By contrast, BMI was significantly different between groups ($p < 0.05$ by unpaired t-test), with DMD patients exhibiting average BMIs of 19.9 (±5.2) compared to 17.2 (±2.5) for healthy controls. For this reason, age, height, weight, and BMI were all used as explanatory variables in multiple regression analyses in a stepwise manner.

3.3. Relationship between MDI and CT indices

Of the 29 DMD patients, 17 underwent muscle CT examination as part of their routine clinical evaluation during the research period. MDI was significantly correlated with both MVI ($r=0.81$; $p < 0.001$) and %MVI ($r=0.79$, $p < 0.001$), as determined by CT (Fig. 2a, b).

3.4. MDI in DMD and healthy controls

MDI scores for the upper arm, thigh, and lower leg were compared between groups using unpaired t-tests (Table 2). MDI was significantly lower in those with DMD than in healthy controls at all sites examined ($p < 0.05$).

3.5. Multiple regression analyses

Multiple regression analyses were applied to examine the contribution of age, height, weight and body mass index for estimating BI index in MFBIA. (Table 3). Age only was selected as the most significant variable for DMD. The F values were shown in Table 3. The rest variables were not selected at all. All coefficients were negative for the DMD group and positive for healthy controls (Fig. 3a). The strong contribution of age is evident based on the high r-square value (Table 3). Table 3 displays the results of the comparison between the GC-treated group to the naïve group. The above-selected variable (age) was treated as forced entry variable in this analysis, in order to match with the analysis method.

3.6. Relationship between MDI and functional abilities

The relationship between MDI and functional abilities is shown in Fig. 3b. Both the Brooke and Vignos scales showed high correlations with MDI scores, suggesting that MDI scores may reflect the severity of muscle involvement in DMD.

3.7. Effect of glucocorticoid medication

Next, we compared DMD patients receiving GC treatment to untreated DMD patients. MDI tended to be higher in the GC-treated group relative to naïve controls in children under 10 years of age, with no difference between groups thereafter (Fig. 4). These results are broadly consistent with our clinical observations, suggesting the potential usefulness of MDI as a measure of clinical outcomes.

4. Discussion

Bioelectrical impedance values of the upper arm, thigh, and lower leg were obtained from male DMD and healthy control subjects over a wide range of ages, from infancy to

Table 2
The results of non-paired t-test between the groups.

	DMD group ($n=29$) Average [95%CI]	t-value	p-value	control group ($n=41$) Average [95%CI]
$MDI_{upper\ arm}$	0.086 [0.025, 0.147]	15.83	<0.001	0.207 [0.144, 0.270]
MDI_{thigh}	0.081 [0.032, 0.130]	25.19	<0.001	0.273 [0.199, 0.347]
$MDI_{Lower\ leg}$	0.136 [0.032, 0.239]	12.73	<0.001	0.281 [0.208, 0.354]
	steroid group ($n=16$) Average [95%CI]	t-value	p-value	non -steroid group ($n=9$) Average [95%CI]
$MDI_{upper\ arm}$	0.086 [0.025, 0.148]	2.2	0.02	0.065 [0.033, 0.098]
MDI_{thigh}	0.082 [0.033, 0.130]	2.48	0.01	0.064 [0.039, 0.088]
$MDI_{Lower\ leg}$	0.155 [0.040, 0.269]	3.29	<0.001	0.098 [0.043, 0.152]

Table 3
The results of regression analysis in the stepwise manner and in the forced entry manner.

	DMD group ($n=29$)				Control group($n=41$)			
	Selected variable (Fvalue)	Estimate	R-square	p-value	Selected variable (Fvalue)	Estimate	R-square	p-value
$MDI_{upper\ arm}$	Age only ($F=22.0$)	-5.40×10^{-3}	$(0.67)^2$	<0.001	Age only ($F=20.5$)	5.60×10^{-3}	$(0.59)^2$	<0.001
MDI_{thigh}	Age only ($F=16.5$)	-4.00×10^{-3}	$(0.62)^2$	<0.001	Age only ($F=33.3$)	7.86×10^{-3}	$(0.67)^2$	<0.001
$MDI_{Lower\ leg}$	Age only ($F=5.55$)	-5.64×10^{-3}	$(0.41)^2$	0.026	Age only ($F=26.0$)	7.12×10^{-3}	$(0.63)^2$	<0.001
	steroid group ($n=16$)				non -Steroid group($n=9$)			
	Forced entry variable (Fvalue)	Estimate	R-square	p-value	Forced entry variable (Fvalue)	Estimate	R-square	p-value
$MDI_{upper\ arm}$	Age ($F=5.02$)	-6.42×10^{-3}	$(0.51)^2$	0.042	Age($F=2.31$)	-2.87×10^{-3}	$(0.50)^2$	0.172
MDI_{thigh}	Age ($F=4.51$)	-4.83×10^{-3}	$(0.49)^2$	0.052	Age($F=0.61$)	0.66×10^{-3}	$(0.15)^2$	0.7
$MDI_{Lower\ leg}$	Age ($F=4.91$)	-11.87×10^{-3}	$(0.51)^2$	0.044	Age ($F=9.61$)	-7.32×10^{-3}	$(0.76)^2$	0.017

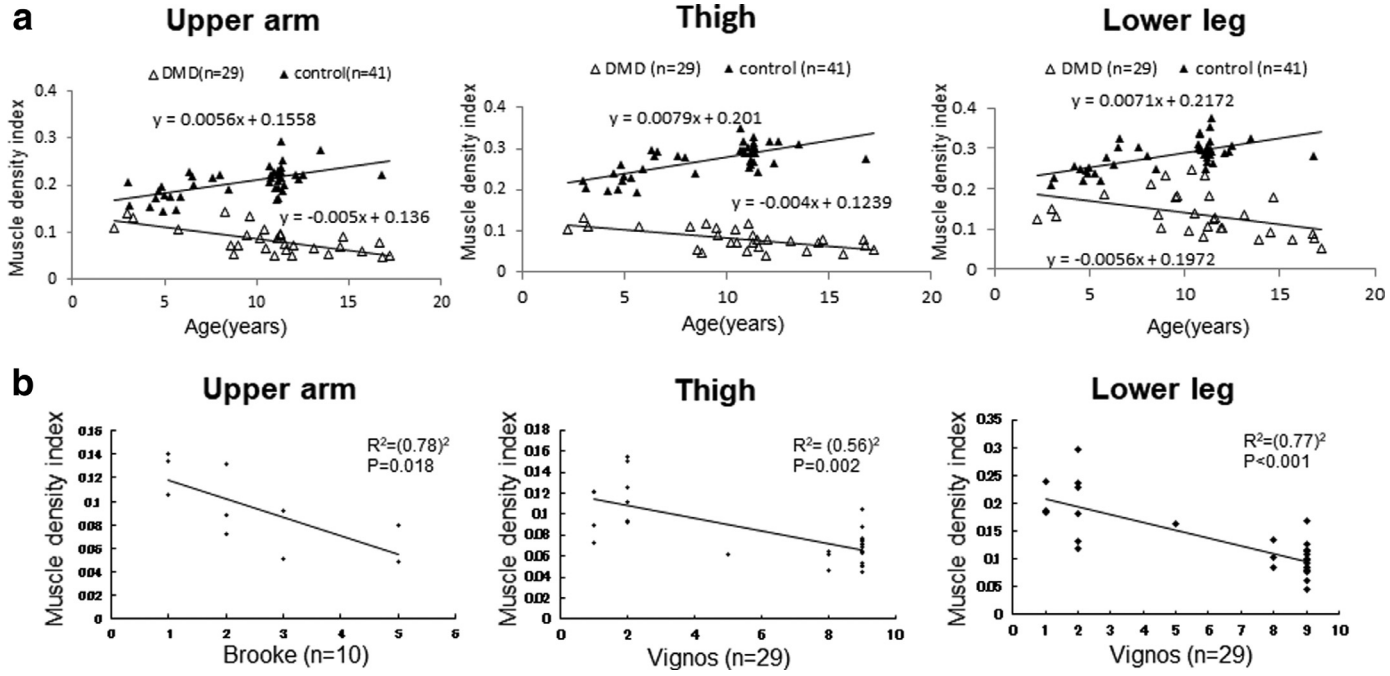

Fig. 3. Relationship between MDI and age (a) and between MDI and functional index(b).

the middle teen years. During this period, drastic muscular changes occur in DMD. The MDI of healthy children showed a significant increase with age, indicative of normal muscle growth and maturation. By contrast, the MDI of DMD patients showed a significant decrease with age, indicating an opposite developmental trajectory over time. Moreover, we observed moderate correlations between MDI and functional abilities across all examined regions. These observations are consistent with the results of Noshiro et al. [17], who showed that membrane capacitance and intracellular resistance are

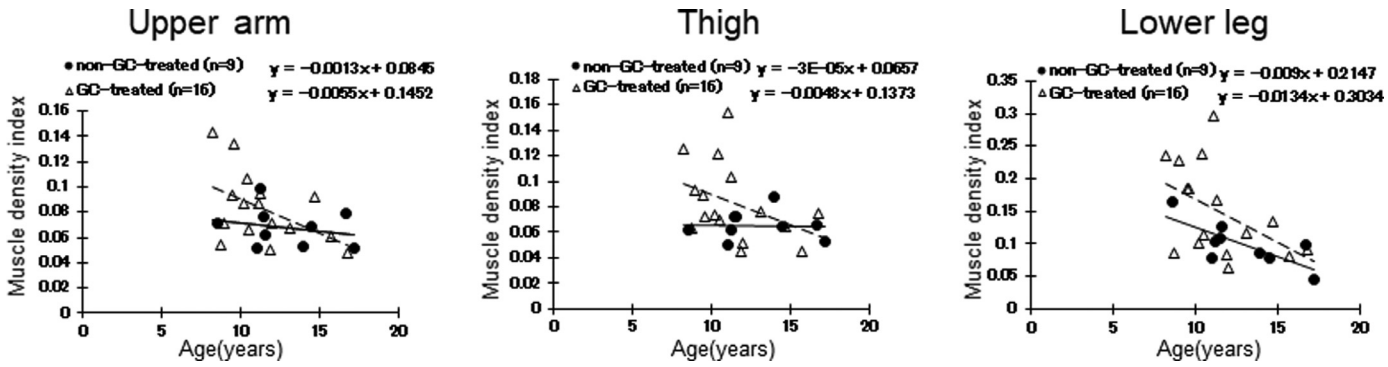

Fig. 4. Relationship between MDI and GC -treated non-GC -treated groups.
GC:glucocorticoid
Because all subjects in the GC -treated group were over 8 years of age, four GC -untreated subjects under 7 years of age were excluded for comparison.

correlated with the stage of disability in DMD patients, suggesting the applicability of MDI as a measure of disease severity in DMD.

MBIA can be used to assess the distribution between extracellular water (ECW) and intracellular water (ICW) based on the assumption that the impedance of low-frequency current primarily reflects ECW, while impedance of high-frequency current reflects the total tissue water (TTW = ICW + ECW) [8,16]. Therefore, MDI is thought to reflect the ratio of ICW to TTW. The results presented here are consistent with previous studies that have demonstrated a significant increase in the ECW:ICW ratio in DMD compared to healthy children [9,16]; however, these data should be interpreted with caution, as MBIA may underestimate lean tissue mass (LTM) compared to DEXA measurement in DMD. This underestimation is likely due to abnormally low ICW levels in dystrophic muscle tissue due to defects in dystrophin, resulting in impaired water homeostasis [9]. Similarly, Mok et al. suggested that it was difficult for MBIA to estimate muscle mass accurately in DMD, although it was an effective method for estimating fat-free body mass [18,19]. To address the potential gaps between these methods, McDonald called for the development of appropriate prediction equations for body composition in dystrophic myopathies [9].

In this study, we measured bioelectrical impedance of segmental muscle, in lieu of the more common whole-body measurements used in previous studies [9,16,18,19]. The severity and rate of progression of muscle involvement in DMD varies from site to site, which makes a segmental muscle assessment more appropriate for DMD analyses. Segmental MBIA is also thought to be more appropriate for assessing muscle involvement in the upper limbs. Furthermore, the segmental four-electrode method used for MBIA has the advantage of minimizing the effects of subcutaneous fat [13]. While electrical impedance myography (EIM) has been used to assess localised muscle [10], there remains an important distinction between EIM and the technique described here in terms of the size the area of interest. We anticipate continued usage of both methods depending on a combination of diagnostic goals and ease of use in the clinical setting.

MBIA and CT indices revealed a strong correlation between MDI, MVI, and %MVI in the thigh, with the most significant correlations observed by MBIA. However, the former measures the whole tissue between the electrodes of segmental muscle, while the latter covers only a single cross-section. Because the two indices represent fundamentally different measurements based on the areas examined, significant care must be taken when comparing the two outcomes.

A significant limitation of this study was the lack of a direct comparison between segmental MBIA and MRI findings, although investigations into such comparisons remain an important topic of discussion. Miyatani et al. reported that the bioimpedance of a limited segment of limbs was significantly correlated with the muscle volume measured using MRI in healthy adults [20], suggesting that direct comparisons between MBIA, CT, and MRI may be possible.

GC medications have a beneficial effect on muscle strength and function in DMD [21,22]; however, defined regimens of GC administration have yet to be established for DMD, due in part to a combination of inadequate outcome measures for monitoring the involvement of skeletal muscle and incomplete efficacy data for GC-treated patients. The present study included both patients with and without GC treatment. We were able to identify a visual difference between groups, indicative of a difference in MDI between DMD patients treated with GCs and those without GC therapy. As described above, segmental MBIA may offer an improved method for determining the efficacy of GC treatment.

Segmental MBIA is an inexpensive, easy to use, and noninvasive measurement tool. Examination with MBIA was well-tolerated, even among boys under 5 years of age. Moreover, it can be performed repeatedly at short intervals because of its portability and convenient setup. This study suggests that segmental MBIA might serve as an outcome measure in DMD. Future longitudinal studies with serial examinations are needed to strengthen the validity of this method for clinical trials.

Acknowledgements

We would like to express our deepest gratitude to Dr. T. Makie of Suzuka National Hospital, who provided valuable comments and advice. This study was supported financially by an Intramural Research Grant (26–6) for Neurological and Psychiatric Disorders from the National Centre of Neurology and Psychiatry.

References

[1] Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447(7140):87–91.
[2] Cirak S, Arechavala-Gomeza V, Guglieri M, et al. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. Lancet 2011;378(9791):595–605.
[3] Kuru S, Sakai M, Tanaka N, Konagaya M, Nakayama T, Kawai M. Natural course of muscular involvement assessed by a new computed tomography method in Duchenne muscular dystrophy. Neurol Clin Neurosci 2013;1(2):63–8.
[4] Wokke BH, van den Bergen JC, Versluis MJ, et al. Quantitative MRI and strength measurements in the assessment of muscle quality in Duchenne muscular dystrophy. Neuromuscul Disord 2014;24(5):409–16.
[5] Mazzone E, Martinelli D, Berardinelli A, et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. Neuromuscul Disord 2010;20(11):712–16.
[6] Zuberi SM, Matta N, Nawaz S, Stephenson JB, McWilliam RC, Hollman A. Muscle ultrasound in the assessment of suspected neuromuscular disease in childhood. Neuromuscul Disord 1999;9(4):203–7.
[7] Lukaski HC. Applications of bioelectrical impedance analysis: a critical review. Basic Life Sci 1990;55:365–74.
[8] Yamada Y, Ikenaga M, Takeda N, et al. Estimation of thigh muscle cross-sectional area by single- and multifrequency segmental bioelectrical impedance analysis in the elderly. J Appl Physiol 2014;116(2):176–82.
[9] McDonald CM, Carter GT, Abresch RT, et al. Body composition and water component measurements in boys with Duchenne muscular dystrophy. Am J Phys Med Rehabil 2005;84(7):483–91.
[10] Zaidman CM, Wang LL, Connolly AM, et al. Electrical impedance myography in Duchenne muscular dystrophy and healthy controls: a multicentre study of reliability and validity. Muscle Nerve 2015;52(4):592–7.
[11] Practical guideline for Duchenne muscular dystrophy (Societas Neurologica Japonica, Japanese society of child neurology, National center of neurology and psychiatry, 2014).
[12] Uchiyama T, Nakayama T, Kuru S. Muscle development in children evaluated by bioelectrical impedance analysis. Brain Dev 2017;39(2):122–9.
[13] Grimnes S, Martinsen OG. Bioimpedance and bioelectricity basics. 3rd ed. Amsterdam: Elsevier/Academic Press; 2015.
[14] Brooke MH, Fenichel GM, Griggs RC, et al. Duchenne muscular dystrophy: patterns of clinical progression and effects of supportive therapy. Neurology 1989;39(4):475–81.
[15] Vignos PJ Jr, Spencer GE Jr, Archibald KC. Management of progressive muscular dystrophy in childhood. JAMA 1963;184:89–96.
[16] Bedogni G, Merlini L, Ballestrazzi A, et al. Multifrequency bioelectric impedance measurements for predicting body water compartments in Duchenne muscular dystrophy. Neuromusc Disord 1996;6(1):55–60.
[17] Noshiro M, Morimoto T, Nagao H, Matsuda H. Electrical impedance in the lower limbs of patients with Duchenne muscular dystrophy: a preliminary study. Med Biol Eng Comput 1993;31(2):97–102.
[18] Mok E, Béghin L, Gachon P, et al. Estimating body composition in children with Duchenne muscular dystrophy: comparison of bioelectrical impedance analysis and skinfold-thickness measurement. Am J Clin Nutr 2006;83(1):65–9.
[19] Mok E, Letellier G, Cuisset JM, Denjean A, Gottrand F, Hankard R. Assessing change in body composition in children with Duchenne muscular dystrophy: anthropometry and bioelectrical impedance analysis versus dual-energy X-ray absorptiometry. Clin Nutr 2010;29(5):633–8.
[20] Miyatani M, Kanehisa H, Masuo Y, Ito M, Fukunaga T. Validity of estimating limb muscle volume by bioelectrical impedance. J Appl Physiol 2001;91(1):386–94.
[21] Takeuchi F, Yonemoto N, Nakamura H, et al. Prednisolone improves walking in Japanese Duchenne muscular dystrophy patients. J Neurol 2013;260(12):3023–9.
[22] Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis and pharmacological and psychosocial management. Lancet Neurol 2010;9(1):77–93.