Cardiopulmonary exercise testing in ambulatory children with Duchenne and Becker Muscular Dystrophy: *a pilot study*

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Introductie

De cardiopulmonale inspanningstest (CPET) is de gouden standaard voor inspanningscapaciteit en is mogelijk van toegevoegde waarde voor de evaluatie van het fysiek functioneren van kinderen met musculaire dystrofie. Er is weinig bekend over de respons van kinderen met musculaire dystrofie op dynamische inspanning vanwege de angst voor mogelijke bijwerkingen. Het doel van deze studie is om de haalbaarheid van de CPET te vergelijken met het Europees aanbevolen standaard test protocol (zes minuten wandeltest, motoriektest, kwantitatieve spierkrachttest) voor kinderen met musculaire dystrofie.

Methode

De CPET en het standaard test protocol werden binnen drie weken op verschillende momenten afgenomen bij een gelegenheidssteekproef van kinderen met Duchenne en Becker musculaire dystrofie die bekend waren in het Spieren voor Spieren-centrum van het Wilhelmina Kinderziekenhuis. De twee protocollen werden vergeleken ten aanzien van inspanningsparameters en uitkomstmaten voor haalbaarheid: testuitvoering, negatieve bijwerkingen en ervaren belasting.

Resultaten

Negen kinderen met Duchenne (N=3) en Becker (N=6) participeerden in deze studie. Vijf deelnemers (55%) voerden een succesvolle maximale of symptoom-gelimiteerde inspanningstest uit. Het standaard testprotocol werd door zes deelnemers goed uitgevoerd (67%). Er waren geen aanwijzingen voor rhabdomyolyse of cardiale afwijkingen na uitvoering van beide protocollen en de mate van spierpijn bleef binnen de vooraf opgestelde grenzen van normaal. De vijf deelnemers die een succesvolle CPET uitvoerden hadden een lage inspanningscapaciteit met cardiale en/of vasculaire beperkingen. Er was een grote variatie in ervaren belasting waarbij de leeftijd en ziekte ernst mogelijk van invloed waren.

Conclusie

De maximale cardiopulmonale inspanningstest lijkt een haalbare en relevante uitkomstmaat voor inspanningscapaciteit en cardiovasculaire beperkingen bij kinderen met relatief milde beperkingen. Toekomstig onderzoek naar de pathofysiologische mechanismen van cardiovasculair beperkingen en ontwikkeling

van veiligheidsmarkers draagt mogelijk bij aan een toenemend inzicht in de inspanningsrespons van kinderen met musculaire dystrofie.

Introduction

The cardiopulmonary exercise test (CPET) is the gold-standard for exercise capacity and could be of additional value to the physical evaluation of children with muscular dystrophy (MD). Knowledge of response to dynamic exercise in MD is scarce because of safety concerns. The purpose of this study was to compare feasibility of the CPET to the current European recommended standard test protocol for physical performance of children with DMD (six minute walk test, motor performance test and quantative muscle testing).

Methods

The CPET and the standard test protocol were separately assessed within three weeks in a consecutive sample of children with Duchenne and Becker muscular dystrophy. Test protocols were compared with regards to exercise outcome and feasibility parameters: ‘measurement completion rate’, ‘adverse events’ and ‘acceptability’.

Results

Nine children with Duchenne (N=3) and Becker (N=6) muscular dystrophy participated in this study. Five children (55%) successfully performed a maximal or symptom-limited exercise test which was slightly less than the standard test protocol (66%). There were no signs of rhabdomyolysis or cardiac events after both visits and muscle pain ratings remained within predefined limits of normal response. The five subjects that successfully performed a CPET showed low values of exercise capacity with cardiac pump and/or vascular limitations. The subjects demonstrated large variability in perceived burden of both test protocols possibly influenced by age and disease severity.

Conclusion

In children with relative mild impairments, the cardio pulmonary exercise test seems to be a feasible and relevant outcome measure for exercise capacity and cardiovascular limitations. To extend our knowledge of exercise response of children with MD further research should be focused on the pathophysiological mechanisms of cardiovascular limitations and development of safety markers for exercise.

Keywords: Duchenne muscular dystrophy, Becker muscular dystrophy, cardio pulmonary exercise test, feasibility, outcome parameter

Introduction

In Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD), a deficiency or reduced expression of the muscular protein dystrophin results in a progressive decline of muscle strength and functional abilities during childhood (1). In addition, ten percent of these patients suffer from a dilated cardiomyopathy (2). In the ambulatory phase of DMD and BMD, pharmaceutical therapies and physical interventions aim at attenuating the disease course and optimising physical functioning and exercise capacity (3, 4). To evaluate the effect of these interventions, feasible outcome measures with good clinimetric properties are imperative (5). European guidelines recommend several functional tests such as the North Star Ambulatory Assessment (NSAA) to assess motor function, quantative muscle testing to assess muscle strength and the six minute walk test (6MWT) to evaluate exercise capacity (1, 6). The six-minute walk test (6MWT) is considered a well-tolerated exercise test reflecting functional ability and has been used as primary outcome measure in large randomized controlled drug studies in ambulatory boys with DMD (7). The clinimetric properties of the 6MWT have been investigated in DMD by several studies and limited evidence is available that it is reliable and able to measure change in walking ability over the time span of one year (8). In other pediatric chronic conditions, the cardiopulmonary exercise test (CPET) is considered the gold-standard for assessing exercise capacity (9). In contrast with the 6MWT, CPET testing includes registration of ECG and respiratory gas exchange, by which it is possible to differentiate between different limiting factors that reduce exercise capacity, i.e. muscular, cardiac or pulmonary impairments. Besides that, the CPET uses standard criteria to evaluate the process of testing (e.g. whether the test results reflect a well performed test (10). These are both of value when evaluating disease progression and therapeutic response.

Despite these advantages, studies including CPET in children with muscular dystrophy are limited because of the fear of exercise induced muscle damage and cardiac events. Nevertheless, no controlled prospective studies have been performed that can underline this statement (11). Sockolov et al. studied the maximal exercise performance of ambulatory boys with DMD and controls and did not report any adverse event (12). More recently, a study in adults with BMD showed

that it is safe to perform a CPET as well as to perform a submaximal exercise training program (13).

Exercise capacity has become an important outcome measure in medical and physical interventions in boys with DMD en BMD(14). Future therapeutic regimes that potentially change disease course will demand adaptation of existing test protocols and develop new functional outcome measures. The possible additional value of the CPET to current test protocols has not been investigated yet because of the limited knowledge regarding the response on maximal exercise testing. Therefore, aim of this study was to determine the feasibility of the CPET compared to the recommended test protocol for physical performance for ambulatory children with DMD and BMD. Feasibility was assessed in terms of: 1) test performance, 2) adverse events and 3) acceptability. More insight into feasibility of maximal exercise testing in children with muscular dystrophy will extend the knowledge of exercise response of these patients, and contribute to the development of appropriate outcome measures for exercise capacity.

Patients and Methods

Patients

A convenience sample was taken of all patients with Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD) visiting the 'Spieren voor Spieren center´ of Wilhelmina Children's Hospital University Medical Centre Utrecht in the Netherlands between January and July 2012. Inclusion criteria were aged between 6-20 years, genetically confirmed diagnosis of DMD or BMD, able to follow test instructions and able to walk ≥ 20 meters without the use of assistive devices (15). Exclusion criteria were: concomitant medical problems that could intervene with the outcomes of the exercise tests, cardiac abnormalities disallowing cardiopulmonary exercise testing (according to the pediatric cardiologist), previous episodes of rhabdomyolysis and insufficient knowledge of the Dutch language. The research protocol was approved by the Medical Ethics Committee of the University Medical Centre Utrecht in The Netherlands. Fifteen subjects met the inclusion criteria and were invited to participate. One subject suffered from an instable heart condition and was excluded. Five subjects were not interested to participate. Eventually, nine subjects were included and informed consent was obtained from all subjects and their parents.

Methods

All measurements were performed within three consecutive weeks during two visits at the Wilhelmina Children's Hospital. The first visit was combined with their annual clinical follow-up appointment with the multidisciplinary team of the 'Spieren voor Spieren-center' and included the standard test protocol as described below ('visit 1').

<u>Demographics</u>

Data on medical history were obtained from medical records and included the type of muscular dystrophy (DMD versus BMD), cardiac involvement, medical treatment and co morbidities. Information on ambulation level, the use of assistive devices and education level were obtained from the participants and their parents at the first visit. Ambulation level was defined according to Hoffer (16). Participants who had independent and unrestricted ambulation without the use of assistive devices were defined as 'normal ambulant' (17).

Anthropometrics

Anthropometric measurements included body mass ((kg) and height (m) using an electronic scale (Seca, Hamburg, Germany) and a stadiometer (Ulmer Stadiometer, Ulm, Germany). Body mass index (kg/m^2) was calculated as BM in kg divided by the squared body height in meters. Standard deviation scores were calculated for BM for age, body height for age and BMI for age using Dutch growth Charts (18). Bioelectrical impedance analysis (Bodystat Quadscan 4000, Isle of Man, British Isles), were used to determine estimated percentage of body fat (19).

Pulmonary function

Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV1) were measured with a standard pulmonary function test. Maximal respiratory pressures were assessed with a Micro Respiratory Pressure Monitor (PT Medical, Leek, the Netherlands). Percentage scores were calculated based on pediatric reference values (20, 21).

Cardiac function

Eight subjects were under medical control of a (pediatric) cardiologist prior to the start of study. Three subjects were evaluated by their cardiologist as part of their annual follow-up by the multidisciplinary team. The most recent medical control of the other four subjects dated from 2010-2011. A resting electrocardiogram (ECG) was performed of all subjects and was evaluated by the same pediatric cardiologist (CB).

Visit 1: standard test protocol

The 6MWT was performed on a 20-metre track in a straight corridor. Test instructions and encouragements were performed according American Thoracic Society (22). A safety chaser was used to insure the safety of the participant during the test. Levels of fatigue and muscle pain were evaluated before and after the test with a validated rating scale for children (23). Six-minute walking distance (6MWD), heart rate, fall frequency and resting periods were recorded as performance outcome measures. Predicted 6MWD was calculated using the formula of Geiger et al. based on age, length and gender (24). Motor function and maximal isometric strength were

measured with ´The North Star Ambulatory Assessment´ (NSAA) and the handheld myometer, both according standardized protocols (25, 26).

Visit 2: cardiopulmonary exercise test

Within two and three weeks after the 6MWT, the subjects performed a progressive cardiopulmonary exercise test (CPET) using an electronically braked cycle ergometer (Lode Corrival Pediatric; Lode BV, Groningen, the Netherlands). The test started with 1-2 min of unloaded peddling. Depending on the individual perceived 6MWD, the work rate was increased with 5-10 Watts each minute until the subject voluntarily stopped because of exhaustion, despite strong verbal encouragement of the test-leader. Subjects breathed through a face mask (Hans Rudolph Inc., Kansas City, MO) connected to a calibrated expiratory gas analysis system (Cortex Metamax B3; Cortex Medical, Leipzig, Germany) (27). A 12-lead electrocardiogram (Hewlett-Packard, Amstelveen, the Netherlands) was recorded continuously throughout the entire test. The presence of ischemic signs or arrhythmic events was assessed during exercise. Transcutaneous oxygen saturation (SpO_2 %) was measured using pulse oximetry (Masimo R9, Marimo BV, Tilburg, the Netherlands) at the index finger. Blood pressure was measured every 2 minutes (SunTech Tango; SunTech Medical, Morrisville, NC, USA). Levels of fatigue and muscle pain were evaluated before and after the test with a validated rating scale for children (23).

Feasibility parameters

Measurement completion

The measurement completion of the CPET was compared to the 6MWT:

The 6MWT was considered successfully completed when the subject followed all ATS-guidelines on standardized instructions and encouragement (22).

Measurement completion of the CPET was determined based on the Rowland's criteria for maximum exercise testing (10) (table 1). The Respiratory Exchange Rate (RER) is the ratio of exhaled carbon dioxide (VCO2) and oxygen uptake (VO_2). RER reflects the peripheral gas exchange and is a useful indicator of effort and anaerobic metabolism. VO_2plateau is reached when the difference between normalized VO_{2peak} and the VO_2 in the last 30 seconds of the minute prior to the last minute, does not exceed 2.1 ml/kg/min. The subject performed a successful cardiopulmonary exercise test when the subjective criterion and at least two objective criteria were reached.

The test was considered a symptom limited exercise test when the subjective criterion a minimal test durance of six minutes was reached. The 6MWT and the CEPT were preliminary ended according ACSM guidelines on adverse events or when severe muscle pain occurred during the test (rating scale score (rss) > 6) (28, 29). The minimal accepted measurement completion rate for both tests was set at 90%.

Table 1. Rowland's criteria to evaluate VO_{2peak} in healthy children

Subjective criteria:
• Signs of intense effort (unsteady walking, running of biking; sweating; facial flushing; clear unwillingness to continue despite encouragement, Borg>8)
Objective criteria:
• Heart rate (HR)>180 beats/min
• Respiratory Exchange Rate (RER) > 1.00
• VO_2 plateau in the last minute

Adverse events

The week before and after each visit, levels of experienced fatigue and muscle pain were assessed. The subjects and their parents reported the level of muscle pain and fatigue each morning and evening on validated rating scales for children. Two and five days after each visit, the subject and his parents were called at home. Clinical features of rhabdomyolysis or other complaints that could be related to the exercise tests were evaluated (30, 31). All exercise ECGs assessed during the CPET were evaluated on ischemic signs or arrhythmic events by the same pediatric cardiologist (CB). A large increase in exercise induced muscle pain (rss > 6), signs of rhabdomyolysis or ischemic signs on the electrocardiogram during the CPET were considered as serious adverse events. The exercise tests were regarded as unsafe if ≥ 2 participants experienced serious adverse events.

Acceptability

After the CPET and 6MWT, subjects were asked to determine their willingness to

perform the test again in the future on a 'Visual Analoge Scale' ranging from 0 ('not at all') to 10 ('completely').

Exercise response

The exercise response of subjects that successfully completed a cardiopulmonary exercise tests were further analysed. Peak values of Oxygen Uptake (VO_{2peak}), normalized Oxygen Uptake (VO_{2peak}/kg), Work Rate (W_{peak}), normalized Work Rate (W_{peak}/kg), were determined based on the average value over the last 30 seconds of the test. The oxygen uptake eliciting the ventilatory threshold (VT) was determined by using the criteria of an increase in both the ventilatory equivalent of oxygen (VE/VO_2) and end tidal pressure of oxygen ($PETO_2$) with no increase in the ventilatory equivalent of carbon dioxide VE/VCO_2 and end tidal pressure of carbon dioxide ($PETCO_2$) (32). O_2-pulse is the amount of oxygen consumed per heartbeat and was calculated by dividing VO_{2peak} by HR_{peak}. Low O_2-pulse reflects either reduced stroke volume or reduced peripheral oxygen uptake (33).
Work efficiency (ΔO_2 /ΔWR) reflects the metabolic cost of performing external work and was calculated by dividing the difference between VO_{2peak} and $VO_{2unloaded}$ by W_{peak}. Low work efficiency might reflect reduced oxygen delivery or local hypo-perfusion. Predicted values were obtained from established reference values (34). The algorithm from Eschenbacher and Maninna, adjusted for the pediatric population was used to distinguish between cardiac, pulmonary, musculoskeletal and motivational factors to explain exercise performance (35, 36).

Data analysis

Quantitative descriptive statistics were used to present baseline characteristics, feasibility parameters and exercise outcome (Mean ± SD).
Group mean scores of reported muscle pain and fatigue over the first three and seven days were compared at baseline between visit 1 and 2 and between weeks pre- and post-visits. The mean change scores of pain and fatigue were compared between visit 1 and visit 2. Data were checked for normal distribution with the Shapiro-Wilk test. Students paired t-test and 95% confidence intervals were used for

normal distributed data. The Wilcoxon signed-rank test was used in case of skewed data. Significance level was set at $P<0.05$.

Results

Inclusion

The study population consisted of nine children and adolescents with DMD (N=3) and BMD (N=6). Demographic and anthropometric data are shown in table 2. None of the subjects previously experienced episodes of rhabdomyolysis. One subject with BMD (6) was known with a dilated cardiomyopathy (DCM) with a reduced left ventricular ejection fraction (28%). One subject showed borderline normal cardiac function (extreme heart-axis, Fraction Shortening=31%, LVEDd=55mm) (37). All other subjects showed normal cardiac function on resting ECG. Four subjects were treated with intermittent prednisone therapy and one subject with ACE-inhibitors. Approval of both the neurologist and the cardiologist was obtained for all subjects before assessment of the CPET. None of the subjects suffered from co morbidities. The level of ambulation was mild to moderately impaired; normal ambulatory (N=5), community ambulatory (N=2) and household ambulatory (N=2). To cover larger distances within the community two subjects used a regular bike, three subjects used an electric bicycle and three subjects used a wheelchair. All but one subject followed regular education.

Table 2. Baseline characteristics

Anthropometrics	**Mean (SD)**
Age (yrs)	10.6 (4.7)
Weight (kg)/Z-scores	37.0 (16.9)/-0.11(1.1)
Height (cm)/Z-scores	140.1 (23.7)/-0.85 (0.6)
BMI (kg/m^2)/Z-scores	17.8 (3.1)/0.46 (1.0)
Fat Percentage (%)	13.0 (8.9)

Pulmonary function	**Mean (SD)**
FEV1 (%)	94.4 (16.4)
FVC (%)	86.9 (13.3)
FEV1 / FVC (%)	108.6 (8.5)
MIP (%)	93.2 (36.5)
MEP (%)	73 (18.2)

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236 Feasibility parameters

237 *Measurement completion (table 3):*

238 Two-third (67%) of the subjects performed the 6MWT according all ATS-guidelines.
239 In two subjects continuous encouragement was required, in addition to the
240 standardized phrases, to motivate them to continue walking. Two subjects had to
241 rest one or two times during the test. The test was ended preliminary in one subject
242 because of extreme fatigue. There were no fall incidents. In one-third (33%) of the
243 subjects the cardiopulmonary exercise test was successfully completed according
244 Rowland's criteria (6,7,9). Three subjects performed a symptom-limited exercise test
245 (2,4,5) and three subjects demonstrated poor effort (1,3,8).

246 Table 3. Individual measurement completion according ATS guidelines (6MWT) and Rowland's Criteria

Participants	**6MWT**		**CPET**						
	instructions	Standardized encouragement	Signs of intense effort	HR_{peak}	RER_{peak}	VO_2peak – VO_2 last minute (ml/kg/min)	Rowland´s Criteria (out of 3)	Test durance (min)	Eschenbacher
DMD									
1 (CA)	+	-	-	126[#]	1.05	- 2.77[#]	1	6.83	Decreased effort.
2 (HA)	+	+	+	136[#]	1.13	0.01	2	2.00	Not applicable
3 (CA)	+	-	-	140[#]	0.87[#]	1.18	1	7.33	Decreased effort
BMD									
4 (NA)	+	+	+	167[#]	1.42	3.24[#]	1	12.4	Cardiac pump limitation and circulatory limitation
5(HA)	+	+	+	146[#]	0.88[#]	1.44	1	6.00	Cardiac pump limitation and circulatory limitation
6 (NA)	+	+	+	174[#]	1.31	1.49	2	15.00	Cardiac pump limitation and circulatory limitation
7 (NA)	+	+	+	178[#]	1.15	0.16	2	8.16	Moderate or severe cardiac 'pump' limitation
8 (CA)	+	+	-	141[#]	1.00	12.46[#]	1	6.00	Decreased effort
9 (NA)	+	+	+	195	1.06	0.10	3	10.33	No obvious cardiac or circulatory limitations

247 Legend: NA = Normal Ambulatory; CA =Community Ambulatory; HA = Household Ambulatory; [#]= not reaching HR 180.

248 (210-age), or RER > 1.00 or VO_2plateau; [##] = not reaching both HR >180, RER >0.99 and VO_2plateau

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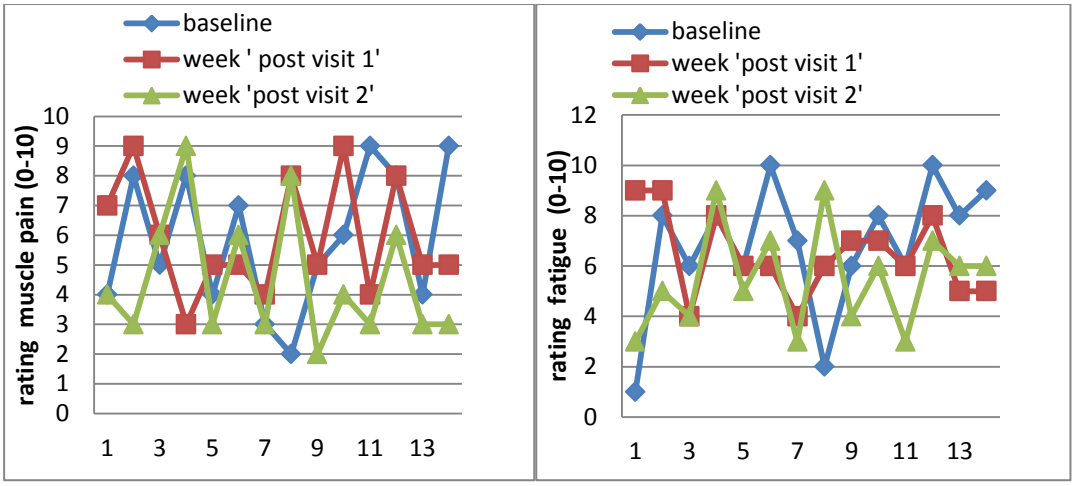
Adverse events:

None of the subjects preceded the predefined cut-off value of an increase of 6 points on the rating scale for muscle pain during both exercise tests. Subject 2 showed a significant different pattern of pain and fatigue scores on the self-reporting rating scales and was therefore analysed separately: Levels of pain and fatigue were remarkably high and showed large fluctuations. There was no clear difference noticeable between measurements at baseline and post visits (figure 1).

Figure 1. Pain and fatigue ratings of subject 2



In the other subjects (N=8), baseline levels of muscle pain and fatigue during the first three and seven days were not significant different between visit 1and visit 2 (p= 0.08-0.17). In comparison to levels at baseline, no significant change in pain or fatigue occurred after visit 1 and visit 2 (p= 0.2-0.9). There was no significant difference between the change score 'muscle pain 3-days' visit 1 versus visit 2 (-0.5 (1.6), -1.9 – 0.99, p=.44), 'muscle pain 7-days' visit 1 versus visit 2 (-0.5 (1.1), -1.5 – 0.5, p=.27), change score 'fatigue 3-days' visit 1 versus visit 2 (0.1 (1.7), -1.5 – 1.7, p=.89) and change score 'fatigue 7-days' visit 1 versus visit 2 (-.1 (0.6), - 0.6 – 0.4, p=.65).

Interview post-Visit 1 (table 4):

One subject (2) experienced muscle pain of the calves, back and neck (rss=6-9), malaise, agitation and fatigue in the first two days. In the following days all complaints improved reaching normal values. One subject (7) reported some muscle pain of his calves the next morning (rss=1). On day 1, 2 and 4 he experienced several incidents of severe muscle pain/cramps (rss=10) of the calves when

performing intensive physical activities such as swimming/walking long distances and playing. One subjects (8) experienced muscle pain of the dorsal side of his knees (rss=5) on day 2 after his tennis and swimming classes. One subject (5) fell one time the evening one day after the visit. His mother related this to general fatigue. Urine colour remained normal in all subjects.

Interview post visit 2 (table 4):
One subject (2) experienced muscle pain of the calves and legs (rss=6), agitation and fatigue in the first two days. In the following days his muscle pain and agitation improved and reached normal values. One subject experienced some muscle pain of his upper legs (rss=3) a couple of hours after the test. The same evening he fell one time. In the past he had experienced several fall incidents during a period of intensive muscle power training. He had no other complaints in the following week. One other subject (5) fell two times the same evening of the test. In the following five days he fell once more the morning after swimming classes. Urine colour remained normal in all subjects.

Table 4. Clinical features of rhabdomyolysis

	1	2	3	4	5	6	7	8	9
Local features									
Muscle pain	-	+	-	-	-	+	+	+	-
Tenderness	-	-	-	-	-	-	+	-	-
Swelling	-	-	-	-	-	-	-	-	-
Bruising	-	-	-	-	-	-	-	-	-
Weakness	-	-	-	-	+	+	-	-	-
Systemic features									
Tea-colored urine	-	-	-	-	-	-	-	-	-
Fever	-	-	-	-	-	-	-	-	-
Malaise	-	+	-	-	-	-	-	-	-
Nausea	-	-	-	-	-	-	-	-	-
Emesis	-	-	-	-	-	-	-	-	-
Confusion	-	-	-	-	-	-	-	-	-
Agitation	-	+	-	-	-	-	-	-	-
Delirium	-	-	-	-	-	-	-	-	-
Anuria	-	-	-	-	-	-	-	-	-

All subjects showed a normal exercise ECG during the CPET. There were no ischemic signs or arrhythmias. None of the subjects reported syncope, chest pain,

dizziness or other complaints. Blood pressure showed normal response during the exercise test. Oxygen saturation remained stable in all subjects.

Acceptability:

The acceptability of both exercise tests showed large variability between the subjects. The willingness to perform the 6MWT again in the future ranged from 1.7-10 with a mean score of 6.1 (3.5). Older children with relative mild physical impairments found the 6MWT not to be representative for their exercise capacity and therefore not useful. The Willingness to perform the CPET in the future ranged from 0-10 with a mean score of 3 (4.3). Younger children in general and children with moderate impairments experienced the extensive measurements during the test (ECG, blood pressure, oxygen mask) as a burden.

Exercise performance

Comparison CPET - 6MWT

Five subjects successfully completed both the 6MWT and the CPET (Table 5). One subject showed normal results on both the 6MWT and the CPET (9). Two subjects demonstrated normal 6MWT values but moderately reduced exercise capacity on the CPET (6,7). One subject showed a mildly reduced 6MWD but severely reduced exercise capacity on the CPET (4). In one subject the 6MWT and CPET were respectively moderately and severely reduced (5). All subjects showed higher peak heart rate and experienced fatigue during the CPET, than during the 6MWT.

Table 5. Exercise response

	4	**5**	**6**	**7**	**9**
6MWT					
6MWD	470	346	584	442	550
6MWD%	66	54	85	83	89
HR_{peak}	109	130	135	145	142
Borg scale Fatigue peak	3	6	6	3	2
CPET					
$VO_{2peak\ \%pred}$	49,7	19,8	58,1	56,6	94,8
$VO_{2peak/kg\ \%pred}$	58,0	19,1	58,6	64,1	110,9
$W_{peak\ \%pred}$	49,5	24,6	41,5	?	99,6

$W_{peak\ \%pred/kg}$	54,6	24,4	47,4	?	106,4
O_2-pulse (ml)	9,52	2,17	11,02	3,18	7,06
O_2-pulse (%pred)	57,4	26,2	64,4	63,7	93,8
ΔO_2/Wpeak	9,8	3,8	9,7	5,8	13
ΔO_2/Wpeak(%pred)	105,6	41	104,2	62,5	139,5
HR_{peak}	167	146	174	178	195
Borg scale Fatigue peak	9	8	9	5	6

Exercise response (CPET, 6MWT): normal (> 82%), mildly reduced (61-81%), moderately ~ (51-60%) (5), severely~ (<50%).

CPET parameters

The mean test endurance including unloaded peddling was 10.4 (3.5) minutes. The reason to end the test was fatigued legs (N=4) and shortness of breath (N=1). Two subjects reported relative low levels of experienced fatigue despite clear signs of intense effort. One subject demonstrated normal maximal exercise capacity and physiological exercise parameters (9). All other subjects showed reduced maximal exercise capacity with possible signs of cardiac pump and/or circulatory limitations (Table 3). The absolute and normalized VO_{2peak} and Work Rate were reduced in all subjects. Three subjects showed a reduced ventilatory threshold and one subject did not reach VT. One subject showed resting bradycardia (6). All four subjects demonstrated low peak heart rate values. There was an increased heart rate response in all four subjects. The O_2-pulse was reduced; one subject did not show any increase of O_2-pulse during the test (5). In the other three subjects there was no initially progressive rise of O_2-pulse at the start of the test (4,6,7). The work efficiency was reduced in two subjects (5, 7). None of the subjects showed pulmonary limitations reflected by normal ventilatory efficiency, ventilatory reserve and stable levels of SpO_2 during the test (table 6).

Table 6. Cutt-off points in the algorithm by Eschenbacher and Maninna

	Used cut off points*	Subjects	Indicative for
VO_{2peak} pred. (%)	<90%	1-8	Low VO_{2peak}
VR	<30%	-	Pulmonary limitation
VE/VCO_2peak	>36	-	Decreased ventilatory efficiency
HRR	>(-6.25 x age)+150	2,4,5,6,7	Cardiac pump limitation (cardiomyopathy/deconditioning)
AT%	<40	4,5,6	Circulatory or 'pump' limitation

VR=Ventilatory Reserve, HRR = Heart Rate Response; AT = anaerobic threshold

*adapted cut off points(36)

Discussion

The feasibility of the cardiopulmonary exercise test (CPET) was investigated in a heterogeneous sample of children and adolescents with Duchenne and Becker muscular dystrophy, by comparing exercise responses to the CPET with the standard protocol of functional tests for this population. No major adverse events occurred with respect to exercise induced muscle pain, signs of rhabdomyolysis or cardiac events and all subjects completed the study without functional deterioration. The measurement completion rate of the CPET was somewhat lower than the six minute walk test (6MWT). Both tests did not reach preliminary defined rates of minimal accepted measurement completion. Most subjects that successfully performed the CPET, showed low values of exercise capacity with signs of cardiac pump and/or vascular limitations. There was a large variability in perceived burden of both exercise tests possibly caused by age and disease severity.

Adverse events

None of the subjects demonstrated evident features of exercise induced rhabdomyolysis. Especially, there were no reports of tea-colored urine, a usually first sign of rhabdomyolysis (30) and previously reported in boys with DMD (38). Moreover, there was no deterioration of physical status at the end of the study. Nevertheless, several subjects did present with physical complaints during the study. One subject, with significant more physical impairments and higher levels of fatigue and muscle pain at baseline in comparison with others, demonstrated increased levels of agitation and malaise after both visits. Several other subjects with minimal complaints at baseline, presented with transient local complaints of muscle pain, cramp and fall incidents. All subjects had experienced these complaints before, while participating in high intensity sports or leisure activities. The variability of complaints between the subjects might indicate a relationship between exercise intensity, functional status and muscle response. The clinical meaning of these complaints and which pathophysiological mechanisms are primary responsible remain uncertain (39). Physical complaints following sport participation, such as muscle pain and muscle cramps, are also frequently reported in the healthy population which implicates a role of normal physiological responses on exercise

stimuli (40). The threshold, at which exercise is no longer beneficial but causes injury or other side-affects, is probably different both between muscular dystrophy patients as in comparison with healthy subjects. Exercise associated muscle cramps (EAMC) for example, is a common complaint in both muscular dystrophy patients and healthy athletes (40, 41). Several authors suggest an altered neuromuscular control as a result of the development of muscle fatigue to be the principal mechanism for the aetiology of EAMC (42, 43). Children with muscular dystrophy are likely to be more proned to develop EAMC given the decreased exercise capacity and altered walking pattern (44, 45) Performing muscular exercise in a shortened position, as children with MD do when walking on their toes, increases the likelihood of inducing cramp(46). This mechanism was striking illustrated by one of the subjects that suffered from muscle cramps in the past which resolved by using ankle splints during the night. During this study muscle cramps reoccurred after two consecutive days of intensive sport participation. The altered neuromuscular control resulting in muscle cramps in this subject and fall incidents in other subjects could primarily be caused by an increased fatigability leading to a 'cramp prone state' after the exercise tests. In this study, a threshold for 'normal versus pathological exercise induced muscle pain was set at in increase of 6 point on a self-rating scale of muscle pain. This cut-off point was chosen based on the study of Robertson et al. which demonstrated that the reported rate of muscle hurt in healthy children after lower extremity resistance exercise ranged from 3.2-6.7 (23). In clinical practice, biological markers such as serum levels of Creatine Kinase (CK) and Myoglobine (Mb) or muscle biopsy are used to examine possible muscle damage (47). Serum levels of muscle proteins in muscular dystrophy patients are higher at baseline and show more fluctuation than in healthy peers and seem therefore less reliable to evaluate exercise-induced muscle damage in this population (48, 49). Because of these validity concerns it was decided not to use biological markers in this study. Therefore conclusive evidence that the CPET can be safely performed in children with muscular dystrophy could not be provided. Nevertheless, intensive clinical monitoring of the subjects by means of questionnaire on muscle pain and fatigue, telephone interviews two and five days post-testing and strict monitoring of cardiac functioning during the exercise tests did not reveal clear signs of exercise induced muscle damage. To extend our knowledge on the exercise response of children with muscular dystrophy and provide patients and caregivers with adequate exercise prescriptions further research is warranted to

409 determine valid and feasible markers for exercise induced muscle damage in this
410 population.

411

412 Completion rate:

413 Two-third of the subjects did not reach the criteria for maximal cardiopulmonary
414 exercise testing as defined by Rowland et al. This can be explained by motivational
415 and physiological reasons. Three subjects ended the CPET premature without signs
416 of intense effort. Two of them, one six and one seven year old boy with DMD, also
417 needed additional encouragement during the 6MWT indicating general test
418 performance difficulties. Behavioral problems are frequently reported in the DMD
419 population (50). The relative young age of these subjects however, might have
420 influenced their performance as well. The acceptability of the CPET was generally
421 low in the younger subjects. They especially experienced the attached measurement
422 equipment such as the face mask and near infra-red spectroscopy probes as a
423 burden. Three subjects did perform a maximal effort but failed to reach all other
424 exercise criteria. This can for a part be explained by the fact that the Rowland's
425 criteria are primarily developed to detect cardiopulmonary limitations in healthy
426 children. In subjects with neuromuscular disorders and progressive muscle
427 weakness, exercise capacity will be increasingly reduced by peripheral limitations
428 without extensively stressing the cardiopulmonary system. For the present,
429 algorithms for the interpretation of exercise data in children with neuromuscular
430 diseases are not available. To further explore the value of maximal exercise capacity
431 as an outcome parameter in this population additional objective criteria to evaluate
432 the exercise response on peripheral level are needed (51).

433

434 Validity

435 Four out of five children with BMD that successfully performed both exercise tests
436 demonstrated lower values of exercise capacity on the CPET in comparison to the
437 6MWT. Two subjects demonstrated a moderate reduced exercise capacity on the
438 CPET despite normal results on the 6MWT. These results support the findings of
439 other studies that the CPET and 6MWT measure different aspects of exercise
440 capacity in less impaired children (52). All subjects with a reduced maximal exercise

capacity demonstrated an increased heart response, reduced anaerobic threshold and low O_2 pulse, all possible signs of cardiac 'pump' or circulatory limitations (35). Nevertheless, only one subject was known with a dilated cardiomyopathy. One subject, with mild functional limitations (community ambulatory) and borderline cardiac abnormalities on echocardiogram, did show a severely reduced maximal exercise capacity. Further research on the additional value of the CPET to detect first cardiac involvement and evaluate disease progression in patient with similar clinical presentation seems therefore valuable. Two other subjects with relative poor muscle function but no cardiac abnormalities, showed reduced mechanical efficiency ($\Delta VO_2/\Delta WR$). Groen et al. reported a reduced mechanical efficiency in children with Juvenile Dermatomyositis and suggested that the $\Delta VO_2/\Delta WR$-slope might be a sensitive marker for local hypo-perfusion (53). Several studies on DMD patients have reported an altered blood flow regulation in exercising skeletal muscle, based on the down regulation of neuronal nitric oxide synthase (nNOS), a dystrophin-associated protein (45, 54). In healthy subjects, nNOS produces the signaling molecule nitric oxide (NO) which modulates the vasoconstrictor response of sympathic reflex activation at the start of exercise. Sanders et al. used near infrared spectroscopy and demonstrated that nNOS deficient DMD patients fail to attenuate normal contraction-induced local vasoconstriction during a repeated isometric handgrip strength exercise which eventually leads to functional muscle ischemia. Tosetti et al. investigated muscle metabolism of mild Becker Dystrophy patients with magnetic resonance spectroscopy and found an increased reliance upon anaerobic metabolism during an incremental isometric strength exercise of the calve muscles but normal aerobic metabolism during recovery (55). Analysis of the NIRS measurements that were performed in this study during the cardiopulmonary exercise tests will hopefully give more insight in the relationship between local hypo perfusion and decreased aerobic exercise capacity in moderate to severely affected children. Future studies on muscle metabolism during aerobic exercise might contribute to a further understanding of exercise limitations in muscular dystrophy.

Limitations

The DMD population was relatively underrepresented in this study sample. Although DMD and BMD share a similar pathophysiological background and some children

with genetic confirmed Becker dystrophy present with a DMD phenotype, caution should be taken when generalising the results of this study to the DMD population (56). Given the fact, that no major adverse events were found in this study or reported in other studies, further research on cardio pulmonary exercise testing in a larger sample of ambulatory children with DMD could be of interest. However, the limited inclusion of children with DMD in this study might also indicate that the CPET is less relevant for this population, in comparison to other chronic diseases. A large part of the children with DMD that regularly visit our medical centre did not meet the inclusion criteria with respect to ambulatory status. The children with DMD that did participate showed motivational problems or advanced disease progression which negatively influenced test performance. Similar test performance difficulties are reported about other functional measures. This might reflect DMD specific behaviour problems what underlines the difficulty of finding feasible and valid outcome parameters for exercise capacity in this population (50). Further research on exercise testing and maximal exercise response should therefore primarily be focussed on the development of appropriate exercise test protocols for this specific population.

Conclusion

In children with relative mild impairments due to muscular dystrophy (MD), the cardiopulmonary exercise test seems to be a feasible and relevant outcome measure for exercise capacity and cardiovascular limitations. To extend our knowledge of exercise response of children with MD further research should be focused on the pathophysiological mechanisms of cardiovascular limitations and development of safety markers for exercise.

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