



Normative data and percentile curves for the three-minute walk test and timed function tests in healthy Caucasian boys from 2.5 up to 6 years old

Jasmine Hoskens^{a,b,*}, Nathalie Goemans^c, Hilde Feys^a, Liesbeth De Waele^c, Marleen Van den Hauwe^c, Katrijn Klingels^{a,b}

^aFaculty of Kinesiology and Rehabilitation Sciences, Department of Rehabilitation Sciences, Neuromotor Rehabilitation Research Group, KU Leuven – University of Leuven, Herestraat 49, box 1510 B-3000 Leuven, Belgium

^bFaculty of Rehabilitation Sciences, Rehabilitation Research Center (REVAL), Hasselt University, Agoralaan gebouw D, B-3590 Diepenbeek, Belgium
^cDepartment of Child Neurology, University Hospitals Leuven, Herestraat 49, Leuven, Belgium

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Abstract

The three-minute walk test (3MWT) and timed function tests (TFTs) (rise from floor, 10 m run, climbing and descending four stairs) are currently used to evaluate functional capacity in young boys with neuromuscular disorders. This study aimed to generate normative data in healthy boys aged 2.5 up to 6 years for these tests and to provide percentile curves according to age and height. The relation between the 3MWT, TFTs and anthropometric variables was investigated. In total 179 boys (mean age: $4.1 \text{ y} \pm 1.0$) were evaluated across four age (2.5 years; 3 years; 4 years and 5 years) and three height groups: ($<100 \text{ cm}$; $100 \text{ to } <110 \text{ cm}$ and $\geq 110 \text{ cm}$). Three-minute walk distance (3MWD) increased significantly, from $168.4 \text{ m} (\pm 18.8)$ at 2.5 years to $214.5 \text{ m} (\pm 26.1)$ at 5 years and from $172.6 \text{ m} (\pm 21.8)$ for children $<100 \text{ cm}$ to $212.7 \text{ m} (\pm 26.2)$ for children $\geq 110 \text{ cm}$. TFTs times decreased significantly with age and height. Significant correlations between the anthropometric values, 3MWD and TFTs were found ($r(s) = 0.55\text{--}0.84$; $p < 0.0001$). These normative data and percentile curves provide a useful tool in the assessment of functional capacity in young boys. This study also confirms the association between functional tests and anthropometric values.

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1. Introduction

Duchenne Muscular Dystrophy (DMD) is a X-linked recessive inherited disorder characterized by a progressive muscle breakdown affecting skeletal, cardiac and respiratory muscles. A delay in motor development is one of the main characteristics of the early stage of this disease. However, several studies have also shown deficits in cognitive and language development, adaptive behavior and social emotional skills and neurobehavioral and neuropsychiatric impairments [1–7]. Even though the first signs show up early

in development, the average diagnosis is mostly only made around the age of four [8]. Usually, concerns only start to rise when children with DMD show signs of delay in global development and/or the achievement of developmental milestones [1,9]. An average delay of 2.5 years between onset of symptoms and final diagnosis has been found, which remained relatively stable over the last decades [8]. Independent sitting, walking and climbing stairs are the three motor milestones most likely to be achieved later compared to healthy and typically developing peers [1]. Other early presenting features of DMD include clumsiness, falling over, poor balance, subtle muscle weakness and an awkward, waddling gait [1,10].

In view of development in therapeutic approaches in DMD, early assessment and diagnosis might have important advantages [11–13]. Therapeutic interventions should start as

* Corresponding author at: Faculty of Kinesiology and Rehabilitation Sciences, Department of Rehabilitation Sciences, Neuromotor Rehabilitation Research Group, KU Leuven – University of Leuven, Herestraat 49, box 1510 B-3000 Leuven, Belgium.

E-mail address: jasmine.hoskens@kuleuven.be (J. Hoskens).

early as possible, before muscle weakness and other clinical features become distinct [11] aiming at preserving important motor milestones such as independent walking. Even though the importance of early intervention and assessment has been highlighted [13], only limited literature is available regarding the clinical presentation of DMD at young age. Subsequently, little is known concerning the assessment of functional capacity in very young boys with DMD.

The six-minute walk test (6MWT) is a widely used clinical measurement scale to assess functional capacity and endurance at submaximal level [14–21]. The 6MWT has already been validated in pediatric populations and has been proven to be a reliable tool for children with DMD [17,21–24]. In young children, the 6MWT can be challenging considering their higher risk of falling and the lack of cooperation due to cognitive or attentional deficits. McDonald et al. found a trend toward greater fall rate in boys with DMD than in healthy controls and the fall rate also tended to be higher in boys younger than 8 years old [24]. Secondly, boys with DMD seemed to require more prompts, reminders, and feedback than healthy boys during walking [24].

Bohannon et al. studied if a two-minute walk test (2MWT) would be a reliable alternative for the 6MWT in children and adults from 3 to 85 years old [25]. The distances covered over 2 and 6 min were highly correlated ($r = 0.968$, $p < 0.0001$), suggesting that the 2MWT would be a reliable alternative for the 6MWT to evaluate functional endurance [25]. However, Vill et al. reported a higher walking speed during the first minute compared to the other 5 min of the 6MWT, during which walking speed remained stable, in 13 boys with DMD between 5 and 11 years old [26]. Furthermore, an increase in test-retest reliability with prolonged time of walking was found in children between 5 and 12 years old [17]. Also in healthy boys between 5 and 6 years old, a moderate to good reliability was only established from three minutes onwards [17]. Therefore, Goemans et al. suggested that a three-minute walk test may be better suitable for younger boys [17].

Four other short functional capacity tests are the rise from floor, 10 m run (10 m run), climbing and descending four stairs. These Timed Function Tests (TFTs), are often used in clinical research and seem to be sensitive to detect changes in disease status in DMD [14,19,27]. The 10 m run and rise from floor test are also incorporated in the North Star Ambulatory Assessment (NSAA), a clinical outcome measure often used to assess gross motor function in ambulatory boys with DMD [23,28]. However, only few normative data are available, especially in young children under the age of four. Pereira et al. established reference values for the 10 m run, 10 m walk and rise from floor test in healthy children from 2 to 12 years old [29]. However, the rise from floor test was assessed starting from sitting position instead of supine [29]. Regarding stair climbing and descending no reference values are available in children.

In previous studies, predictive factors for walking distance, such as age and anthropometric variables have been explored [20,21,30,31]. Significant correlations between age, height

and weight and the 6MWT were found [20,21,30,31]. No studies investigated the relation between the anthropometric variables and the TFTs.

In summary, the 3MWT and the TFTs provide important information about endurance and other functional aspects crucial in everyday life of young boys with DMD. Still there is a need for adequate reference values in large samples of healthy children.

Therefore, the first aim of this study was to collect reference values and provide percentile graphs for the 3MWT and four TFTs (time to rise from the floor, 10 m run and climb and descend four stairs) in a large sample of healthy boys aged 2.5 up to 6 years old. A second aim was to explore the correlations between the different functional tests and relations with anthropometric variables. These insights are crucial when interpreting results of the functional capacity measurements in young boys with DMD, to monitor their motor evolution and to evaluate early interventions.

2. Material and methods

2.1. Participants

Healthy boys of 2.5 to 5.9 years of age were recruited in nine different schools in Belgium. Parents were asked to fill in a questionnaire in order to identify health-related problems. Children with developmental, cardiovascular, neurological or musculoskeletal disorders were excluded. All children had to understand and fully cooperate with the test procedure. Participants were divided into four age categories (2.5 years, 3 years, 4 years, 5 years) and three height categories (<100 cm, 100 to <110 cm, ≥ 110 cm).

Sample size calculation was performed by the Leuven Biostatistics and Statistical Bioinformatics Centre (L-BioStat). They sought to establish the precision of the estimated percentiles when 40 children per age group were included in the study. Hereto the 6MWT data obtained in a previous study in 442 children aged between 5 and 12 years old were analyzed [21]. A quantile regression was performed on the 6MWT results including the age categories as factors in the model. Estimated percentiles (p) and their standard errors (σ) were obtained for all age categories. Then, the standard error for the 3MWT (σ_{3MWT}) when only 40 patients are included, was calculated for each percentile and age category as follows:

$$\sigma_{3MWT}^2 = \sigma^2 \times \frac{n}{40} \times \frac{1}{2^2}$$

where n is the actual number of children in the age category. The factor $n/40$ corrects the standard error for the change in the number of children, the factor $1/2^2$ for the fact that walking is performed for three minutes rather than six. This implicitly assumes that a child walks half the distance when given half the time. Given the fairly constant pace with which children perform the test, this assumption was deemed reasonable. The predicted percentiles and recalculated standard errors were plotted and analyzed. The standard errors

were very small in comparison with the predicted values and they were fairly constant across the age categories. Extrapolating these results to the younger age groups between 2.5 and 5.9 there can be assumed that the estimates of the percentiles of the 3MWT in these age groups will be fairly precise.

To investigate the feasibility of the 3MWT and TFTs in young boys with DMD, 3MWT and TFTs were also performed in a small patient group. Boys, diagnosed with DMD aged between 2.5 and 6 years old were recruited via the Neuromuscular Reference Center of the University Hospital of Leuven. Ethical approval was obtained from the Ethics Committee Research UZ/ KU Leuven (S60777, S59068). Also, the institutional board of each school gave permission to perform the tests at their school and written informed consent was obtained from all parents.

2.2. Test procedure

Prior to the assessment, anthropometric parameters (length and weight) were measured using a standardized procedure. Subsequently, the 3MWT and TFTs were conducted by one out of three experienced physiotherapists. The total test procedure took about 15 min per child.

The 3MWT is a submaximal functional capacity test measuring the distance walked in a time span of 3 min. A standardized test protocol was used. The 3MWT was performed outdoors or indoors on a flat surface. Children had to wear comfortable clothes and shoes. A tape-measure of 25 m was placed on the ground with a cone at the beginning and end of the 25 m. At approximately 40 cm around both cones, tape was used to indicate the turning circle. The children walked in counterclockwise direction along the tape line for three minutes. Children were instructed to walk up and down along the tape line without stopping or running. Every boy underwent one practice trial to ensure he had understood the instructions. A stopwatch was used to record the time. Every minute, the distance covered was noted. During the test, the investigator walked behind the child as a ‘safety chaser’ and encouraged him in order to achieve the best possible performance, for example: “You are doing very well, keep up the good work,...”. Goemans et al. evaluated the test-retest reliability of the 6MWT in 90 healthy boys aged 5–12 years [17]. High values were found for the total group (ICC= 0.95) as well as for the different age categories (ICC= 0.80–0.94) [17]. From three minutes onward, this test has shown to be very reliable (ICC > 0.90) for the total group and also good reliability was found for the 5 year olds (ICC= 0.76) [17].

All participants also performed four timed tests: rise from floor test, 10m run, and climbing and descending four stairs. A chronometer was used to measure the time to complete each test. The quality of performance was also observed.

For the rise from floor test, starting position was supine with both arms next to the body and legs on the floor. This test was performed without shoes or socks. The instruction

was to get up to a standing position as fast as possible with arms along the body [32].

For the 10 m run test, the boys were asked to run as fast as possible from one point to another over a distance of 10 m. Each boy had to start with both feet behind the starting line. The time was stopped the moment the second foot cleared the finish line [29,32]. One practice trial was performed to ensure good understanding of the instructions. Continuous encouragements were given.

For the rise from floor test and 10 m run test, the quality scores of the North Star Ambulatory Assessment (NSAA) were used: 2 – normal, achieves goal independently; 1 – modified method but achieves goal without physical assistance; 0 – unable to perform the task independently [32].

The stairs tests were performed at stairs with handrails on both sides. To climb four stairs, the child was instructed to climb the stairs as fast as possible but in a safe manner until both feet were placed on the fourth step. To descend the stairs, the instruction was to descend the stairs as fast as possible in a safe manner until both feet were placed on the ground. The investigator was positioned closely to the child in order to ensure the safety.

For the stairs test following quality scores were used: 1 – impossible; 2 – both feet on one step, both arms holding the handrails; 3 – both feet on one step, one arm holding the handrails; 4 – both feet on one step, no use of handrails; 5 – alternating, with use of handrail; 6 – alternating, no use of handrails [16].

Test-retest reliability was evaluated by McDonald et al. in DMD boys aged 5–20 years and was found to be very high for the different timed items: rise from floor test: ICC=0.87; 10 m run: ICC=0.85; climb four stairs: ICC=0.91; descend four stairs: ICC=0.83 [16].

2.3. Statistical analysis

Descriptive statistics were applied for age, anthropometric variables, 3MWT and TFTs according to the different age and height categories. Mean and standard deviation or median and interquartile range were used depending on data distribution. To check the data for normality, the Shapiro–Wilk test and visual inspection of distribution plots were used. Parametric one-way analysis of variance (ANOVA) with post hoc Tukey HSD tests was used to analyze differences in three-minute walk distance (3MWD) and velocity between the different age and height categories. For the TFTs, a non-parametric one-way ANOVA (Kruskal–Wallis test) was used with post hoc pairwise comparisons using the Dunn–Bonferroni tests on each pair of groups.

To construct percentile curves, the 5%, 10%, 25%, 50%, 75%, 90% and 95% percentiles of the 3MWT and TFTs outcomes were estimated for different values of age and height using quantile regression analysis.

Furthermore, Pearson-product moment and Spearman rank correlation coefficients were calculated to determine correlations between the 3MWD and velocity, TFTs, age and height. Correlation coefficients of >0.70 were considered as

Table 1
Participants characteristics (mean values \pm standard deviation).

		N	Age (y)	Height (cm)	Weight (kg)
Age	2.5 years	44	2.8 \pm 0.1	93.9 \pm 3.3	14.6 \pm 1.8
	3 years	44	3.5 \pm 0.3	99.9 \pm 4.9	16.0 \pm 1.8
	4 years	46	4.5 \pm 0.3	107.8 \pm 4.4	18.6 \pm 1.8
	5 years	45	5.4 \pm 0.2	113.3 \pm 4.6	20.4 \pm 2.3
Height	<100 cm	69	3.0 \pm 0.4	94.8 \pm 3.3	14.7 \pm 1.6
	100 to <110 cm	56	4.2 \pm 0.6	105.2 \pm 2.9	17.6 \pm 1.3
	\geq 110 cm	54	5.2 \pm 0.4	114.0 \pm 3.5	20.7 \pm 2.0
Total group		179	4.1 \pm 1.0	103.8 \pm 8.6	17.4 \pm 3.0

high, between 0.50 and 0.70 as good or moderate, between 0.30 and 0.50 as fair and of <0.30 as weak or no correlation [33]. An alpha level of less than 0.05 was considered significant.

3MWD and TFTs results of the DMD boys were individually compared to the normative data and percentile curves.

The statistical analysis was performed using SPSS 24.0.

3. Results

3.1. Participants

Parents of 308 boys, were invited to participate in this study. In total 192 written informed consents and medical questionnaires were returned. Thirteen children were excluded. One boy was excluded because of a clubfoot, 10 boys were absent or refused to cooperate and two boys were excluded because of deviating values due to running or exhaustion during the 3MWT. Finally, 179 boys completed the full test procedure. Mean age, height and weight of the total group were 4.1 years (\pm 1.0), 103.8 cm (\pm 8.6) and 17.4 kg (\pm 3.0), respectively. All boys were healthy, Dutch-speaking and Caucasian. Overall, the mean Body Mass Index (BMI) of all children was 16.1 (SD: 1.2). Table 1 reports age and anthropometric data per age and height subcategory and for the total group.

Eleven DMD boys (mean age: 4.9 years; SD: 0.6; range: 3.9–5.9 years) also performed the 3MWT and TFTs. The mean height and weight was 106.5 cm (\pm 6.2) and 19.7 kg (\pm 2.8).

3.2. 3MWT and TFTs according to age and height

Descriptive data for the 3MWT and TFTs are given in Tables 2A and 2B.

The mean three-minute walk distance (3MWD) was 191.2 m (\pm 30.4 m). An increase in mean 3MWD was seen from 168.4 m (\pm 18.8 m) at the age of 2.5 years to 214.5 m (\pm 26.1 m) at 5 years. The largest increase in distance between two subsequent age groups was 23.5 m, between the 3 and 4 year olds. The average velocity remained fairly constant during the 3MWT. The mean distance covered after one minute was 33.8% (\pm 1.9%) of the total distance and 66.8% (\pm 1.7%) after two minutes. For the total group, a significant

difference in meters walked was found between the first (mean= 64.6 m; SD= 11.1 m) and third minute (mean= 63.4; SD= 9.9 m). The mean difference was 1.2 m ($p=0.01$). Also for the five year olds, a small but significant difference in favor of the first minute was found (mean difference 2.0 m; $p=0.04$). For the first three age groups, comparable distances were walked in the first and third minute ($p=0.07$ – 0.59). Velocity increased with age from 56.1 m/min (\pm 6.3 m/min) at 2.5 years to 71.5 m/min (\pm 8.7 m/min) at 5 years. The 3MWD also increased according the three height categories from 172.6 m (\pm 21.8) in children <100 cm to 212.7 m (\pm 26.2) in children \geq 110 cm. In parallel, an increase in velocity was seen from the shortest group (57.5 m/min (\pm 7.3)) to the tallest group (70.9 m/min (\pm 8.7)). Fig. 1(A) shows the mean 3MWD and standard deviation per age and per height group.

One way ANOVA revealed significant differences in 3MWD between age groups ($p < 0.0001$). Post hoc analyses showed significant differences between 2.5 and 4 years ($p < 0.0001$), 2.5 and 5 years ($p < 0.0001$), 3 and 4 years ($p < 0.001$) and 3 and 5 years ($p < 0.0001$). No significant difference was found between the 2.5 and 3 ($p=0.21$) and the 4 and 5 year age groups ($p=0.08$). According to height, one way ANOVA and post hoc analysis showed significant differences between all different groups ($p < 0.0001$) (Fig. 1(A)).

All children were able to perform the four TFTs independently. Almost all children were able to rise from the floor without any components of the Gower's maneuver. Only in the youngest age groups very few children rolled spontaneous to prone to stand up or used a hand on the thigh to come up. All children were able to run with an appropriate swing phase and without double stance phase. The median quality scores (range) to climb 4 stairs evolved from 3.5 (2–6) at 2.5 years to 6 (5–6) at 5 years of age; and to descend 4 stairs from 3 (2–6) in the youngest children and 6 (3–6) in the oldest children.

The median times (IQR) to complete the four TFTs for the total group were: 2.63 s (2.12–3.47 s) to rise from the floor; 4.06 s (3.62–4.75 s) to run 10 m, 2.31 s (1.89–3.46 s) to climb and 2.89 s (2.15–4.62 s) to descend four stairs. Fig. 1(B) shows boxplots with IQR for each TFT, according to the different age and height groups. An overall decrease in median time was seen with increasing age and height.

Significant differences were found for all TFTs (rise from floor, 10 m run, climbing and descending 4 stairs) between

Table 2(A)

Distance and velocity after one, two and three minutes for the different age and height categories (mean values and standard deviations).

	Age				Height			Total group
	2.5 years	3 years	4 years	5 years	<100 cm	100 to <110 cm	≥110 cm	
1 min distance (m)	56.9 ± 6.2	60.3 ± 9.5	68.4 ± 10.3	72.4 ± 10.3	58.1 ± 8.2	65.5 ± 10.4	71.9 ± 10.3	64.6 ± 11.1
%3MWD after 1 min	33.9 ± 2.5	33.7 ± 1.8	33.8 ± 1.6	33.7 ± 1.6	33.7 ± 2.3	33.9 ± 1.6	33.7 ± 1.6	33.8 ± 1.9
2 min distance (m)	112.2 ± 12.6	118.8 ± 17.1	135.2 ± 19.1	144.1 ± 18.9	114.8 ± 15.3	129.1 ± 19.6	142.9 ± 19.0	127.8 ± 21.2
%3MWD after 2 min	66.7 ± 1.8	66.4 ± 1.7	66.9 ± 1.4	67.1 ± 1.7	66.5 ± 1.8	66.8 ± 1.3	67.1 ± 1.8	66.8 ± 1.7
3MWD (m)	168.4 ± 18.8	178.6 ± 23.8	202.1 ± 27.8	214.5 ± 26.1	172.6 ± 21.8	193.2 ± 29.1	212.7 ± 26.2	191.2 ± 30.4
Velocity (m/min)	56.1 ± 6.3	59.5 ± 7.9	67.4 ± 9.3	71.5 ± 8.7	57.5 ± 7.3	64.4 ± 9.7	70.9 ± 8.7	63.7 ± 10.1

m: meters; 3MWD: three-minute walk distance; min: minutes; cm: centimeters.

Table 2(B)

Timed function tests results for the different age and height categories (median values and interquartile ranges).

	Age				Height			Total group
	2.5 years	3 years	4 years	5 years	<100 cm	100 to <110 cm	≥110 cm	
Rise from floor (s)	3.7 (3.2–4.9)	3.0 (2.6–3.6)	2.4 (2.1–2.8)	2.0 (1.8–2.3)	3.5 (2.9–4.7)	2.6 (2.1–3.0)	2.1 (1.9–2.5)	2.6 (2.1–3.5)
10 m run (s)	5.1 (4.5–5.8)	4.5 (4.0–4.9)	3.8 (3.5–4.1)	3.5 (3.3–3.9)	4.9 (4.3–5.6)	3.9 (3.6–4.4)	3.6 (3.3–4.0)	4.1 (3.6–4.8)
Velocity 10 m (m/s)	2.0 (1.7–2.2)	2.2 (2.0–2.5)	2.6 (2.5–2.8)	2.8 (2.5–3.0)	2.1 (1.8–2.3)	2.5 (2.3–2.8)	2.8 (2.5–3.0)	2.5 (2.1–2.8)
Climb 4 stairs (s)	3.7 (3.0–5.0)	2.8 (2.3–3.6)	2.1 (1.9–2.4)	1.8 (1.6–1.9)	3.5 (2.7–4.6)	2.2 (1.8–2.8)	1.9 (1.6–2.1)	2.3 (1.9–3.5)
Velocity climbing (steps/s)	1.1 (0.8–1.3)	1.4 (1.1–1.7)	1.9 (1.7–2.1)	2.2 (2.1–2.5)	1.2 (0.9–1.5)	1.9 (1.4–2.2)	2.1 (1.9–2.4)	1.7 (1.2–2.1)
Descend 4 stairs (s)	6.1 (5.0–7.1)	3.7 (3.0–4.4)	2.4 (2.1–2.8)	2.0 (1.7–2.4)	5.0 (3.8–6.6)	2.6 (2.1–3.3)	2.1 (1.8–2.5)	2.9 (2.2–4.6)
Velocity descending (steps/s)	0.7 (0.6–0.8)	1.1 (0.9–1.3)	1.7 (1.4–1.9)	2.0 (1.7–2.3)	0.8 (0.6–1.1)	1.5 (1.2–1.9)	1.9 (1.6–2.2)	1.4 (0.9–1.9)

s: seconds; m: meters; cm: centimeters.

age and height categories ($p < 0.0001$). Post hoc tests revealed significant differences between 2.5 and 4; 2.5 and 5; 3 and 4 and 3 and 5 year olds for all four TFTs. No significant difference was found for the rise from floor test between age groups 2.5 and 3 years ($p = 0.06$). Between age groups 4 and 5 years no significant differences were found for the 10 m run and descending 4 stairs ($p = 0.65$ and 0.09 , respectively). According to height, the three groups differed significantly for all four TFTs (p -values ranging between $p < 0.0001$ and $p=0.03$) (Fig. 1(B)).

Statistical comparison according to age and height revealed similar results regarding velocity on the 3MWT, 10 m run, climbing and descending 4 stairs tests. The corresponding figures can be found in Appendix A.

Percentile curves with percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 3MWD and times to complete the different TFTs according to age are presented in Fig. 2(A) and (B). Appendix B shows percentile curves regarding height for the 3MWD. Percentile curves for velocity on the 10 m run, climbing and descending four stairs are provided in Appendix C.

3.3. Correlations between 3MWT, TFTs and anthropometric values

Significant correlations were found between the 3MWD, four TFTs and the anthropometric values (age, height and weight) ($p < 0.0001$) (Table 3). Correlations between the

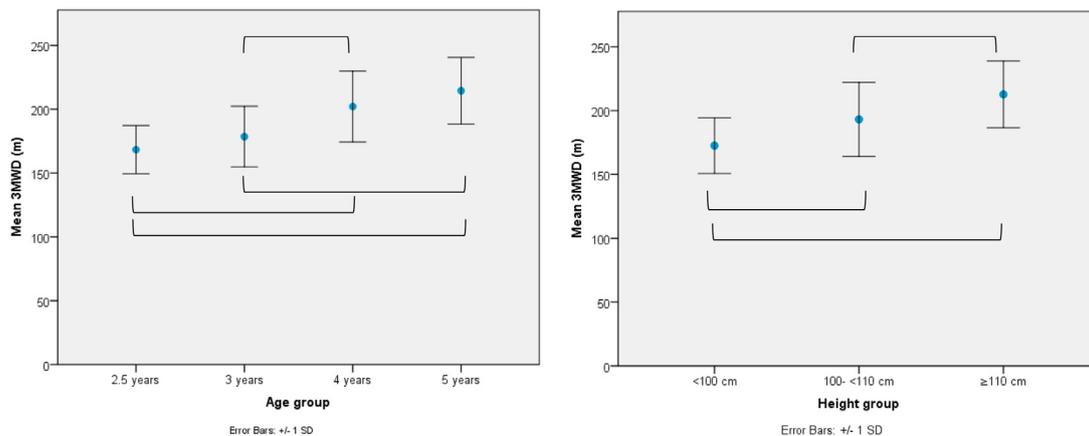


Fig. 1(A). Mean 3MWD and standard deviation of the 3MWD per age and height group. (the brackets indicate significant post-hoc differences).

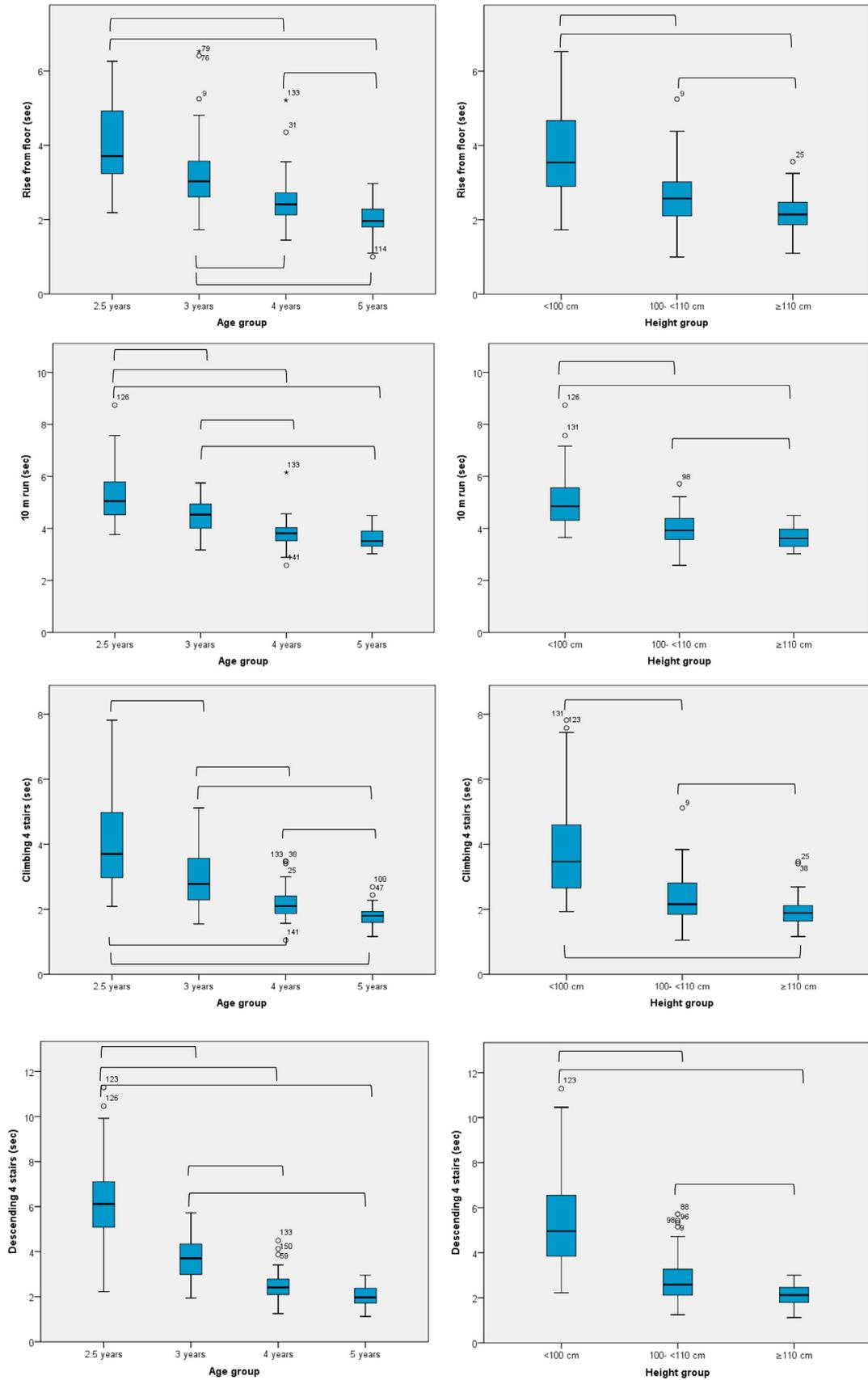


Fig. 1(B). Box plots of median time and Interquartile Range (IQR) with T-bars of the different timed function tests per age and height group. (the brackets indicate significant post-hoc differences).

Table 3
Correlations between 3MWT, TFTs and the anthropometric values.

	3MWD (<i>r</i>)	Rise from floor (<i>rs</i>)	10 m run (<i>rs</i>)	Climb 4 stairs (<i>rs</i>)	Descend 4 stairs (<i>rs</i>)
Age	0.61*	−0.72*	−0.73*	−0.79*	−0.84*
Height	0.57*	−0.65*	−0.69*	−0.73*	−0.78*
Weight	0.55*	−0.58*	−0.64*	−0.67*	−0.70*
3MWD		−0.65*	−0.60*	−0.67*	−0.62*
Rise from floor			0.75*	0.82*	0.74*
10 m run				0.78*	0.74*
Climb 4 stairs					0.84*

3MWD: 3-minute walk distance; *r*= Pearson-product moment correlation coefficient; *rs*: Spearman rank correlation coefficient.

* Significant at the *p* < 0.0001 level.

functional tests and age were all above 0.70 except for the 3MWD (*r* = 0.61). For height, correlations with the functional tests ranged between 0.57 and 0.78 and for weight between 0.55 and 0.70. The 3MWD was moderately correlated with the four TFTs (*rs*= −0.60 to −0.67). All four TFTS were highly intercorrelated with coefficients ranging from 0.74 to 0.84.

3.4. Data on DMD patients

The DMD boys walked a mean distance of 184.9 m (±25.4) in three minutes. Compared to the age percentile graphs, the 3MWD of six boys was between percentile (P) 5 and 25. Three boys walked a distance between P25 and 50, and only two boys walked a distance just above P50 for their age. Regarding height, one boy walked a distance below P5; three boys between P5–P25; three other boys between P25–P50 and four boys walked a distance above P50.

The median times (IQR) on the TFTs for the DMD boys were: rise from floor: 3.65 s (2.74–4.59); 10 m run: 5.71 s (3.98–6.50); climbing 4 stairs: 2.78 s (1.96–4.93) and descending 4 stairs: 3.47 s (2.30–3.87). Compared to the age percentile graphs, times to rise from floor and 10 m run of seven boys and times to climb and descend 4 stairs of six boys were below P5. Two boys showed times above P50 on the rise from floor test and three boys on the climbing 4 stairs test. One boy ran 10 m very fast (>P95) and another boy outperformed in descending 4 stairs (±P95). All other times ranged between P5 and P50.

4. Discussion

The aim of this study was to provide normative data and percentile curves for the 3MWT and TFTs as a reference for use in young DMD boys. Age- and height-specific data were established in healthy Belgian boys aged 2.5 up to 6 years old. Furthermore, the relation between the functional tests was investigated as well as the relation with anthropometric

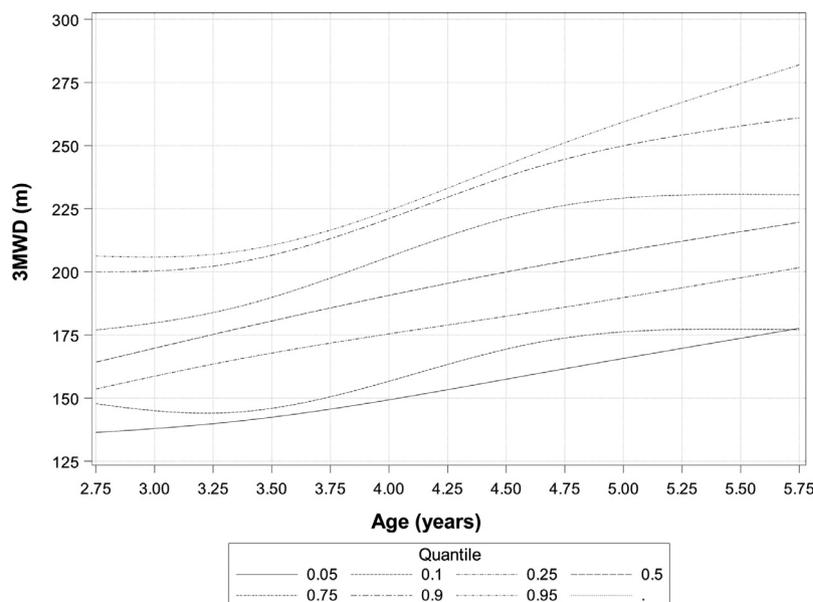


Fig. 2(A). Plot of estimated percentiles of three-minute walk distance versus age. Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 3MWD were estimated for different values of age.

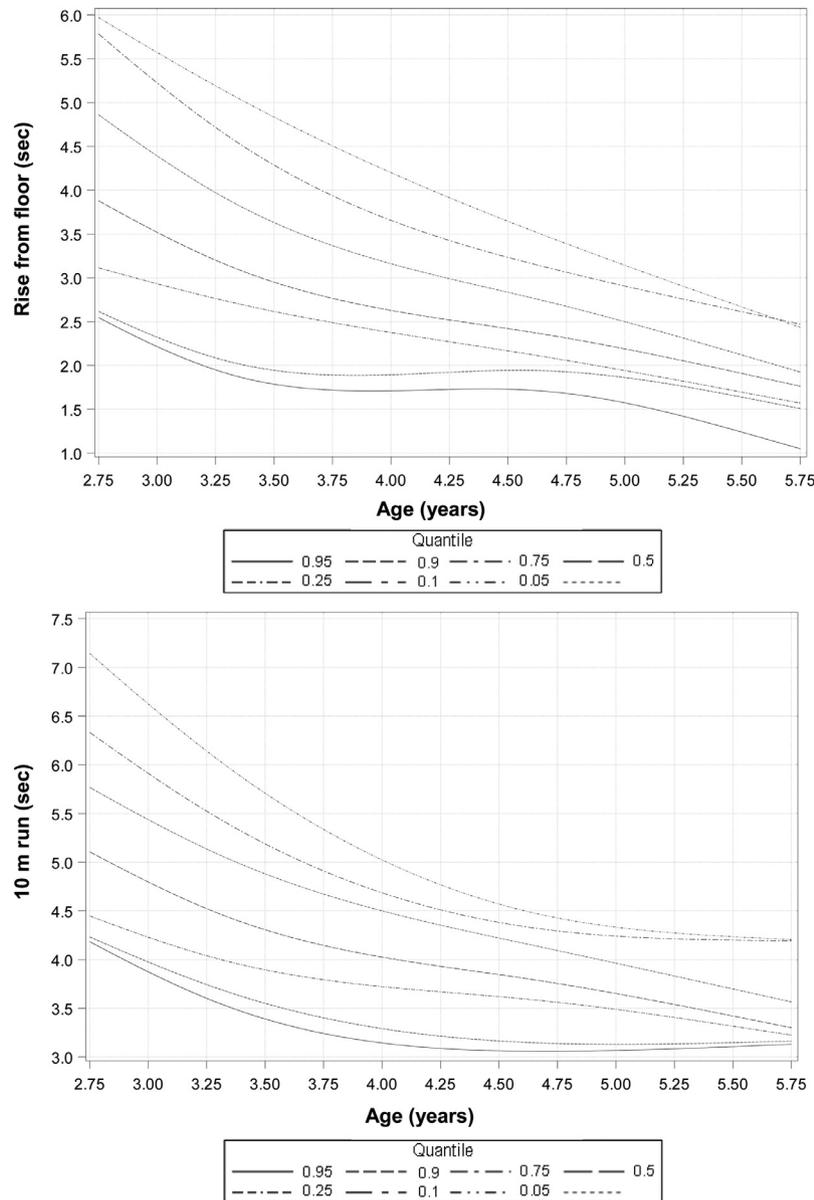


Fig. 2(B). Plot of estimated percentiles for the four timed function tests versus age. Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% were estimated for different values of age.

variables. Finally, to confirm the clinical feasibility of the tests and the usefulness of the percentile curves in young boys with DMD, the tests were performed in 11 young DMD boys.

In early childhood, between 2 and 6 years old, children expand their movement repertoire by acquiring fundamental movement behaviors, such as balance, walking, running, jumping, throwing, catching. [34]. This period of movement foundation is the basis for the formation of more complex motor programs, which are acquired at a later age. Around this age, boys with DMD also still improve in their motor skills [14,22]. Comparison with normative values of healthy boys as established in this paper allows the interpretation of motor abilities of young DMD boys against normal growth and development.

For the 3MWT, an increase in distance and consequently in velocity with age and height was seen. However, this increase was not linear across age categories. The largest and significant increase in distance between two subsequent age groups was seen between the 3 and 4 year olds. Step length, cadence and walking velocity show evidence of both central nervous system maturation and growth until approximately 4 years of age [35]. For example, younger children walk with a wider base of support. As they mature, they gain better control over their body mass on the supporting limb, because of greater coordination and improved balance [35,36]. Around the age of 4 to 5 years, children also establish the possibility to create a controlled forward propulsive force by pushing on the leg that supports their body weight rather than falling from one leg into the other [37]. This results in a positive

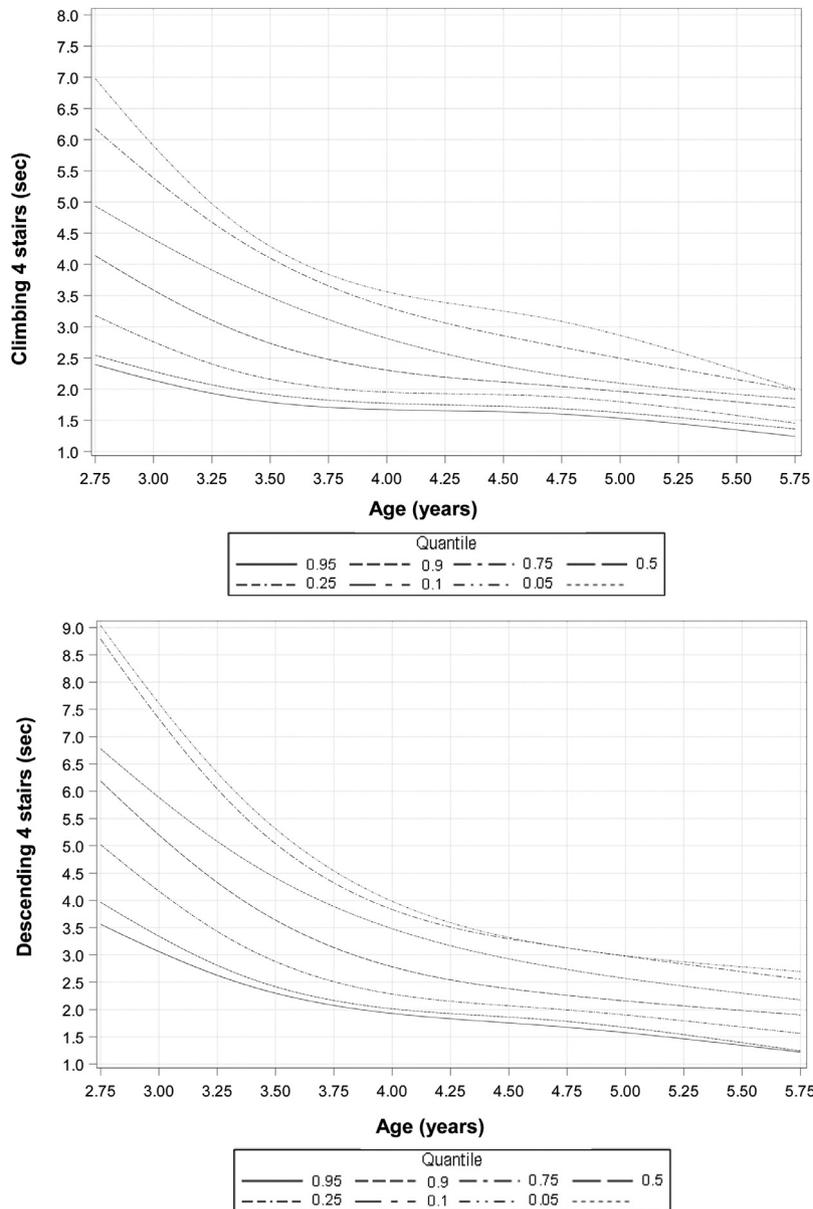


Fig. 2(B). Continued

vertical acceleration of the center of mass [37]. Differences in power generation are also seen between the 3 and 4 year olds. The youngest walkers use their hip flexor and extensor more than their ankle plantar flexor for power generation [35]. The gain in control and more efficient power generation might explain the significant increase in walking distance between the 3 and 4 year olds in our study. According to height, a more steady increase in 3MWD was seen with significant differences between each subsequent height group. This might be explained by the fact that total body height has a strong positive correlation with leg length and step length. However, both growth and the maturation process influence the time-distance parameters in children up to the age of 4 years old [35].

Overall, the 3MWT test was feasible for all participants. However, the youngest children needed more encouragement,

especially at the turning point. It could be questioned whether the two-minute walk test would be an alternative for the youngest children. However, the excluded children started running or refused to cooperate at the beginning of the test procedure and none of the children were excluded due to problems in the last minute of the test procedure. Moreover, Goemans et al. [17] found only good reliability from three minutes onwards (ICC= 0.76) in five year old children, whereas for the first two minutes lower ICC's were found (ICC= 0.39 and 0.42, respectively). Vill et al. [26] studied the 6MWT in 13 boys with DMD aged 5 to 11 years old. They also found a lower ICC after two minutes (ICC= 0.61), which became higher with longer duration of the test up to 0.84 after 6 min [26]. They did not report the ICC after three minutes [26]. Furthermore, they reported a higher walking speed during the first minute compared to the other five

minutes of the 6MWT. We also found small significantly longer distances covered in the first minute compared to the third minute for the total group and the five year olds. On the other hand, Bohannon et al. [25] compared the walking performance over the first 2 min and the full 6 min of the 6MWT. They found high correlations between the distance covered after 2 and 6 min ($r = 0.97$, $p < 0.0001$), suggesting that the 2MWT would be a reliable alternative for the 6MWT to evaluate functional endurance. Altogether, despite these high correlations between 2MWD and 6MWD [25], we would still recommend to use a three-minute protocol. The 3MWT has a good feasibility, increases the reliability and minimizes the effect of the increased walking speed in DMD boys during the first minute.

Similar to the 3MWT, a better performance on the TFTs was observed with increasing age and height, resulting in a decrease in time to perform the rise from floor test, 10 m run, descending and climbing 4 stairs. *The rise from floor test* showed a significant decrease in time, starting from the age of 3. Between the age groups 2.5 and 3 years, a smaller decrease in time only reaching borderline significance was seen. Almost all boys were able to rise from the floor without showing Gower's symptoms. However, some boys in the youngest age categories rolled over to prone or needed to take support with one hand on a leg to stand up. This can also be explained by the possible maturation process of this functional skills. The study of Pereira et al. showed similar results, but there was a difference in test protocol [29]. They started from a sitting position, while we started from supine.

Regarding the 10m run, all participants were able to run with a swing phase (both feet clearing the ground) and without a double support phase. Our findings are consistent with other data concerning running age, where a swing phase can be seen starting from the second birthday [38]. An overall decrease in time to run 10 m was seen in children from 2.5 to 5 years old. This decrease was also significant between age groups until the age of four. We found a smaller non-significant decrease between age groups 4 and 5 years. Pereira et al. reported the same evolution in time for children between 2 and 6 years old [29].

The climbing 4 stairs test was the only TFT which showed significant differences between all consecutive age groups with a gradual decrease from 3.7 s at 2.5 years to 1.8 s at 5 years. Healthy children are assumed to walk upstairs alone with both feet on each step at an average age of 20–24 months [38]. All children included in our study were able to climb stairs independently. We also observed how the boys climbed the stairs, using quality scores. Normally, children will be able to climb stairs by placing one foot on each step around approximately 35–36 months, or the age of 3 [38]. From the 2.5 and 3 year olds in our study, respectively, 36% and 70% of the children placed one foot on each step. Most of the 2.5 year olds still needed the handrail. All 4 and 5 year old children climbed the stairs alternating and most of them did not need the handrail. We have to remark that the boys were free to choose how to climb the stairs. The only instruction

given was to do it as quickly as possible, but in a safe way.

We used the same procedure and instructions for *descending the stairs*. To walk downstairs, you need eccentric contractions, which are more difficult than concentric contractions during climbing. Overall, children achieve the descending stair milestones later than the corresponding climbing milestones. Walking downstairs alone with both feet on each step occurs on average at 25 months, or 2 years of age [38]. Placing one foot on each step is achieved at 43–44 months, or 3.5–4 years of age [38]. Quality scores of the boys in our study were overall lower for descending stairs compared to the climbing, but increased with age. Of the 2.5 year olds, 91% of the boys placed both feet on each step and most of them also needed the handrail, while at 5 years, 71% of the children descended the stairs alternating and most of them without using the handrail. As expected, the time needed to descend four stairs was higher compared to the time needed to climb four stairs, and the timed recorded evolved on average from 6.1 s to 2.0 s according to the age subcategories.

Our study is the first to describe reference values according to height for the 3MWT and TFTs. Three-minute walk distance increased significantly with increasing height. In several studies regarding walking distance, height was described as a relating factor to walking distance [21,30,31,39,40]. However, only two studies also reported specific reference standards for the 6MWT according to height [21,41]. No studies reported height related reference values for the four TFTs. We found a significant decrease in time to perform the different TFTs with increasing height. It is known that boys with DMD are shorter than their healthy peers and the introduction of corticosteroid has beneficial effects on muscle function but slows growth further [42]. This emphasizes the importance of height specific reference values next to age related reference values for young boys with DMD.

For the TFTs, the percentile graphs show higher variability in scores for the younger children compared to the older children. This is much less the case for 3MWT. The TFTs are short snapshots of functional skills, which might result in variability between children, while the 3MWT is more consistent, especially at a young age. The variability for the TFTs might be explained by the fact that children at this young age are still more in initial stages of skill acquisition [38]. Subsequently, cognitive and behavioral factors, such as understanding of the test procedure and attention can cause possible variation in scores, especially in very young children.

We found significant moderate to high correlations between the 3MWD and all TFTs, as well as with the anthropometric values. This is in line with the results of other studies including walking protocols and timed tests in children [17,23,25,27]. The 3MWT is moderately correlated to all four TFTs indicating that both tests still assess different aspects of motor development. The 3MWT requires more endurance, whereas the TFTs are more related to explosive strength. Our

results point to the fact that both tests evaluate complementary aspects of motor development and therefore the combination of these tests is useful to have a broad view on the functional capacity of the child.

The 3MWT and TFTs were also feasible in young boys with DMD. Overall, variable results were found. Although a few boys achieved low scores on the 3MWT, 3MWD seemed to range more within the normal range in most of the DMD patients. While obviously slower times were found regarding the TFTs in several boys. Other studies confirm these findings by stating that the TFTs are more sensitive than muscle testing or gait assessment to assess disease progression [27,43–45]. Our findings show the clinical usefulness of the percentile curves to interpret the individual test results and to monitor disease progression over years.

This study is prone to a few limitations. The reliability of the 3MWT and TFTs has not yet been investigated in children from 2.5 to 6 years old. Goemans et al. performed the 6MWT in healthy boys from 5 years onward [17]. They reported a good reliability of the test after three minutes [17]. This indicates that the 3MWT can be reliable in young boys, but further examination is needed to assess the test retest reliability. Secondly, the assessments of the boys were done by three experienced assessors. Slight differences in encouragements, enthusiasm and instructions might have influenced the performance of the boys. However, to limit inter-rater variability a standardized protocol was followed. Subsequently, variability in the test scores can also be explained by child related factors. Cognitive and behavioral factors can cause possible variation in scores, especially in young children and may also be a challenge in young boys with DMD. Other possible influencing factors are participation in sports activities, genetic predisposition or movement experience. In view of the comparison with DMD boys our study only included boys. Future studies should also include young girls to enable the use of the 3MWT and TFTS in the evaluation of other not gender-related childhood disorders. Normative data in young children would also be useful for other endpoints such as the 2MWT, 6MWT and 100 m run/walk.

Despite the limitations, this study was the first to establish age and height specific reference data for the 3MWT and TFTs in a cohort of healthy Belgian boys aged 2.5 up to 6 years. Based on the results of this study, previous findings [17,24] and our clinical expertise, we would recommend to use the 3MWT in children up to 5 years old. The 3MWT and TFTs are feasible and safe in young boys and provide important information about the functional capacity.

5. Conclusions

The 3MWT and four TFTs (rise from floor test, 10 m run, climbing and descending 4 stairs) proved to be feasible, cheap and safe measures of submaximal endurance and functionality, especially in young children. This study generated normative data and percentile curves, which are crucial when evaluating the disease progression, response to therapeutic treatment and natural history of boys with DMD.

Acknowledgments

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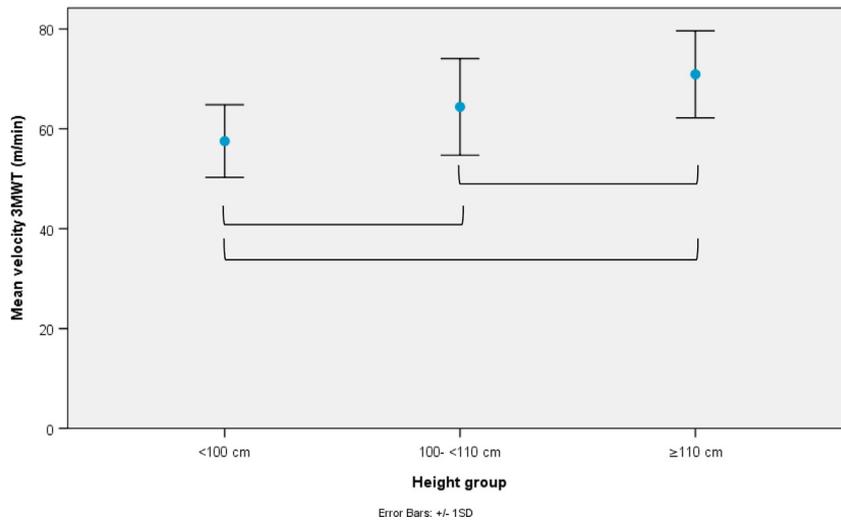
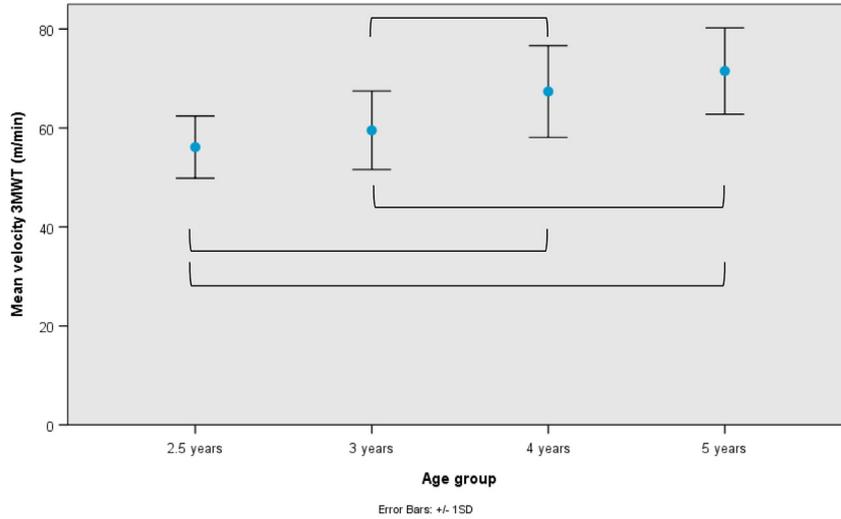
Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.nmd.2019.06.597](https://doi.org/10.1016/j.nmd.2019.06.597).

Appendix A

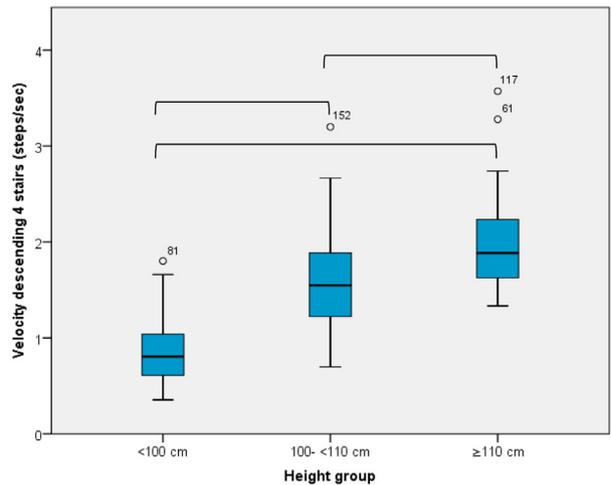
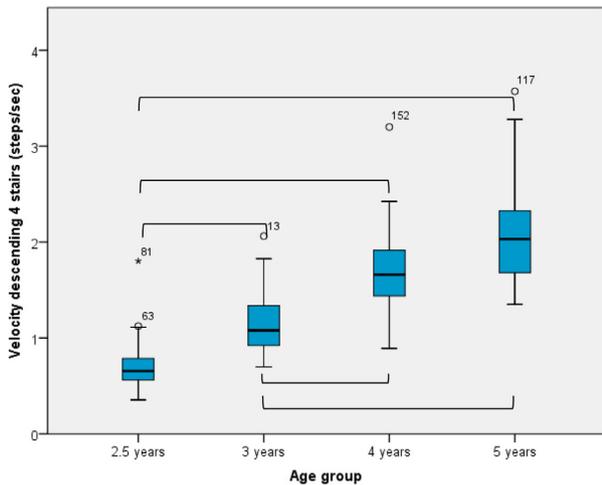
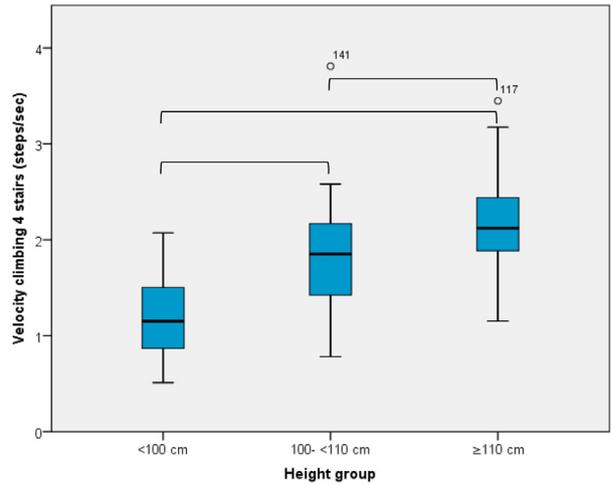
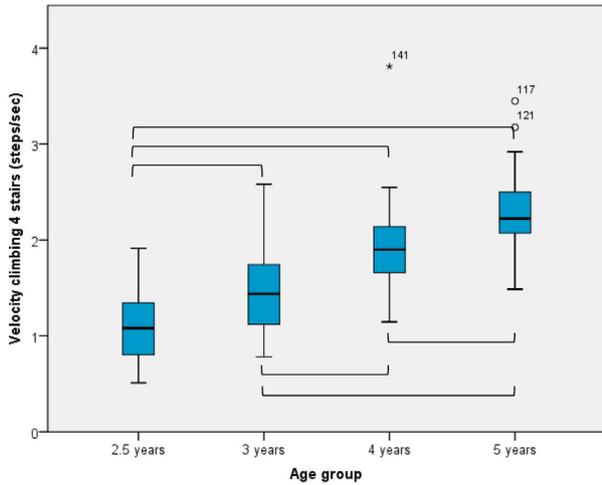
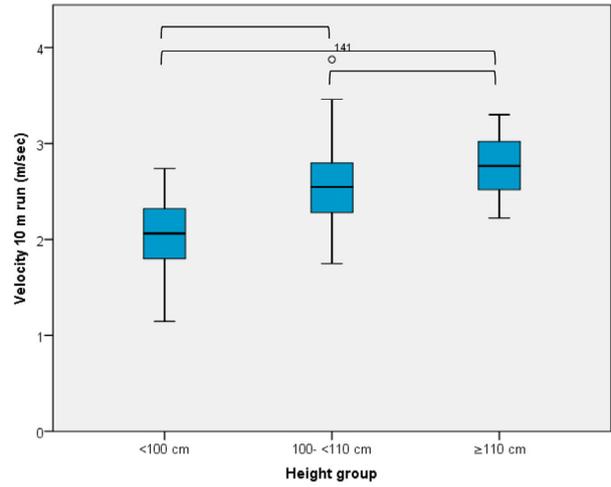
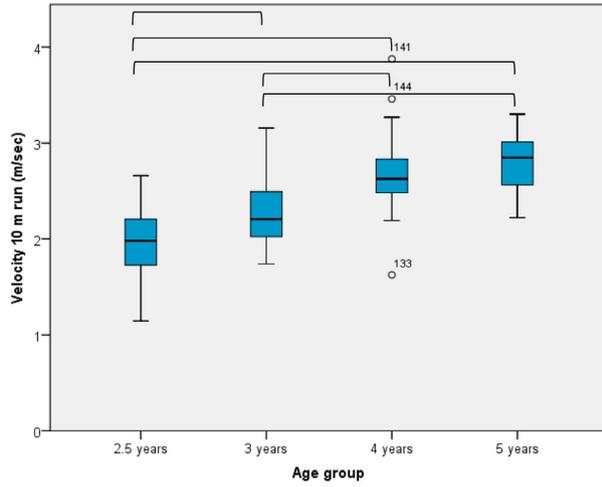
A. Mean velocity on the 3MWT and standard deviation per age and height group.

(the brackets indicate significant post-hoc differences)



B. Box plots of median velocity and Interquartile Range (IQR) with T-bars of the 10 m run, climbing and descending 4 stairs per age and height group.

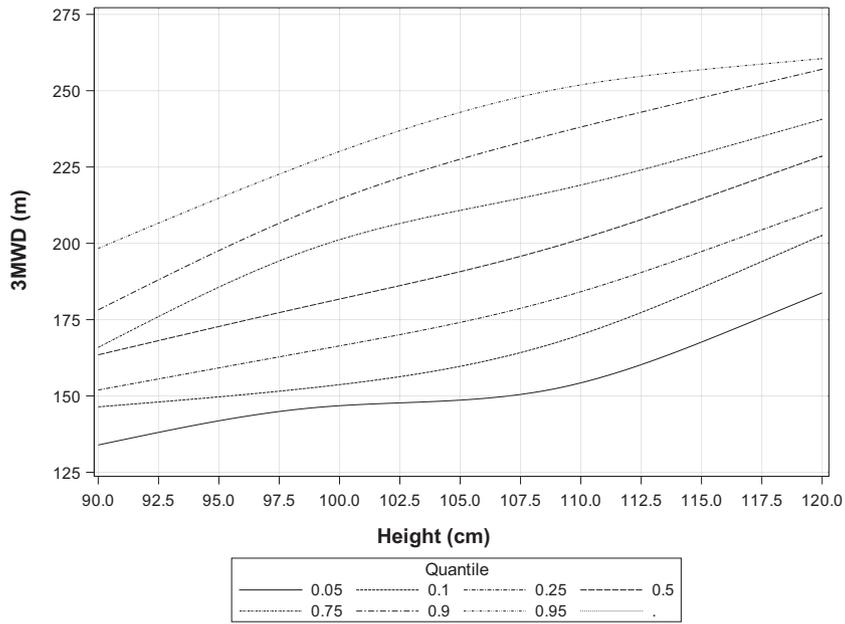
(the brackets indicate significant post-hoc differences)



Appendix B

Plot of estimated percentiles of three-minute walk distance versus height.

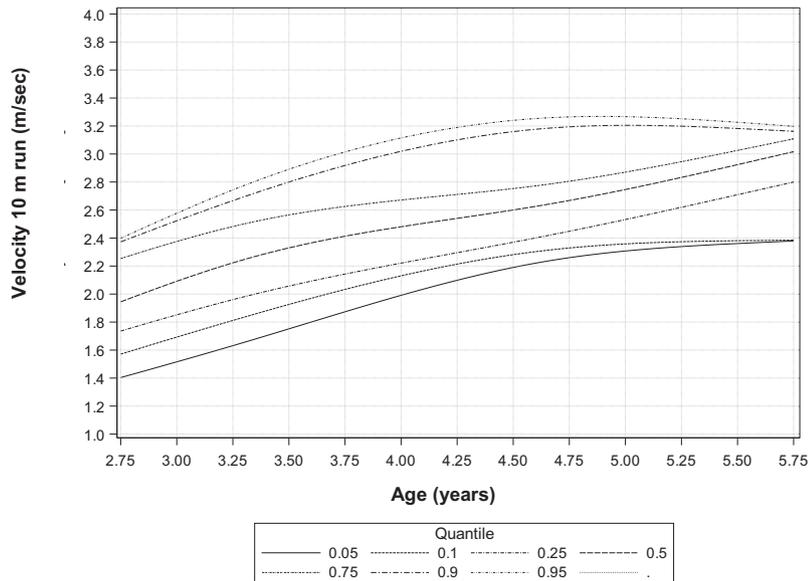
Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 3MWD were estimated for different values of height.

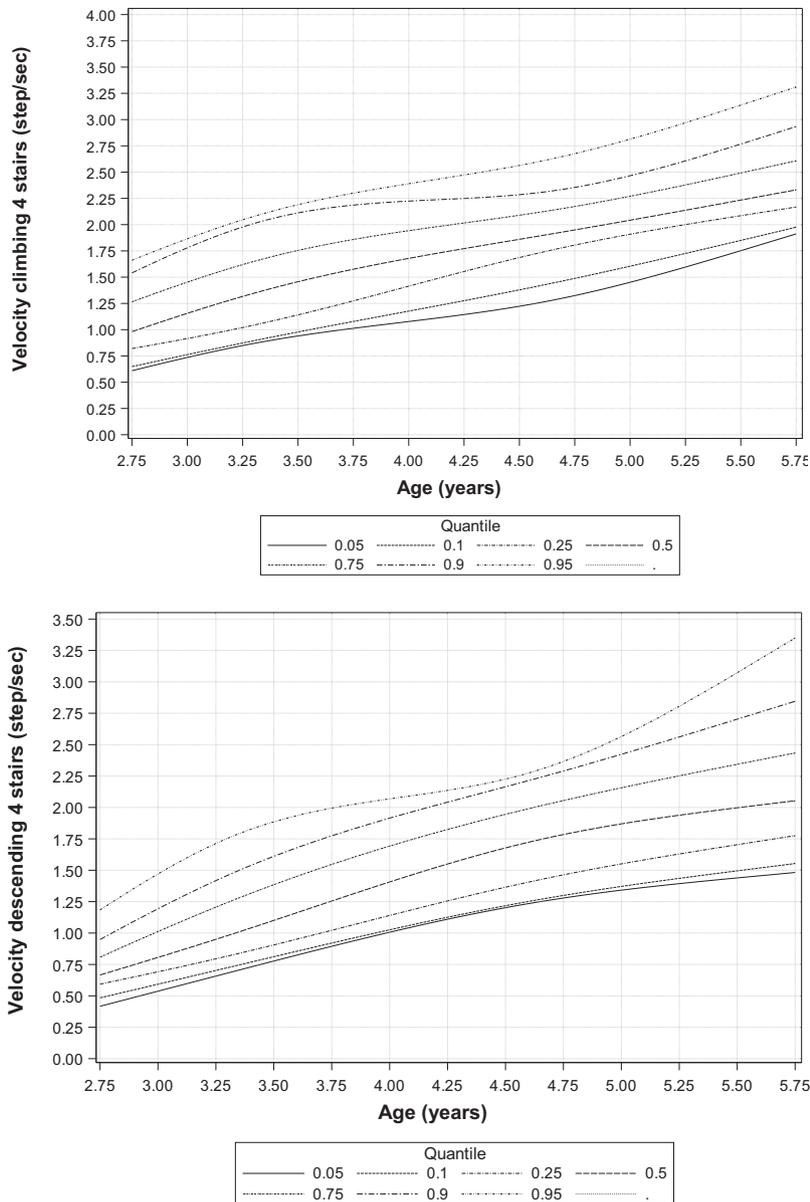


Appendix C

Plots of estimated percentiles for velocity on 10 m run, climbing and descending four stairs versus age.

Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% were estimated for different values of age.





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