THE ASSISTED 6-MINUTE CYCLING TEST TO ASSESS ENDURANCE IN CHILDREN WITH A NEUROMUSCULAR DISORDER

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ABSTRACT: *Introduction*: For late- or non-ambulant children with a neuromuscular disorder no suitable endurance tests are currently available. We developed the assisted 6-minute cycling test (A6MCT) for the legs and arms and investigated its psychometric properties in healthy boys and boys with Duchenne muscular dystrophy (DMD). *Methods*: Ninety-nine healthy boys and 30 boys with DMD (12 wheelchair-dependent) performed the A6MCT. Seventy healthy boys also performed the 6-minute walk test (6MWT), and 23 boys performed the A6MCT twice within 2 weeks. Boys with DMD also performed the Motor Function Measure (MFM). *Results*: The A6MCT was feasible for >90% of all boys. Boys with DMD achieved fewer cycling revolutions than controls. The A6MCT was positively correlated with the 6MWT and was reproducible in healthy boys, and it correlated with disease severity in boys with DMD. *Conclusions*: The A6MCT is a promising outcome measure for the follow-up of non-ambulant children with a neuromuscular disorder.

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Neuromuscular disorders (NMD) in childhood are heterogeneous, but all have the general feature of progressive loss of muscle function. Although there is currently no cure for most NMD, promising treatments are now becoming available.[1] To monitor disease progression and the effect of such new treatments,[2] clinically meaningful outcome measures are needed in relation to children's abilities to function in daily life. Endurance tests are often used as such an outcome measure in many clinical trials in children with NMD,[1] because they have been proven to correlate with functioning in daily life.[3,4]

Endurance exercise protocols for children with NMD can be divided into maximal ("all-out") and submaximal tests.[5] During all-out tests, children are motivated to reach their maximal mechanical power and aerobic peak, that is, the highest volume of oxygen that can be consumed by the body per time unit. Such a maximum test is often not feasible for children with NMD, who cannot reach their aerobic peak capacity or maximum heart rate because of muscle weakness and local fatigue. A submaximal test that predicts rather than directly assesses the maximal exercise capacity is better suited to this group and has the additional advantages of being safer, more comfortable, and less exhausting.

Currently, the most frequently used submaximal test for children with NMD is the 6-minute walk test (6MWT),[3] which assesses the distance that a patient can quickly walk, without running, in 6 minutes. The 6MWT is easy to perform and feasible for ambulatory children with NMD,[6] although its responsiveness to measure changes over time may be limited, as shown by the relatively large standard deviations of the within-subject change in distance walked.[7] However, for children with progressive NMD who are at the end of their ambulatory phase or are already wheelchair-dependent, no suitable submaximal endurance test is currently available. This is problematic for long-term follow-up, in a time that controlled treatment trials are being developed for these older, more severely impaired patients there is a need for a feasible and reliable clinical outcome measure.

The aim of our study was to develop a submaximal endurance test for both legs (leg-cycling) and arms (arm-cranking) for children who are expected to lose their walking ability in the near future as well as children who have recently become wheelchair-dependent and have problems with lifting and reaching with their arms. We chose to develop a cycling test for both the arms and legs that could be performed by both ambulatory and wheelchair-confined children and that has the additional advantage that participants are at less risk for falls. Although regular bicycle ergometers have the ability to adjust their mechanical power to the capacity of children with severe muscle weakness, the initial load and further increments are often still too hard to sustain for these patients, for when the bicycle ergometer displays no resistance the child is still cycling at >5 watts.[5] We therefore chose to use a motor-assisted device that would allow even very weak patients to perform the movements required for the tests.

In the first part of this study we describe the development of our assisted 6-minute cycling test (A6MCT), a bicycle test for the legs and arms

Abbreviations: A6MCT, assisted 6-minute cycling test; DMD, Duchenne muscular dystrophy; ICC, intraclass correlation coefficient; MFM, Motor Function Measure; NMD, neuromuscular disorder; NUD study, No Use is Disuse study; 6MWT, 6-minute walk test

Key words: assisted 6-minute cycling test, Duchenne muscular dystrophy, neuromuscular diseases, physical endurance, validation study

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using a motor-assisted mobility trainer. In the second part we investigate the feasibility of the A6MCT in healthy boys and boys with Duchenne muscular dystrophy (DMD), which is the most common inherited muscular dystrophy affecting young boys and results in wheelchair-dependency at a mean age of 10 years.[8] Girls were not recruited to participate in this study, because DMD primarily affects males. For healthy boys, we also examined the relationship between the A6MCT and the commonly used 6MWT (construct validity), and then investigated whether the same test results would be obtained when the test is repeated within a short period under the same circumstances (test–retest reliability). For boys with DMD, we assessed the relationship between the A6MCT and disease severity.

METHODS

This study was approved by the medical ethics committee of Arnhem–Nijmegen, The Netherlands. All parents, and participants who were >12 years of age, gave their written informed consent.

Part 1: Development of the A6MCT. ***Rationale and Device.*** The A6MCT was developed in collaboration with several experts from the neuromuscular center of the Radboud University Nijmegen Medical Centre, including 2 rehabilitation physicians, one physiotherapist, 3 neurologists and clinical neurophysiologists, and 2 biomedical engineers. For the motor-assisted bicycle ergometer device we chose to use a mobility trainer (KPT Cycla; Kinetec, France) that could be used with a patient's personal (electric) wheelchair if necessary, and thus would not need to be transferred to a regular home trainer. The decision for the specific type of mobility trainer used in our validation study was based on the availability of motor assistance, the constant assistance and resistance provided, the stability of the device on the floor layer, and the possibility of easy alternation between leg and arm testing. Motor assistance was expected to allow for bicycle testing even when muscle strength was insufficient to achieve fully active movements. Furthermore, the device could be adapted to body height, and the pedal distance could also be adjusted to limb length.

Duration and Assistance. We aimed to develop a submaximal test (heart rate about 70% of maximum) for both the legs and arms in a single-stage protocol. We chose a protocol length of 6 minutes in which children were free to choose their own speed to match the commonly used 6MWT, which has been shown to be feasible and relevant for children with NMD.[7] A protocol length of >6 minutes could diminish the chance of a suboptimal performance due to the short attention span in some children.

To determine a feasible level of resistance (Wmax) for children with severe muscle weakness, we conducted a pilot study in 3 boys with DMD (2 community walkers aged 9 and 10 years, and 1 household walker aged 9 years). Participants cycled in periods of 1 minute with a continuous speed of 65 rounds per minute (RPM) at several resistance levels ranging from level 1 (7.7 W) to level 10 (51.3 W). The same procedure was used for both legs and arms. A feasible resistance level was defined as a level that could be sustained for 1 minute of cycling at 65 RPM without exhaustion. Overall physical fatigue was assessed using the OMNI Scale for Perceived Exertion, which grades perception of physical exertion on a scale from 0 (not tired at all) to 10 (very, very tired) and contains both verbal and pictorial descriptions.[9]

Results from the first part of this pilot study showed that boys with DMD were able to cycle with both legs and arms with a continuous speed of 65 RPM only at the lowest resistance level (7.7 W) for 1 minute without becoming exhausted (OMNI Scale score $\leq$6). However, in the second part of the pilot study, we asked the boys to continue cycling at their own speed for another 6 minutes, and they all had to terminate the test after approximately 3 minutes. Therefore, we decided to adjust the protocol and to use fixed motor assistance (passive mode 1, no-load speed 7 RPM) instead of resistance. With this protocol, the boys were able to complete 6 minutes of cycling at their own constant velocity. Our pilot study among the 3 boys with DMD confirmed the feasibility of this A6MCT for both legs and arms.

Final A6MCT Protocol. The A6MCT for legs and arms was performed using the motor-assisted mobility trainer (KPT Cycla) in passive mode 1 with a no-load speed of 7 RPM for each participant. After a short demonstration, the participants were instructed to cycle as fast as possible and keep this up for 6 minutes. Starting positions for both the leg-cycling and arm-cranking protocols were standardized (see Fig. 1). For the A6MCT for the legs, the hip and knee of the bended leg were held in $\backsim$90° flexion, while the knee of the other leg was submaximally extended. For the A6MCT of the arms the pedal axis was a few centimeters (with a maximum of 5 cm) below shoulder level when the pedals were horizontal. The distance from the chair to the bicycle was determined by allowing participants to move their legs and arms over the submaximal range of motion, which produced a feeling of stretch but not pain (Fig. 1). Participants were seated comfortably with the back supported by the back of the seat. Verbal encouragement comments from the instructor to maintain attention and complete the test with the best

FIGURE 1. Starting position for the assisted 6-minute cycling test for legs and arms. (This figure has been published previously in Jansen et al.[10] Permission was obtained from the original publisher, BioMedCentral, to reproduce the figure.) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

possible effort were given every 15 seconds throughout the exercise (see Appendix). The assessor was seated next to the participant and informed the participant about the time completed and left, and about the amount of revolutions cycled so far. Participants were allowed to rest if they were not able to continue cycling due to fatigue. In that case, they were also motivated to continue cycling as soon as possible. The primary test outcome was the number of revolutions achieved in 6 minutes. Revolutions per minute (cumulative) and resting periods were also recorded.

Part 2: Validation of the A6MCT in Healthy Boys. ***Participants.*** Healthy boys, without limitations in arm or leg function, aged 6–16 years, were recruited from 3 Dutch primary schools from between May 2010 and April 2011. Age (years), height (meters), and body weight (kilograms) were recorded.

Feasibility. Healthy boys from the 3 different schools performed the A6MCT for legs and arms and cycled at passive mode 1 with a no-load speed of 7 RPM. The tests for legs and arms were performed in random order to exclude bias due to fatigue. Participants rested 10 minutes in a chair prior to each test to recover from the expected submaximal effort and to start each test with a resting heart rate. Heart rate (beats per minute, bpm) was assessed using a standard heart rate monitor (Onyx Classic; Sigma, Germany) before and after the A6MCT. We also assessed the perceived overall physical exertion using the OMNI Scale for Perceived Exertion (see Duration and Assistance subsection) and recorded any signs of exercise intolerance (i.e., excessive muscle pain, extreme fatigue, dizziness, or an uncomfortable feeling). All assessments took place at the primary schools of the participants and were conducted by 4 researchers (M.d.J., H.C., F.E., and M.J.).

Construct Validity. A subgroup of healthy boys from 2 primary schools also performed the 6MWT in addition to the A6MCT in order to examine the relationship between both endurance tests. The 6MWT was performed according to an adjusted protocol for DMD patients, as described by McDonald et al.[7] Participants were instructed to walk as fast as possible without running. The assessor walked $\backsim$1.5 m behind the participant and encouraged the participant every 15 seconds to keep exercising using standardized phrases. As the corridors of the primary schools involved in this study were only 20 m long, participants performed the 6MWT in a corridor with a marked test area of 20 m instead of the usual 25 m. Participants were allowed to rest or stop if they were not able to continue. The distance walked at the end of the 6-minute period was recorded in meters, together with the cumulative distance walked (per minute), rest periods needed, and number of falls.

Test–Retest Reliability. To study the test–retest reliability of the A6MCT, boys from the third primary school performed the A6MCT for legs and arms twice with a 2-week interval. Participants and researchers involved in this part of the study were blinded to previous test results during the second test day.

Part 3: Validation of the A6MCT in Boys with DMD. ***Participants.*** For validation of the A6MCT in DMD we obtained test results from age-matched boys with DMD from the randomized controlled No Use is Disuse (NUD) study.[10] We used only the baseline data from this study, meaning that participants had not yet received any physical training intervention. The NUD study included boys with DMD who were at the end of their ambulatory phase (needing $\geq$5 seconds to get up from the floor, unable to get up from the floor, unable to bicycle without assistance, and dependent on a wheelchair to move over a distance $>$500 m), or had recently (within 1–2 years prior) begun full-time use of a wheelchair. Full-time users of a wheelchair, in this study, defined as wheelchair-dependent boys, had to be able to touch the top of

their head with both hands, or had to be able to use a hand-operated wheelchair, indicating a moderate-to-good arm–hand function. Age (years), height (meters), and body weight (kilograms) were registered.

Feasibility. Procedures were similar to those described for healthy boys, and all boys intended to perform the A6MCT for both legs and arms. However, in the NUD study the test order was standardized, and boys first performed the A6MCT for legs and then for arms. Furthermore, all assessments were performed by 1 researcher (M.J.) and took place at the Department of Rehabilitation, Radboud University Nijmegen Medical Centre.

Relationship between the A6MCT and Disease Severity. The boys with DMD also performed the Motor Function Measure (MFM) as part of the NUD study, and the baseline results were used to assess the relationship between the A6MCT and disease severity. The MFM is a valid and reliable scale to assess motor function in both ambulant and wheelchair-dependent patients with a neuromuscular disorder.[11] The scale consists of 32 items in 3 dimensions: dimension 1—standing position and transfers (D1); dimension 2—axial and proximal motor function (D2); and dimension 3—distal motor function (D3). Each item is scored on a 4-point Likert scale (generic grading: 0—does not initiate movement or starting position cannot be maintained; 1—partially completes the exercise; 2—completes the exercise with compensations, slowness, or obvious clumsiness; and 3—completes the exercise with a standard pattern). The scale has a maximum score of 96, and a higher score indicates better motor function. We calculated a percentage of the maximum score for the total MFM score.

Statistical Analysis.. Statistical analyses were performed using SPSS (version 16.0) for Windows (SPSS, Inc., Chicago, Illinois). $P < 0.05$ was considered significant.

Performance and Feasibility. Performance on the A6MCT is expressed as mean ± standard deviation (SD) for continuous data (number of revolutions achieved and heart rate) and as median with range for ordinal data (OMNI Scale) for healthy boys, the total group of boys with DMD, ambulatory boys with DMD, and wheelchair-dependent boys with DMD. Healthy boys and boys with DMD, and also ambulatory and wheelchair-dependent boys with DMD, were compared using independent *t*-tests for continuous data (age, height, body weight, A6MCT, and heart rate) and Mann–Whitney *U*-tests for ordinal data (OMNI Scale). The feasibility of the A6MCT for legs and arms was determined by calculating the percentage of boys who were able to perform the test. We established that the test was feasible when at least 95% of the healthy boys and 90% of the boys with DMD were able to perform the A6MCT. The correlation between the A6MCT and age was investigated by calculating the Pearson correlation coefficient (r) for both healthy boys and boys with DMD. Pearson correlation coefficients were interpreted as follows: 0–0.25 = little to no correlation; 0.26–0.49 = low correlation; 0.50–0.69 = moderate correlation; 0.70–0.89 = high correlation; and ≥0.90 = very high correlation.[12]

Construct Validity in Healthy Boys. The relationship between the A6MCT and the 6MWT was examined by calculating Pearson correlation coefficients (r) and using stepwise linear regression analysis, and parametric normal distributions were confirmed by the Kolmogorov–Smirnov test ($P > 0.05$). The r-values were interpreted as described previously.[12] Regression coefficient (β) and 95% confidence interval (CI) data were calculated from stepwise linear regression analyses. The 6MWT was the only independent variable in step 1 of the regression analysis, whereby the A6MCT was the dependent variable. Height was added as a potential confounder in the second step of the regression analysis, because the 6MWT is positively related to height in healthy boys.[7] We expected a moderate-to-high positive correlation between the A6MCT and the 6MWT, as both tests aim to measure submaximal endurance.

Test–Retest Reliability in Healthy Boys. The test–retest reliability of the A6MCT (legs and arms) was first explored by examining whether any changes existed between the first and second assessment by using paired *t*-tests. Next, intraclass correlation coefficients (ICCs) were calculated and Bland–Altman figures, including 95% limits of agreement ($Mean_{Difference} \pm 1.96 * SD_{Difference}$) were generated.[13,14] ICCs were considered acceptable when they were >0.70.[15]

Relationship of the A6MCT with Disease Severity in Boys with DMD. The correlation between the A6MCT and disease severity as expressed by the MFM was examined by calculating Spearman correlation coefficients (ρ). Correlation coefficients were calculated for the total group of boys with DMD, but also separately for ambulatory and wheelchair-dependent boys. We expected a low-to-moderate positive correlation, as both outcome measures were expected to assess different aspects of disease severity (i.e., endurance vs. motor function).

RESULTS

Validation of the A6MCT in Healthy Boys. ***Participant Characteristics.*** Ninety-nine healthy boys with a mean age of 9.9 years participated in this study. Characteristics of the healthy boys are shown in Table 1.

Table 1. Subject characteristics and test results of healthy boys and boys with Duchenne muscular dystrophy.

	n	Healthy ($n = 99$)	*n*	DMD ($n = 30$)	*P*-value
Demographics					
Age (y)	98	9.9 ± 2.0 (6.4–16.8)	30	10.5 ± 2.6 (6.4–16.6)	0.02*
Body weight (kg)	98	35.0 ± 8.9 (18.0–63.7)	30	45.5 ± 19.4 (18.3–92.4)	<0.01*
Height (m)	98	1.4 ± 0.1 (1.2–1.9)	30	1.4 ± 0.2 (1.1–1.8)	<0.01*
A6MCT (revolutions)					
Legs	95	843 ± 82 (604–1016)	29	405 ± 152 (118–714)	<0.01*
Arms	95	778 ± 111 (492–1003)	28	370 ± 120 (142–574)	<0.01*
6MWT (distance)	70	623 ± 72 (411–829)	NA	NA	NA
MFM (%)	NA	NA	30	66.2 ± 14.2 (32.3–91.7)	NA

Data expressed as mean ± SD (range). DMD, Duchenne muscular dystrophy; A6MCT, assisted 6-minute cycling test; 6MWT, 6-minute walk test; NA, not assessed; MFM, Motor Function Measure.
**Significant difference between healthy boys and boys with DMD at the 0.05 level.*

Feasibility and Performance. Of the 99 healthy boys included in this study, 95 performed the A6MCT for legs, and another 95 performed the A6MCT for arms. The reason for the missing data was mainly a lack of time to perform the 2 tests. Only 1 boy was unable to complete the A6MCT for legs, because his feet tended to slip off the pedals. This means that the A6MCT was feasible for 99% of the healthy boys, without any signs of exercise intolerance.

The mean number of revolutions achieved during 6 minutes of cycling was 843 ± 82 for legs and 778 ± 111 for arms (Table 1). Figure 2 shows the constant cycling velocity (rounds per minute) that participants selected and maintained during the A6MCT for legs and arms. Figure 3 shows the positive correlation between the number of revolutions achieved with the A6MCT for legs and arms ($r = 0.64$, $P < 0.01$), indicating that boys who achieved a high number of revolutions with the arms also achieved a high number of revolutions with the legs and *vice versa*. The mean heart rate prior to the A6MCT was 95 bpm for the legs and 96 bpm for the arms, and increased to a maximum of 161 bpm and 163 bpm, respectively (Table 2). Perceived exertion increased from 0 (not tired at all) to 4 (getting more tired) on a maximal scale of 0 (not tired at all) to 10 (very, very tired) during the A6MCT for both legs and arms. As expected, the number of revolutions achieved with the A6MCT for legs ($r = 0.38$, $P < 0.01$) and arms ($r = 0.60$, $P < 0.01$) increased moderately with age in healthy boys.

Construct Validity. Seventy healthy boys performed the A6MCT for both legs and arms and the 6MWT. Two boys were excluded: 1 fell during the 6MWT and walked carefully thereafter (no injuries occurred), and the other ran instead of walking. The mean walked distance at the 6MWT was 623 ± 72 m, with a maximum heart rate of 160 bpm and an OMNI Scale score of 3 (Table 2). Heart rate and OMNI Scale scores at the end of both the A6MCT and 6MWT were comparable (Table 2).

The A6MCTs for legs ($r = 0.58$, $P < 0.01$) and for arms ($r = 0.65$, $P < 0.01$) were moderately correlated with the 6MWT (Fig. 4). This means that boys who walked further during the 6MWT also achieved a higher number of revolutions with the A6MCT compared with boys who walked a shorter distance.

Results from the linear regression analysis confirmed the positive relationship between the A6MCTs for legs ($\beta = 0.6$, $P < 0.01$) and for arms ($\beta = 1.0$, $P < 0.01$) and the 6MWT. After correction for height, the findings were slightly different (A6MCT for legs: $\beta = 0.7$, $P < 0.01$; A6MCT for arms: $\beta = 0.7$, $P < 0.01$). Height was also positively related to the A6MCT for arms ($P = 0.01$), indicating that tall boys achieved more revolutions with their arms than small boys, but not to the A6MCT for legs ($P = 0.08$).

Test–Retest Reliability. Twenty-three (leg-cycling) and 22 (arm-cranking) boys performed the A6MCT twice within 2 weeks. With respect to A6MCT for legs, the mean number of revolutions was 870 ± 93 for the first assessment and 875 ± 92 for the second assessment (mean difference: 4 ± 46, $P = 0.70$). For the A6MCT for arms, boys achieved, on average, 33 ± 46 revolutions more during the second assessment (mean number of revolutions = 840 ± 118) compared with the first assessment (mean number of revolutions = 807 ± 117; $P < 0.01$). Nevertheless, a high correlation was found between the 2 assessments for both legs (ICC = 0.88, 95% CI 0.72–0.95) and arms (ICC = 0.89, 95% CI 0.76– 0.95). Bland–Altman plots confirmed the good test–retest reliability of the A6MCT for both legs and arms by showing that only 1 or 2 data points (4.3–9.1%) were beyond the 95% limits of agreement and also by showing that the difference in the number of revolutions achieved between the first and second assessment was not correlated with the mean number of revolutions for

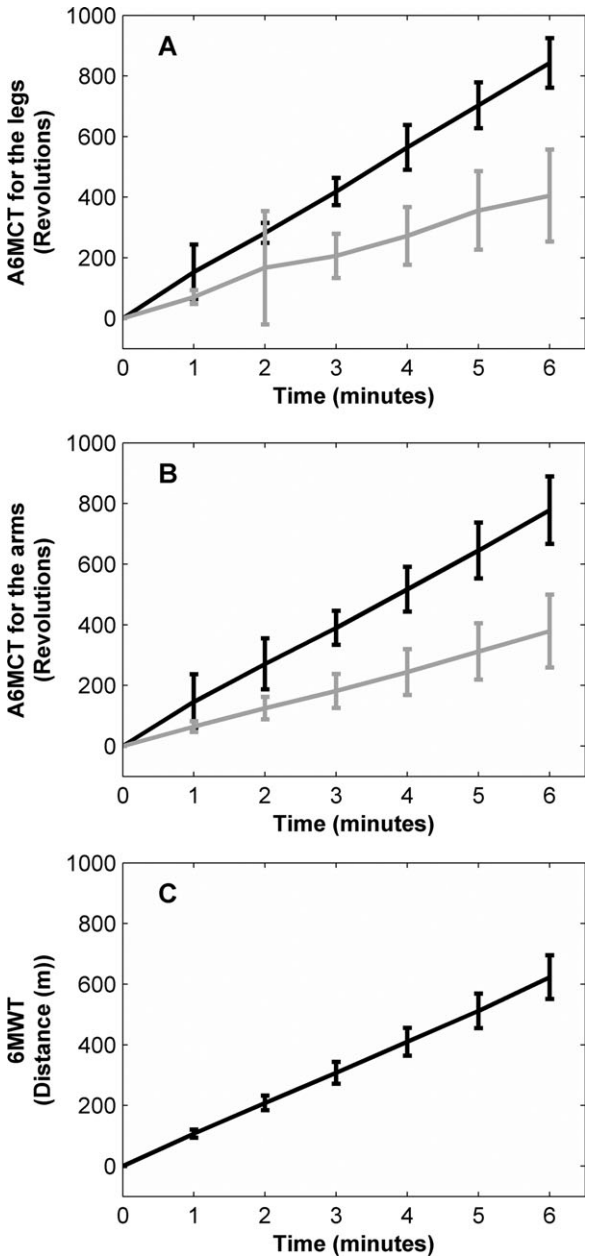

FIGURE 2. Cumulative number of revolutions for the legs (**A**) and arms (**B**) assessed using the A6MCT, and cumulative distance (meters) assessed using the 6MWT (**C**) per minute. Mean and SD are shown. Black lines: healthy boys; gray lines: boys with Duchenne muscular dystrophy. A6MCT, assisted 6-minute cycling test; 6MWT, 6-minute walk test.

the 2 tests (legs: $r = -0.04$, $P = 0.87$; arms: $r = -0.31$; $P = 0.18$) (Fig. 5).

Validation of the A6MCT in Boys with DMD. ***Participant Characteristics.*** Thirty boys with DMD (18 ambulant, 12 wheelchair-dependent) with a mean age of 10.6 years were included in this study. Characteristics of the total group of boys with DMD are shown in Table 1 and in Table 3 for ambulatory and wheelchair-dependent boys separately. As expected, wheelchair-dependent boys were older and taller than ambulatory boys ($P < 0.01$).

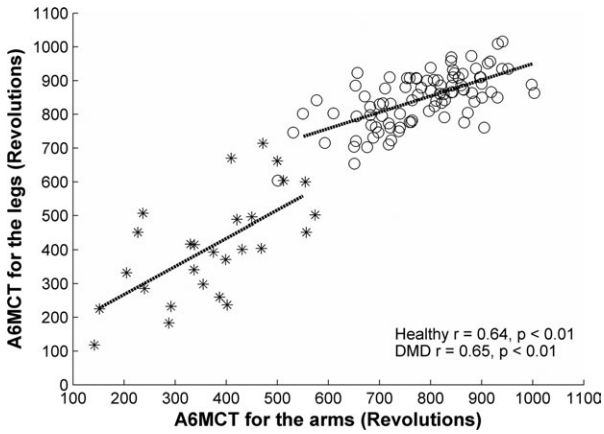

FIGURE 3. Correlations between the A6MCT for legs (**A**) and arms (**B**). The A6MCTs for legs and arms were found to be positively correlated in both healthy boys (circles) and boys with DMD (stars). A6MCT, assisted 6-minute cycling test; DMD, Duchenne muscular dystrophy.

Feasibility and Performance. Of the 30 boys with DMD who participated in this study, 29 (97%) performed the A6MCT for legs, as 1 wheelchair-dependent boy, with an MFM score of 47%, had insufficient muscle strength, particularly of the hip flexors, to be able to perform the test. Of the other 29 boys who were able to perform the A6MCT for legs, 93% (i.e., 27 of the 29 boys) had an MFM score of $\geq$50%. The 2 boys who scored $<$50% on the MFM (32% and 41%, respectively) achieved a relatively low number of revolutions with their legs ($<$225). Another 2 boys did not perform the A6MCT for arms due to problems with attention span. All other boys (100%) were able to complete the A6MCT for arms. No periods of rest were needed during the tests, and no signs of exercise intolerance were reported.

Table 2. Endurance parameters of the A6MCT and 6MWT.

	Healthy	DMD	*P*-value
A6MCT legs			
HR start	95.4 ± 12.3	100.5 ± 12.3	0.11
HR end	161.0 ± 21.5	155.4 ± 17.9	0.27
OMNI start	0 (0–6)	1 (0–3)	0.07
OMNI end	4 (0–10)	6 (0–10)	0.03*
A6MCT arms			
HR start	95.9 ± 12.0	107.28 ± 12.3	<0.01*
HR end	163.0 ± 22.3	149.3 ± 17.5	<0.01*
OMNI start	0 (0–10)	2 (0–8)	<0.01*
OMNI end	4 (0–10)	6 (0–10)	0.02*
a6MWT			
HR start	100.0 ± 13.9	NA	NA
HR end	160.1 ± 20.4	NA	NA
OMNI start	0 (0–7)	NA	NA
OMNI end	3 (0–10)	NA	NA

A6MCT, assisted 6-minute cycling test; 6MWT, 6-minute walk test; HR, heart rate; OMNI, OMNI Scale for Perceived Exertion; DMD, Duchenne muscular dystrophy; NA, not assessed.
**Significant difference between healthy boys and boys with DMD at the 0.05 level.*

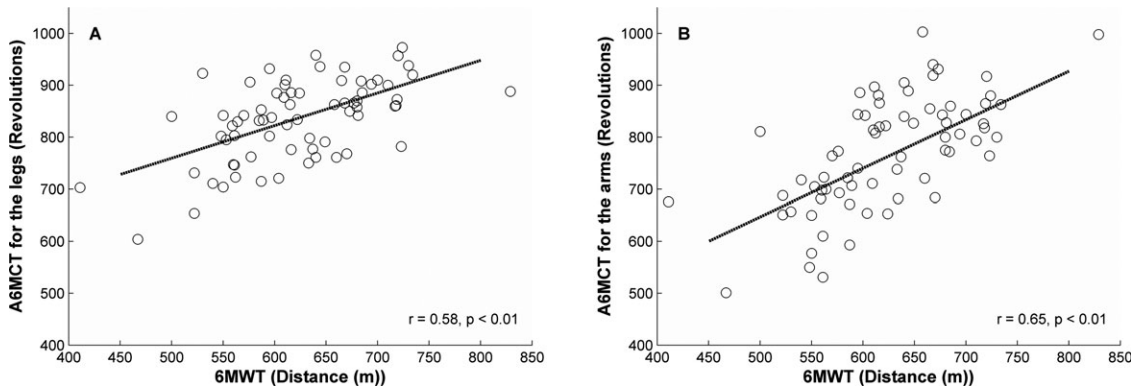

FIGURE 4. Correlations between the 6MWT and A6MCT for legs (**A**) and arms (**B**) in healthy boys. The A6MCT for legs and the A6MCT for arms are positively correlated with the 6MWT. A6MCT, assisted 6-minute cycling test; 6MWT, 6-minute walk test.

As expected, boys with DMD achieved fewer revolutions with their legs (mean number of revolutions: 405 ± 152) and arms (mean number of revolutions: 370 ± 120) compared with healthy controls, with only slight overlap ($P < 0.01$) (Table 1). However, boys with DMD also cycled at a constant velocity throughout the tests (Fig. 2), and the correlation between the A6MCT for legs and arms was comparable to that of healthy boys (Fig. 3). Regarding the endurance parameters (Table 2), heart rates and OMNI Scale scores for perceived exertion prior to the A6MCT for arms were increased in DMD boys compared with healthy controls. After the A6MCT for legs, the heart rate response (maximum of 155 bpm) and increase in OMNI Scale scores (mean OMNI score = 6) in DMD boys were comparable to healthy controls. Maximum heart rate at the end of the A6MCT for arms was slightly lower in boys with DMD (maximum of 149 bpm) compared with healthy boys ($P < 0.01$). No signs of exercise intolerance were observed. The number of revolutions achieved with the A6MCT for legs ($r = -0.18$, $P = 0.34$) and arms ($r = 0.22$, $P = 0.25$) was not significantly correlated with age and was not significantly different for ambulatory and wheelchair-dependent boys (Table 3).

Relationship with Disease Severity. The mean MFM score (age percentage) was 66.2 ± 14.2 (range 32.3–91.7). Wheelchair-dependent boys (mean MFM = 52.8 ± 8.5) had lower MFM scores than ambulatory boys (mean MFM = 75.0 ± 9.3) ($P < 0.01$; Table 3). As shown in Figure 6, the number of revolutions achieved with the A6MCT for legs was positively correlated with the MFM in the total group of boys with DMD ($\rho = 0.65$, $P < 0.01$), the ambulatory boys ($\rho = 0.72$, $P < 0.01$), and the wheelchair-dependent boys ($\rho = 0.74$, $P = 0.01$). This means that the number of revolutions achieved with the A6MCT for legs decreased with a decrease in motor function. A similar positive correlation between the A6MCT for arms and motor function was found for wheelchair-dependent boys ($\rho = 0.84$, $P < 0.01$). A trend for a positive correlation between the A6MCT for arms and motor function was also found for the total group of DMD patients ($\rho = 0.32$, $P = 0.94$) and for ambulatory boys ($\rho = 0.46$, $P = 0.07$), but this was not significant (Fig. 6).

DISCUSSION

In this study we have shown that the A6MCT for legs and arms is a feasible, valid, and reproducible endurance test for children and adolescents aged

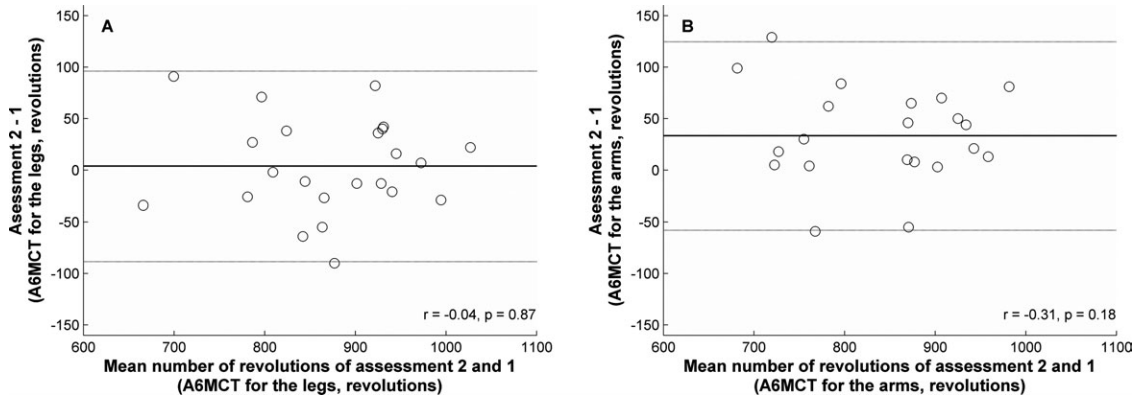

FIGURE 5. Bland–Altman plots for the A6MCT for legs (**A**) and arms (**B**) with the mean (solid line) and 95% limits of agreement (dotted lines) for comparisons between assessments 1 and 2 in healthy boys. A6MCT, assisted 6-minute cycling test.

Table 3. Subject characteristics and test results of ambulatory and wheelchair-dependent boys with Duchenne muscular dystrophy.

	n	Ambulatory (*n* = 18)	*n*	Wheelchair (*n* = 12)	*P*-value
Demographics					
Age (y)	18	9.3 ± 1.7 (6.4–11.6)	12	12.4 ± 2.6 (8.7–16.6)	<0.01*
Body weight (kg)	18	33.6 ± 8.6 (18.3–49)	12	63.3 ± 17.3 (35.7–92.4)	<0.01*
Height (m)	18	135.0 ± 13.6 (114–166)	12	158.3 ± 16.2 (131–181)	<0.01*
A6MCT (revolutions)					
Legs	18	431.44 ± 147.9 (183–714)	11	361.6 ± 154.4 (118–670)	0.24
Arms	16	371.88 ± 110.8 (205–555)	12	368.3 ± 135.6 (142–574)	0.94
MFM (%)	18	75.0 ± 9.3 (54.2–91.7)	12	52.8 ± 8.5 (32.3–61.5)	<0.01*

Data expressed as mean ± SD (range). DMD, Duchenne muscular dystrophy; A6MCT, assisted 6-minute cycling test; 6MWT, 6-minute walk test; NA, not assessed; MFM, Motor Function Measure.
**Significant difference between healthy boys and boys with DMD at the 0.05 level.*

6–16 years. The A6MCT is the first ergometer test that can also be performed by wheelchair-dependent children with a progressive neuromuscular disorder and shows comparable characteristics of endurance testing in healthy boys. The motor assistance enables children with severe muscle weakness to perform a submaximal endurance test that is related to their disease severity.

Our results demonstrate that the A6MCT with a fixed motor assistance level (no-load, speed 7 RPM) is feasible for both healthy boys and boys with DMD. Ninety-nine percent of the healthy boys and 97% of the boys with DMD were able to perform the A6MCT for legs. Our preliminary results suggest that the threshold value for being able to perform the A6MCT for legs is a total MFM score of around 50% for boys with DMD. With respect to the A6MCT for arms, all healthy boys and boys with DMD were able to perform the test. This is unique, as other ergometer tests are often too hard to sustain for children with severe muscle weakness due to their initial load and increments in resistance.[16,17]

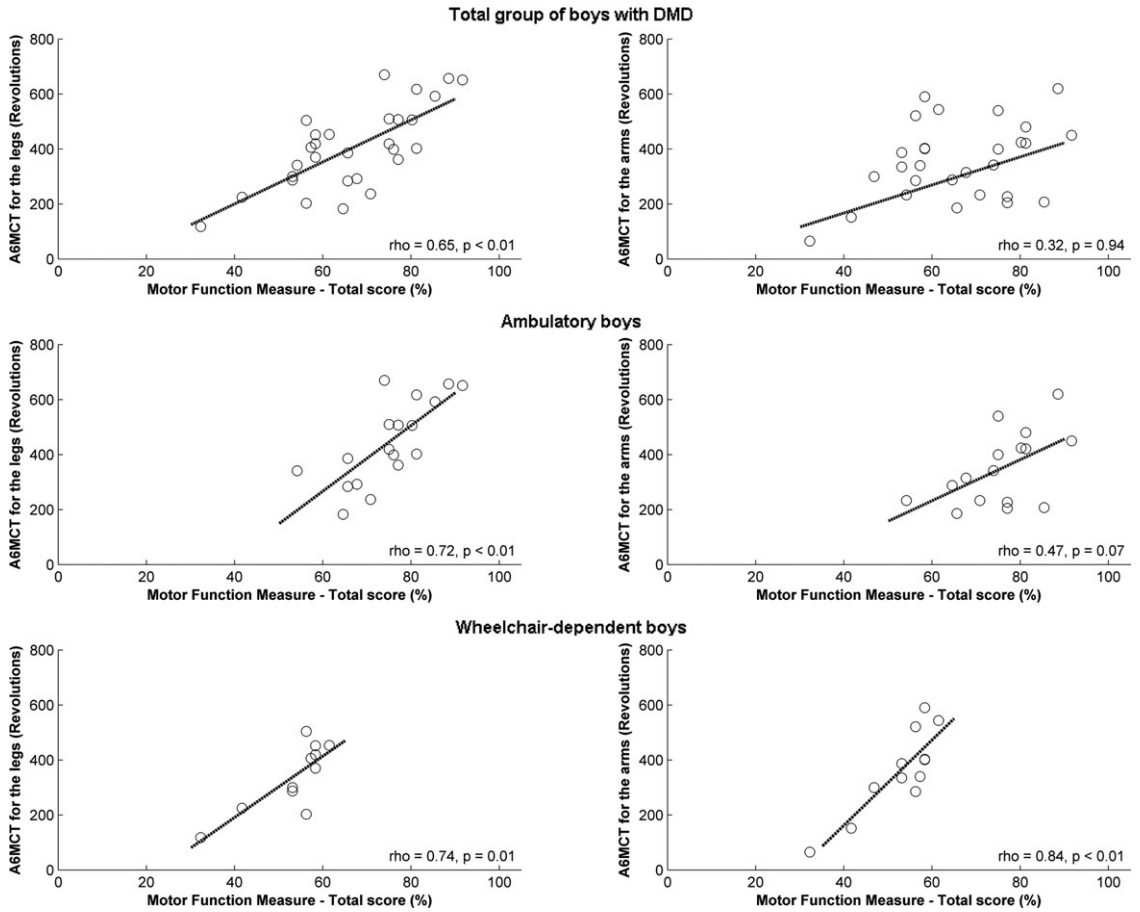

FIGURE 6. Correlations between the A6MCT for the legs and arms and the total score of the motor function measure (percent) in boys with DMD. A6MCT, assisted 6-minute cycling test.

The A6MCT provided a sensitive outcome measure to distinguish between healthy boys and boys with DMD. Boys with DMD achieved significantly fewer revolutions with their legs and arms compared with healthy, age-matched controls. There was very little overlap in performance; just 2 boys with DMD performed within 2 SD of the mean number of revolutions achieved by healthy boys for both the legs and arms. Our findings corroborate earlier evidence that boys with DMD have a lower functional capacity than healthy boys.[7]

Although wheelchair-dependent boys achieved slightly fewer revolutions with the A6MCT than ambulatory boys with DMD, we did not find a significant correlation with age, as was shown by previous studies of the 6MWT.[7] The absence of a negative correlation between the A6MCT and age in our study could be explained by the relatively heterogeneous phenotype of DMD in our study; that is, we included ambulatory as well as wheelchair-dependent patients. Even within these 2 groups, we found wide variability in motor function with age. For example, two 8-year-old boys participated in this study: 1 was ambulatory and relatively fit, and the other was already confined to a wheelchair. Previous studies also confirmed the heterogeneity of the DMD phenotype from the motor, respiratory, and survival points of view.[18] Longitudinal research is needed to investigate the responsiveness of the A6MCT.

The validity of the A6MCT was confirmed by its relationship to the often-used 6MWT in healthy boys and its correlation with disease severity, as shown by the MFM in boys with DMD. Although one could speculate that the motor assistance may be unnecessary and would limit the performance of healthy boys, we found that healthy boys who walked further also achieved a higher number of revolutions with the A6MCT compared with boys who walked a shorter distance. This means that the distance cycled was related to the submaximal level of functional capacity. Reproducibility data from healthy boys show that boys achieved, on average, 33 more revolutions during a second assessment for the arms, which was performed within 2 weeks after the first assessment. This can be explained by a learning effect. Dutch boys, even those with an NMD, are accustomed to bicycling with their legs (thus being trained), but not with their arms (thus being untrained).

For boys with DMD, the distance cycled with the legs decreased with a lower motor function, indicating that the A6MCT is also able to measure clinically meaningful changes over time.

The findings from the wheelchair-dependent boys with DMD confirmed the positive correlation between the A6MCT for arms and the MFM. The absence of a relationship between the number of revolutions cycled with the arms and the MFM within the total group of boys with DMD and the subgroup of ambulatory boys was not entirely unexpected, as both tests assess 2 different aspects of disease severity (i.e., endurance and motor function). Furthermore, the MFM measures proximal motor functions, but the number of items that assess gross arm motor functions is limited, and it is well known that fine arm motor functions (such as writing) are relatively preserved in boys with DMD.[19] No other arm function tests, except the practical but nonsensitive general Brooke scale for upper limb function,[20] exist for DMD patients. The A6MCT for arms could therefore become a first test for assessment of arm function in NMD patients.

The submaximal nature of the A6MCT for legs and arms was confirmed by the maximum heart rate of about 160 bpm and the perceived exertion score of "getting more tired" in healthy boys. The relatively high starting heart rates could be explained by the excitement of the healthy boys to participate in the study. Maximal heart rates were comparable to those reached during the 6MWT, and also to those reached by boys with DMD during the A6MCT for legs (155 bpm) and arms (149 bpm). As no studies have yet reported the aerobic peak of boys with DMD, one could speculate that the A6MCT is more "all-out" for boys with DMD than for healthy boys. Maximum perceived exertion scores were only "tired" and not "very, very tired," indicating that boys exercised at a submaximum level.[21] Future trials should investigate the relationship between the A6MCT and cardiac and pulmonary function. Increases in perceived exertion were comparable to those of healthy boys, and no signs of exercise intolerance were found, confirming the feasibility and safety of the A6MCT for boys with DMD.

Outcome measures for use in clinical trials on DMD, especially those investigating the effectiveness of new drug treatments, should be clinically relevant and present how participants feel and function in a proper time period. With respect to the A6MCT, the meaningfulness for the boys and their parents could be limited by the fact that cycling is less critical to human performance in daily life than walking. Arm-cranking, however, could be correlated with arm abilities and should be studied further. Overall, the results of this first validation study are promising and support further research on the A6MCT for children with NMD, especially during a time that treatment trials are being developed for more severely impaired NMD patients. We recommend investigation of the relationship between the A6MCT and clinically relevant endpoints (such as the age at which ambulation or the

ability to put on a t-shirt are lost) and the ability of the A6MCT to measure changes over time. In addition, we recommend investigation of the relationship between the A6MCT and the 6MWT in a larger group of NMD patients and a feasibility study among older, even more severely disabled children and young adults with NMD. We further suggest establishment of normative data for healthy girls, although we do not expect any gender differences from the literature on the 6MWT.[20] Finally, we recommend a practice test to reduce measurement errors due to an improvement in coordination and a reduction in anxiety.[3] Assessors using the A6MCT should always remember the influence of the child's motivation to participate in the test and adhere to the standardized procedures regarding encouragement.

In conclusion, our results show that the A6MCT for legs and arms is a promising objective outcome measure to monitor disease progression and to evaluate the effectiveness of treatments in children with severe muscle weakness who are either restricted walkers or wheelchair-dependent.

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APPENDIX 1: ASSISTED 6-MINUTE CYCLING TEST

General procedures

Equipment:

1 Mobile trainer (KPT Cycla; Kinetec).
2 Stopwatch.
3 Polar band.
4 OMNI Scale for Perceived Exertion.
5 Height-adjustable chair.
6 Height-adjustable table.
7 Footstool.

Set up:

1 The test should be performed in a quiet room, preferably without any family or friends in the room.
2 The mobile trainer should be placed against the wall to prevent moving.
3 Participants should be seated comfortably with their back supported by the back of the seat
4 Assessor sits next to the participant.
5 For the A6MCT for the legs, the hip and knee of the bended leg should be hold in $\backsim 90^\circ$ flexion, while the knee of the other leg is submaximally extended.
6 For the A6MCT of the arms the pedal axis should be a few centimeters (with a maximum of 5 cm) below shoulder level when the pedals are horizontal.
7 The distance from the chair to the bicycle should be determined by allowing participants to move their legs and arms over the submaximal range of motion, which may cause a feeling of stretch but not pain.
8 Passive mode 1 of the mobility trainer should be used; that is, no-load speed of 7 RPM.
9 Show the number of revolutions at the mobility trainer display.

Test procedure:

1 Briefly demonstrate the cycling exercise and allow a short practical test.
2 A 10-minute rest period should be given prior to the test.
3 Record heart rate and level of perceived exertion.
4 Set stopwatch to zero.
5 Read the following statement: "*You will cycle as fast as possible and keep this up for 6 minutes. Try to continue until I tell you to that you have finished. You are allowed to rest, but try to continue for the whole 6 minutes.*"
6 The assessor should inform the participant about the time completed and left, and about the amount of revolutions cycled.
7 The assessor records the number of revolutions every minute.
8 The assessor should give verbal encouragements to maintain attention and to complete the test 15 s throughout the exercise.
9 Encouragement comments could be:
   (a) "*You're doing a great job! Keep going!*"
   (b) "*Wow, you're doing great! Keep going!*"
   (c) "*You're doing great, only 3 minutes left! Keep going!*"
10 At the final 10 seconds of the test, the assessor should count down.
11 Record the total number of revolutions cycled, heart rated and perceived exertion at 6 minutes.

Name:		Date of test:	
Date of birth:		Examiner:	
Time (minutes)	**Revolutions (cumulative)**	**Heart rate (beats per minute)**	**Fatigue (OMNI score)**
1			
2			
3			
4			
5			
6			
Number of revolutions at six minutes:		**Comments:**	

FIGURE 7. Scoring sheet.

Determining a valid test:

1 A test is valid if the participant completes or discontinues the test due to fatigue.
2 A test is invalid if the participant:
   (a) Discontinues the test due reasons other than fatigue (such as noncompliance or injury).
   (b) Did not follow the instructions.

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