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Aerobic fitness in youth with chronic disease and mobility impairments: assessment and treatment

Aptidão aeróbia de jovens com doenças crônicas e mobilidade reduzida: avaliação e tratamento

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Gabriela Barroso de Queiroz Davoli

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Postgraduate Program in Rehabilitation and Functional Performance

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Doctoral Thesis presented to the Ribeirão Preto Preto Medical School, University of São Paulo to obtain the title of Doctor in Health Sciences.

Area: Physiotherapy

Supervisor: Professor, PhD Ana Claudia Mattiello-Sverzut.

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“...Serenidade para aceitar as coisas que não posso mudar, coragem para mudar as coisas que posso e sabedoria para discernir uma da outra...”

- Reinhold Niebuhr

DAVOLI GBQ. Aerobic fitness in youth with chronic disease and mobility impairments: assessment and treatment. 2023. 189p. Thesis – Ribeirão Preto Medical School, University of São Paulo, Brazil.

Introduction: Primary or secondary changes in the skeletal muscle system are observed in most childhood chronic diseases, which in this thesis included spina bifida (SB), spinal muscular atrophy (SMA), Charcot-Marie-Tooth disease (CMT), Duchenne and Becker muscular dystrophy (DMD e BMD), congenital muscular dystrophy (CMD), and myotonic dystrophy type 1 (MD1). These changes mainly affect the patient's lower limbs impairing their mobility. It increases the risk of a sedentary lifestyle and deconditioning, creating a "vicious cycle" of activity discouragement and worsening overall conditioning. Obesity and cardiovascular disease are additional inactivity problems, and aerobic fitness is reported as the main associated factor. Nevertheless, limited evidence is available about assessing and improving aerobic fitness in youth with chronic disease. **Aim:** to explore and create insight for alternative tests to assess the aerobic fitness of youth with chronic diseases which are non-ambulatory or have a mobility impairment and to test the effects of an upper-limb combined program on this group's aerobic fitness and muscle strength. **Methods:** In chapter two, three different cardiopulmonary exercise tests (CPET) were used: the arm-crank CPET, the 10 meters shuttle ride test (10m-SRT), and the short-time continuous push-test (ST-CPT) to test the validity and reliability of the ST-CPT for wheelchair-users patients with SB, and to develop a prediction model of absolute oxygen uptake (VO_{2peak}) for this test. In chapter three, a systematic review of literature following the PRISMA guidelines was performed to synthesize evidence about the quality and feasibility of CPET in NMDs and patients' aerobic fitness. One independent reviewer searched pubMed/MEDLINE, EMBASE, SCOPUS, and Web of Science databases between September and October 2020. At chapter four, two CPETs were used: the arm-crank CPET and the anti-gravity treadmill CPET to assess the quality and feasibility of these CPETs in mobility-impaired patients with SMA and BMD and to compare the exercise responses of the patients in the two tests. In chapter five, non-ambulatory and mobility-impaired patients with SB, CMT, CMD, and MD1 were randomized and submitted to a 14-week upper limb combined program consisting of a high-intensity-interval training - HIIT (8 weeks) and strength training (6 weeks) twice a week. **Results:** Chapter two shows that the ST-CPT is a valid and reliable maximal field test to assess aerobic fitness in wheelchair-users patients with SB. The distance covered in this test and the patient's body mass may estimate 72% of the patient's absolute VO2 peak. Chapter three presents that CPET is feasible for ambulatory patients with NMDs when their functional level and exercise modality are considered. However, there is still a vast potential for standardizing and designing disease-specific CPET protocols for patients with NMDs. Chapter four indicates that the anti-gravity treadmill CPET is a feasible and accepted test to assess the aerobic fitness of SMA and BMD patients. Conversely, the arm-crank CPET needs some adaptations to be feasible for this group. Chapter five shows that the upper limbs 14-week combined program improves aerobic fitness, performance, and flexor muscle strength of youth with SB, CMT, CMD, and MD1. **Conclusion:** This thesis brought a new perspective on pediatric rehabilitation regarding aerobic fitness assessment and treatment for chronic childhood diseases. Nevertheless, there are still many unanswered questions to be responded by future studies.

Key-words: aerobic fitness, combined training, children, adolescents, chronic disease.

DAVOLI GBQ. Aptidão aeróbia de jovens com doenças crônicas e mobilidade reduzida: avaliação e tratamento. 2023. 189p. Tese – Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil.

Introdução: Alterações musculares primárias ou secundárias são observadas nas doenças crônicas infantis, que nesta tese incluíram espinha bífida (EB), atrofia muscular espinhal (AME), doença de Charcot-Marie-Tooth (CMT), distrofia muscular de Duchenne e Becker (DMD e DMB), distrofia muscular congênita (DMC) e distrofia miotônica tipo 1 (DM1). Essas alterações afetam principalmente os membros inferiores dos pacientes prejudicando sua mobilidade e aumentando o risco de sedentarismo, descondicionamento físico, obesidade e doenças cardiovasculares. A aptidão aeróbia é o principal fator relacionado a isso, contudo, há pouca evidências sobre métodos de avaliação e intervenções terapêuticas para melhorar a aptidão aeróbia deste grupo. **Objetivo:** explorar e criar *insights* de testes alternativos para avaliar a aptidão aeróbia de jovens com doenças crônicas que não andam ou têm dificuldade de locomoção e testar os efeitos de um treino combinado com foco nos membros superiores (MS) sobre a aptidão aeróbia e força muscular deste grupo. **Métodos:** No capítulo dois, três diferentes testes de esforço cardiopulmonar (TECP) foram usados: o TECP em cicloergômetro de membro superior (CMS), o teste *shuttle* de 10 metros com propulsão da cadeira de rodas e o teste contínuo de curta duração com propulsão da cadeira (TC-CD-PCR) para testar a validade e confiabilidade do TC-CD-PCR para pacientes com EB não deambuladores, e desenvolver um modelo de predição do consumo absoluto de oxigênio (VO_{2pico}) para este teste. No capítulo três, uma revisão sistemática da literatura seguindo as diretrizes do PRISMA sintetizou evidências sobre a qualidade e viabilidade do TECP em DNMs e avaliou o condicionamento aeróbio desses pacientes. Um revisor independente pesquisou nas bases de dados PubMed/MEDLINE, EMBASE, SCOPUS e *Web of Science* entre setembro e outubro de 2020. No capítulo quatro, dois TECPs foram usados: o CMS e o TECP em esteira antigravidade para avaliar a sua viabilidade e qualidade em pacientes com AME e DMB e comparar as respostas de exercício obtidas nos dois testes. No capítulo cinco, pacientes não deambuladores e com mobilidade reduzida (EB, CMT, CMD e MD1) foram randomizados e submetidos a um treino combinado com foco nos MS (14 semanas) que consistiu em um treino intervalado de alta intensidade (8 semanas) e treino de força (6 semanas) 2x/semana. **Resultados:** O capítulo dois mostrou que o TC-CD-PCR é válido e confiável para avaliar a aptidão aeróbia de EBs não deambuladores. A distância percorrida neste teste e a massa corporal do paciente puderam estimar 72% do $VO_{2\ pico}$ absoluto. O capítulo três apresentou que o TECP é viável para pacientes deambuladores com DNMs quando o nível funcional dos pacientes e a modalidade de exercício foram consideradas. No entanto, ainda existe um vasto potencial para padronizar e projetar protocolos de TECP específicos para DNMs. O capítulo quatro indicou que o TECP em esteira antigravidade é viável e aceito para avaliar a aptidão aeróbia de pacientes com AME e DMB. Por outro lado, o CMS precisa de algumas adaptações. O capítulo cinco mostrou que o programa combinado de 14 semanas para MS melhora o condicionamento aeróbio, o desempenho e a força muscular flexora de jovens com EB, CMT, CMD e MD1. **Conclusão:** Esta tese trouxe uma nova perspectiva para a área da reabilitação pediátrica quanto à avaliação e tratamento da aptidão aeróbia em doenças crônicas infantis.

Palavras-chave: aptidão aeróbia, treinamento combinado, crianças, adolescentes, doenças crônicas.

Contents

1

General Introduction

GENERAL INTRODUCTION

Childhood chronic diseases

Primary or secondary changes in the skeletal muscle system are observed in most of the youth with chronic disease, which in this thesis included spina bifida (SB), spinal muscular atrophy (SMA), Charcot-Marie-Tooth disease (CMT), Duchenne and Becker muscular dystrophy (DMD e BMD), congenital muscular dystrophy (CMD), and myotonic dystrophy type 1 (MD1).

For lower motor neuron diseases, open spina bifida (SB), for example, is the most severe and common congenital defect of the neural tube (less than 1:1,000 born). Because of the spinal cord dysplasia, the patients' present signs of motor and sensory dysfunction below the spinal level of the lesion[1,2]. Therefore, secondary changes in the skeleton muscle system, such as muscle weakness, paralysis, hypotrophy, and shortening, are observed, mainly below the level of the lesion. These changes impair the patients' mobility skills from patients who are independent to walk, walk with aids such as orthosis, canes, walkers, or are wheelchair-users[3].

Spinal muscular atrophy (SMA) is also a cause of lower motor neuron disease (1 in 11,000 live births)[4]. Nevertheless, unlike SB, SMA principally affects the spinal cord anterior horn cells[4]. The defect in the survival motor neuron 1 gene (SMN1), responsible for encoding the survival motor neuron protein (SMN), leads to the degeneration of the motor neuron from the spinal cord[5,6]. The motor neuron degeneration causes general weakness, hypotrophy, muscle fatigue, and muscle shortening in this group. Thus, the secondary impairment at the skeleton muscle affects the patient's motor abilities and the achievement of motor milestones during childhood, such as sitting with or without support and ambulation[5].

Despite its classification as a lower motor neuron disease, the SMA also fits into the heterogeneous group of neuromuscular diseases (NMDs)[7,8]. NMDs comprise disorders characterized by the involvement of one or more components of the motor unit (motor neurons, peripheral nerves, neuromuscular junctions, and skeletal muscle)[4,7,8]. Regarding this group, many other inherited childhood chronic diseases that, primary and secondary, affect the skeleton muscle system can be highlighted.

Charcot-Marie-Tooth (CMT), for example, is a peripheral neuropathy and the most common NMD of childhood (1:2,500 live births)[4,9]. Mutation in more than 80 different genes affects the synthesis of some essential proteins for the structure and function of peripheral axons or Schwann cells leading to the degeneration of sensory and motor fibers[4]. Muscle weakness, hypotrophy and fatigue, are secondary to these damages and might impair the patient's ability to walk. Nevertheless, unlike the other already cited chronic infant diseases, few patients with CMT lose ambulation, less than 5%[10,11].

Unlike the conditions mentioned above, muscular dystrophies is a heterogeneous group of disorders contained in NMDs group and which primarily affect the skeleton muscle system[12]. It occurs because several proteins associated with the sarcolemma are affected disrupting the muscle cell and extracellular matrix interaction[13]. Duchenne muscular dystrophy (DMD), for example, is a dystrophinopathy that most affect children, specifically boys (1:5,000 boys live). In DMD, the non or reduced production of the sarcoglycan protein dystrophin, responsible for protecting the muscle fiber during contraction, leads to a continuous cycle of muscle degeneration and regeneration (by stem cells) ending in muscle replacement by collagen and fatty tissue[8]. This muscle remodeling causes weakness and fatigue, which in addition to the fast progression of the disease, result in ambulation loss around 9-12 years old. Other examples of muscular dystrophies are congenital muscular dystrophy (CMD) and myotonic dystrophy type 1 (MD1).

However, because of their many subcauses and a range of signs and symptoms, the function and mobility of CMD and MD1 patients are affected in different degrees[4].

Aerobic fitness in childhood chronic diseases

Despite the different spectrums of childhood chronic diseases, it is possible to distinguish from the above section that the primary or secondary impairment of the skeletal muscle system limits the patient's mobility and function to many degrees. Mobility limitations increase the risk of a sedentary lifestyle, favoring deconditioning and creating a "vicious cycle" of activity discouragement and worsening overall conditioning [14,15]. Obesity and cardiovascular disease are additional problems of reduced activity in healthy and disabled youths[16-19].

Studies exploring cardiovascular disease risk in non-disabled and disabled children and adolescents found aerobic fitness to be the main factor associated with cardiovascular risk[16,18]. Low aerobic fitness has been described for ambulatory[17,20-22] and wheelchair users patients with SB[17,20,23-,25], ambulatory youth and adults with SMA[26,27], and ambulatory children and adolescents with DMD[28,29]. To the best of our knowledge, no study assessed this physical fitness component of youth with CMT, CMD and MD1. Nevertheless, reduced aerobic fitness for these conditions has been described for ambulatory adults with CMT[30-34], CMD[30] and MD type 2, the adult manifestation form of MD[31].

During childhood, healthy and active habits are formed, and the family is open to lifestyle changes[18,35]. Moreover, sedentary children and adolescents tend to be sedentary adults. Therefore, monitoring aerobic fitness and introducing strategies to increase it is a fundamental aspect of pediatric rehabilitation to reduce cardiovascular risk in chronic childhood diseases.

Assessment of aerobic fitness in childhood chronic diseases

The main indicator of aerobic fitness in the pediatric population is peak oxygen uptake (VO_{2peak})[36,37]. The gold-standard method to obtain it is through an incremental cardiopulmonary exercise test (CPET) with gas exchange measurement and performed to tolerance limit or until indications for termination[38]. Nevertheless, because of the variability in mobility and function impairment from the different childhood chronic disease, numerous devices and protocols have been elected for CPET performance in this group.

For ambulatory youth with SB patients, for example, a treadmill[21,39] and lower limb cycle ergometer[17,18,20] have been used. Different, for ambulatory SMA recumbent lower limb cycle ergometer was the elected option[26,27], while for ambulatory DMD again, the lower limb cycle ergometer was used[28,29]. Moreover, for wheelchair user SB the arm-crank ergometer[17,18,20,25,40,41] and the wheelchair propulsion ergometer[24,42] were the elected devices.

Regarding the CPET protocols, step increments were used for SB patients performing the CPET on the treadmill[39,43]. Based on the underlying disease, step, and ramp-wise increments were used for lower limb cycle ergometers. Specifically, for SB patients, for example, only the step increment was used[17,18,20], while for patients with DMD, one study used the step increment[28] and the other the ramp-wise increment[29]. Concerning patients with SMA, only the ramp-wise increment was elected[26,27]. Similar to what was observed for lower limb cycle ergometers, both types of increments were used in the arm-crank ergometer studies. Five studies used the step increment[17,18,20,40,41] and three the ramp-wise increment[25,42].

However, the speed or workload used for the increment varies from 0.25 km/h to 0.50 km/h on the treadmill[39,43]. At the lower limb cycle ergometer, it was from 5 to 20

watts per minute[17,18,20,26-29]. Similarly, in the arm-crank ergometer, the range was from 5 to 15 watts per minute[17,18,20,40,41], while in the wheelchair propulsion ergometer, it was set at 0.1 torque per minute[24,42].

This variety of devices, types, and rates of increments make it difficult for professionals to analyze the results and select the best option to provide for the patients to have their best capacity at the CPET in clinical practice and research. Distinct physiological responses are generated by different devices and protocols in youth. Rowland et al.[44], for example, found lower values of heart rate peak (HR_{peak}) and higher values of peak respiratory exchange rate (RER_{peak}) in the CPET performed in a lower limb cycle ergometer than at the treadmill. Armstrong et al.[37] obtained different values of HR_{peak}, RER_{peak}, and peak minute ventilation (VE_{peak}) in two different CPET protocols performed on a treadmill.

In addition, another two points deserve attention when assessing aerobic fitness: (1) the muscle mass involved in the task and (2) the specialization and expensiveness of CPET. Regarding the first one, the amount of active muscle mass impacts the physiological responses obtained in the CPET[45,46]. Studies performed in healthy adults have shown that the physiological values obtained during an arm-crank CPET are approximately 70% lower than those obtained in a lower limb cycle ergometer[47,48].

Considering the second factor, CPET requires specialized and expensive equipment and professionals, which are not always available in clinical practice[41,49]. Thus, some authors proposed maximal field tests such as the 10 meters shuttle run and ride test (10m-SRunT and 10m-SRideT, respectively) as alternatives to assess ambulatory[50] and wheelchair-users patients[24]. Nevertheless, these field test protocols also show reservations about the measured main variable: aerobic fitness, agility, or anaerobic performance[24,49].

Aerobic fitness intervention in childhood chronic diseases

Considering the elevated risk of cardiovascular disease for chronic childhood disease, emerging strategies to improve physical fitness, mainly aerobic fitness, are important for this group[45]. Few studies have tested the beneficial effects of exercise on the physical fitness of children and adolescents with chronic diseases[23,26,40,43,51]. Regarding aerobic exercise, the Groot et al.[43], for example, reported an increment in the distance covered during the six-minute walking test (6MWT) and a moderate increase of VO_{2peak} and maximum speed achieved at the CPET for ambulatory youth with SB after a 12-week home-based aerobic program[43]. A rise in the distance covered during the 6MWT was also found for ambulatory patients with DMD after a 12-week home-based aerobic training[51], and gains at the maximum workload achieved at the CPET for wheelchair-user children and adolescents with SB, after a 16-week home-based aerobic program[40]. In a less specific-disease group, Zwinkles et al.[23] observed increment at anaerobic performance (mean power, peak power and agility), and aerobic performance (number of shuttles achieved at the 10m-SRunT and 10m-SRideT), although no significant changes in the VO_{2peak}, after a 8-weeks high-intense-interval training designed for wheelchair and ambulatory chronic youths.

To the best of our knowledge, no study examined the effect of strength training alone on the physical fitness of children and adolescents with chronic disease. Aerobic fitness benefits have been reported from strength training in healthy children and adolescents[52]. Moreover, additional healthy gains may be reach with combined programs of strength and aerobic training[52]. Montes et al.[26], for example, checked the benefits of a 6-months home-based combined program (strength and aerobic), in ambulatory children, adolescents and adults with SMA. These authors found a significant improvement in the

VO_{2peak} of patients despite no changes at muscle strength, motor function and distance covered at the 6MWT.

Many different variables contribute to physical fitness gains after aerobic, strength, or combined programs in healthy and chronically disabled youth, including training environment, type, specificity, frequency, intensity, and duration[15,45,52]. Most studies cited above opted for home-based programs[26,40,43,51], focused on the lower limbs of ambulatory chronic disease youth[26,23,43,51]. The intensity of the training programs varied from 70-130% of the maximal speed[43], 60% of HR_{peak}[51], anaerobic threshold HR[40], moderate intensity based at OMNI perception exertion scale (aerobic training) and 60-80% of the one maximum repetition (strength training)[26] to maximal effort based at subjective perception[23]. The training frequency ranges from two times a week[23,43] to three times a week[40,51]. Only the study of Montes et al.[26] used a high frequency of five times a week for aerobic exercise, despite a three times a week frequency for strength exercise.

This variety in frequency, intensity, and duration of the training makes it difficult for clinicians to analyse the beneficial effects of these programs and choose one to implement in clinical practice. Moreover, because most programs have been designed for patients with slight impairments in mobility and focused on the lower limbs, there is a gap in the literature regarding exercise for non-ambulatory patients and patients with large impairments in mobility. Those patients would benefit from training directed at the less disease-affect members, such as the upper limbs.

Considering the above sections, this thesis has two general aims: (1) to explore and create insight for alternative tests to assess the aerobic fitness of chronic disease youth who are non-ambulatory or have mobility impairment, (2) to test the effects of a combined program (aerobic and strength training) on the physical fitness of this group. Therefore, the second chapter will present the psychometric properties (validity and reliability) of a

newly designed field test, entitled "short-time continuous push-test," to assess the aerobic fitness of wheelchair users with SB, together with a prediction model of the VO_{2peak} to facilitates this test implementation in clinical practice. The third chapter brings a literature review of the available CPET protocols used to assess patients with NMDs, and a careful examination of these protocols' quality and feasibility, guiding research and clinicians to better options. A pilot study about the feasibility of two different CPET protocols and devices (lower body positive pressure treadmill and the arm-crank ergometer) to assess the aerobic fitness of youth with SMA can be found in chapter four. The fifth chapter brings the effects of a 14-week combined program (aerobic and strength training) on the aerobic fitness and muscle strength of children and adolescents with chronic disease. We expect this thesis to bring a new perspective on pediatric rehabilitation regarding aerobic fitness assessment and treatment for chronic childhood diseases.

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2

Short-Time Continuous Push-Test to Assess and Predict the Aerobic Fitness of Wheelchair Users Youth with Spina Bifida: Criterion Validity and Test-Retest Reliability

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Abstract

Objective: To estimate the validity and reliability of the short-time continuous push-test to assess the aerobic fitness of wheelchair users with spina bifida (SB). Secondarily, to develop a prediction model for the oxygen uptake (VO_{2peak}) in this test.

Design: Cross-sectional observational study, including the test-retest design.

Setting: Specialized rehabilitation units at two Brazilian hospitals.

Participants: Children and adolescents with SB (n=24), wheelchair-user for the distance of 500m of functional mobility scale. Median (95%CI) age of 10.0 (10.0-12.4) years.

Interventions: Not apply

Outcomes: The arm-crank CPET, the 10 meters shuttle run test (10m-SRT) and short-time continuous push-test were performed to assess validity. For reliability, 50% of the patients performed the short-time continuous push-test twice (test-retest). Thirteen patients participated in the prediction model development.

Results: No significant differences were found between the arm-crank CPET, 10m-SRT and short-time continuous push-test, except for the respiratory exchange ratio (RER_{peak}) between CPET and short-time continuous push-test and distance between 10m-SRT and short-time continuous push-test. Correlations between the three tests for most variables were strong to moderate (r=0.55-0.89). Excellent to good reliability (ICC=0.75 to >0.90) and agreement (small LOA, SEM and CV) were found for all variables between the test and retest. The short-time continuous push-test distance and patient body mass could predict 72% of VO_{2peak} (SE: 176.7 ml/min, CV: 7.4%).

Conclusion: The short-time continuous push-test is a valid and reliable maximal field test to assess aerobic fitness in wheelchair-users patients with SB. The distance covered in this test and the patient's body mass may estimate the patient's VO_{2peak}.

Key words: field test, aerobic fitness, prediction model, wheelchair, upper limbs

Introduction

Open spine bifida (SB) is the most severe and common congenital defect of the neural tube (less than 1:1000 born)[1,2]. Because the spinal cord dysplasia, signs of motor and sensory dysfunction below the spinal level of the lesion are observed early and influence the patients' mobility skills[1,2]. Mobility limitations increase the risk of a sedentary lifestyle, obesity and cardiovascular disease in this group[3,4]. In SB wheelchair-user patients, additional factors such as lower limb hypomobility and deconditioning place them at a higher cardiovascular risk than their ambulatory SB peers[4-6].

A study exploring cardiovascular disease risk in adolescents and young adults with SB found aerobic fitness to be the only factor associated with cardiovascular risk in this group[4]. Low aerobic fitness has been described in wheelchair-users patients with SB compared to their ambulatory peers with SB[3,6-8] and other childhood diseases such as cerebral palsy[8,9]. Thus, evaluating aerobic fitness and introducing strategies to increase it is a fundamental aspect of pediatric rehabilitation to reduce cardiovascular risk in this group[4].

The main indicator of aerobic fitness in the pediatric population is peak oxygen uptake (VO_{2peak})[10,11]. For wheelchair users, the gold-standard method to obtain it is through an incremental cardiopulmonary exercise test (CPET) using an arm-crank or wheelchair propulsion ergometer and gas analysis[12-14]. Nevertheless, the CPET requires specialized and expensive equipment and professionals, which are not always available in clinical practice[5,12]. In order to facilitate the implementation of aerobic fitness assessment as a routine, maximal field tests such as the 10m-shuttle test (10m-SRT) and the 12-minute wheelchair propulsion test have been proposed for children and adolescents with SB[5,8].

In general, these field tests proved to be more practical and functional tests assessing the aerobic fitness of wheelchair patients in more common and specific muscle activities for them[5,8,9]. The 10m-SRT is a maximal and incremental field test. Its measurement properties were tested, and excellent reliability and validity were found for children and adolescents with cerebral palsy-CP[9], osteogenesis imperfect-OI and SB when compared to CPET[8,15]. Despite being a valid test to assess aerobic fitness, the frequent deceleration, accelerations and turnings during the 10m-SRT require great anaerobic performance and agility[8,9,16]. For patients with SB, for example, Bloemen et al.[8] found high correlations between the number of shuttles achieved during the 10m-SRT and two agility tests (10×5 meter sprint test and the slalom test) and moderate correlation with an anaerobic power test.

In 2018, our research group studied an alternative to that. The feasibility and validity of the maximal continuous "12-minute wheelchair propulsion test", proposed by Franklin et al.[17], was tested in wheelchair-users children and adolescents with SB comparing the peak heart rate (HR_{peak}) achieved in this test with the one reached in the CPET[5]. Of the 11 patients assessed, only four completed the test (12 minutes). However, the authors observed that patients who stopped the test in the sixth minute (n=6) presented HR_{peak} corresponding from 88 to 109% of the HR_{peak} achieved in the CPET, where another objective criterion of maximal effort was achieved (peak respiratory exchange ratio - RER_{peak}>1.0). This finding led the authors to suggest that the 12-minute wheelchair propulsion test could be a good test to assess the aerobic fitness of wheelchair-user children and adolescents with SB; however, protocol adaptations are needed[5].

Therefore, considering the importance of having valid and reliable maximal field tests to evaluate and follow the aerobic fitness of wheelchair-users patients with SB to prescribe and design rehabilitation protocols, this study has two aims. First, to estimate

the validity and reliability of the adapted 12-minute wheelchair propulsion test, we will entitle here a “short-time continuous push-test” to assess the aerobic fitness of wheelchair users with SB. Secondarily, to develop a prediction model for the VO_{2peak} in this test to favor its use in clinical practice. We expect the cardiac, pulmonary and performance variables of the short-time continuous push-test not to differ and present a moderate to strong correlation with the same variables obtained from the CEPT and 10m-SRT. We also hypothesize that the distance from the short-time continuous push-test is a good predictor of VO_{2peak}.

Methods

Study design and participants

It is a cross-sectional observational study, including the test-retest design, performed from September 2017 to December 2019. During this period, 78 children and adolescents with SB followed at the Rehabilitation Centre from the Clinical Hospital of Ribeirão Preto Medical School, University of São Paulo (CER-HC-FMRP-USP), and Pediatric Urology of the Children's Institute from the Clinical Hospital of Medicinal School, University of São Paulo (ICr-HC-FMUSP) were invited to participate. Only 36 of those patients attended the study assessments. However, 12 patients had just participated in one of the three tests used in this study (see *Criterion validity)* and had to be excluded. Therefore, 24 patients completed at least two tests and composed the final sample size. The inclusion of patients followed the criteria: confirmed diagnostic of SB, age range from 8 to18 years old, wheelchair users at least for the 500 meters distance of the functional mobility scale – FMS 500m (community), and able to understand test instructions. Patients who had fractured the upper limbs one year before the study or presented hydrocephaly resulting in motor incoordination were excluded. The Ethical

Committee of CER-HC-FMRP-USP and ICr-HC-FMUSP approved this study (CAEE nsº: 66503017.6.0000.5440 and 66503017.6.3001.0068). All participants and their legal guardians signed the informed consent.

Study protocol

Criterion validity

Three different tests assessed the patients' aerobic fitness. Patients performed all tests in the same period (afternoon), but only one test was carried out per day, with a 48-interval time applied between the tests. Due to safety reasons, the CPET conducted in a hospital environment at the Ergospirometry Laboratory of CER-HC-FMRP-USP, or the Clinical Research Laboratory of ICr-HC-FMUSP, was the first test performed by the patients. The other two field tests, the 10m-SRT and the short-time continuous push-test, were conducted in a sports court environment in the School of Physical Education and Sport of Ribeirao Preto, at the Healthy Science Department of Ribeirao Preto Medical School, or the Associations Athletic Academic Oswaldo Cruz in São Paulo. The patients performed the two field tests on the same sports court and under similar conditions. Moreover, the execution order of the field tests was randomized. Before the first test, a researcher drew lots one of two identical folded papers containing the tests' names.

Before the CPET, patients had their body mass (ZTFI LD1050, Sao Paulo, Brazil), arm span (distance between the third finger from one hand to another), and body composition (Biodynamics-450, Sao Paulo, Brazil) determined[18,19]. Patients and their caregivers were also interviewed about their functional mobility level using the Brazilian version of FMS[20], answered two questionnaires to determine their level of physical activity[21] and sexual maturation status[22]. Moreover, an expert physical therapist tested their level of spinal injury[23].

Test retest reliability and agreement

Fifty percent of the patients who performed the short-time continuous push-test were randomized and invited to do this test again after a one-week interval. Twelve patients composed the reliability and agreement study. They performed the second short-time continuous push-test in the same sports court, day period, and similar condition (e.g., wheelchair, evaluator) from the first time.

Measures

Arm-crank CPET

The CPET was performed using an electro-magnetically braked arm cranking ergometer (Lode Angio, Lode BV, Groningen the Netherlands) following the same protocol and interruption criteria described in the study of Tuijtelaars et al.[18] and Leonardi-Figueiredo et al.[19].

10m-SRT

The 10m-SRT followed the same protocol and interruption criteria described in the study of Bloemen et al.[8].

Short-time continuous push-test

For the short-time continuous push-test, the patients were positioned beside a cone at the start of a 10 meters linear track delimited by two cones. After two minutes of resting, a trained physiotherapist (GBQD) explained and demonstrated the test. The short-time continuous push-test consisted of (a) warm-up: 2 minutes propelling their wheelchairs in a comfortable self-selected speed; (b) effort: 6 minutes propelling their wheelchairs from one cone to another as fast as possible to cover the greater distance possible; (c) cool-down: 2 minutes propelling their wheelchairs in a comfortable self-

selected speed. Every 2 minutes, the physical therapist gave a standardized stimulus for the patients to maintain a fast speed and performance. Completed the six minutes, the test ended. Reasons for earlier test interruption included: patients' inability to continue the test because of arm pain or exhaustion.

During the three tests, patients wore a facemask and a heart rate monitor strap (Polar®. V800, EUA or GARMIN®, EUA) to continuously measure and monitor the gas exchange and heart rate, respectively. Patients in the CPET also wore an electrocardiogram monitoring electrode (3M™ Red Dot™ with foam tape and sticky gel, EUA). Calibration was performed before each test. In 54% (n=13) of the tests, we used the Oxycon mobile device (Carefusion, Yorba Linda, CA, EUA), and in 46% (n=11), the K5 (COSMED, Rome, Italy).

Cardiorespiratory responses

The mean value obtained in the last 30 seconds of each test indicated the peak of VO_2, minute ventilation (VE_{peak}), breath frequency (BF_{peak}), respiratory exchange ratio (RER_{peak}), and oxygen pulse (O_2/HR_{peak}). The HR_{peak} was the highest HR obtained in each test. The criteria of maximal effort followed the achievement of at least one objective criteria ($HR_{peak} \geq 180$ bpm a $RER_{peak} \geq 1.0$) and all subjective criteria (sweating, facial flushing, apparent unwillingness to continue the test despite encouragement)[8].

Statistical analysis

The analysis was performed in the software SPSS® (version 23) and Microsoft Excel. Histogram and box plot checked the normal distribution of the variables. The data did not follow a normal distribution. Thus, the numerical variables were reported as a median and confidence interval of 95% [95%CI]. Ordinal data were shown as frequency and percentage. Criterion validity of the short-time continuous push-test was assessed by

ANOVA of Friedman's test, followed by a posthoc analysis for paired comparisons using the Wilcoxon signed-rank test with Bonferroni correction and the Spearman correlation coefficient test. A p-value<0.016 was considered for significance, and a very strong and strong correlation was indicated by correlation coefficient (r) ≥ 0.90 and 0.70 ≥ r ≤ 0.89, respectively[24]. Excellent and good reliability (test-retest) of short-time continuous push-test considered an intraclass correlation coefficient (ICC)>0.90 and ICC= 0.75-0.90[25]. The ICC was calculated based on one measurement (K=1), absolute agreement and a 2-way mixed-effects model[25]. For absolute agreement (test-retest), the Bland Altman plot and the standard error of measurement (SEM) with the smallest detectable change (SDC) were used[26,27]. In the case of heteroscedasticity, the coefficient of variation (CV) was elected[27]. To develop the prediction model of VO_{2peak}, scatterplots checked the linear relationship between the absolute VO_{2peak} obtained from the first short-time continuous push-test performed and independent variables. Stepwise univariate linear regression analysis determined which independent variables significantly correlated with the absolute VO_{2peak} (Table 4), and the stepwise multivariate linear regression checked the best model. Autocorrelation in the residuals was evaluated by the Durbin-Watson statistic[24]. Multicollinearity and highly influential points (outliers) were checked[24]. Homoscedasticity was verified, and histogram and P-P plots assessed the normality of residuals[24]. To validate the prediction model, the Wilcoxon signed-rank test, ICC, the Bland Altman plot, SEM or CV assessed the measured and predicted values of absolute VO_{2peak}. A p-value<0.05 was considered for statistical significance.

Results

Participants

Of the 24 patients included, 15 achieved the criteria of maximal effort in the CPET, 14 in the short-time continuous push-test and, 12 in the 10m-SRT. The table 1 summarizes the patients' characteristic. The patients achieving maximal effort in at least one of the three tests (n=18) presented a median age (95% confidence interval) of 10.5 (10.0-13.0) years old, a body mass of 39.6 (34.3-50.3) Kg and an arm span of 142.0 (135.3-153.0) cm. Most patients (56%) were male, in the pubertal sexual maturation status (83%), and wheelchair users for mobility in the school (FMS 50 m: 88%). Thirty-nine percent of patients had the highest level of spinal injury (thoracic), and 33% had a sedentary level of physical activity. In the 10m-SRT and short-time continuous push-test, the patients had a higher percentage of predicted HR_{peak} and delta of HR_{peak} (ΔHR - HR_{peak}-HR_{rest}) (Table 1.).

Table 1. Patients characteristic

Variables	**All patients Median n=24**	**Patients with maximal tests n=18**	**Patients with submaximal tests n=6**
		Median (95%CI)	
Age (years)	10.0 (10.0-12.4)	10.5 (10.0-13.0)	10.0 (8.0-13.0)
Body mass (Kg)	39.6 (34.5-47.0)	39.6 (34.3-50.3)	38.5 (26.0-46.5)
Arm span (cm)	142.0 (137.2-150.9)	142.0 (135.3-153.0)	144.5 (132.4-156.0)
BMI (Kg/m^2)	18.1 (15.9-20.2)	19.0 (16.2-21.4)	15.4 (11.4-21.0)
Fat-free mass (%)*	65.3 (63.5-74.5)	65.2 (62.5-75.0)	67.0 (52.0-88.3)
Fat mass (%)*	34.6 (25.4-36.5)	35.0 (25.1-37.4)	33.0 (11.5-49.0)
BMR*	808.5 (710.3-1067.9)	827.0 (699.0-1151.0)	727.0 (405.4-1158.5)
	n (%)	**n (%)**	**n (%)**
Sex (m/f)	14/10 (58/42)	10/8 (56/44)	4/2 (67/33)
Sexual maturation			
pre-pubertal	5 (21)	3 (17)	2 (33)
pubertal	19 (79)	15 (83)	4 (67)
FMS 5m			
crawling	9 (37)	5 (28)	4 (67)
wheelchair	10 (42)	9 (50)	1 (17)
crutches	1 (4)	-	1 (17)

independent on level surfaces	4 (17)	4 (22)	-
FMS 50m			
wheelchair	21 (88)	15 (83)	6 (100)
sticks	1 (4)	1 (6)	-
independent on level surfaces	2 (8)	2 (11)	-
FMS 500m			
wheelchair	24 (100)	18 (100)	6 (100)
Level of lesion			
thoracic	10 (42)	7 (39)	3 (50)
high lumbar	9 (37)	6 (33)	3 (50)
low lumbar	4 (17)	4 (22)	
sacral	1 (4)	1 (6)	
Level of physical activity			
extremely sedentary	6 (25)	6 (33)	4 (67)
sedentary	9 (38)	5 (28)	-
moderately active	8 (33)	7 (39)	1 (17)
active	1 (4)	-	1 (17)
% predicted of HR_{peak}			
CPET (bpm)	79 (73-85)	87 (78-90)	65 (55-78)
10m-SRT (bpm)	87 (81-90)	93 (84-96)	77 (74-82)
Continuous push-test (bpm)	90 (82-91)	92 (85-96)	79 (72-83)
ΔHR (HR_{peak}-HR_{rest})			
CPET (bpm)	68 (53-74)	70 (63-83)	35 (15-61)
10m-SRT (bpm)	67 (60-78)	84 (67-90)	52 (43-71)
Continuous push-test (bpm)	72 (64-82)	86 (70-92)	59 (49-70)
CPET workload (watts)	35 (29-46)	39 (30-52)	23 (16-41)
Test duration			
CPET (s)	425 (381-579)	454 (397-648)	329 (217-515)
10m-SRT (s)	597 (525-669)	710 (544-769)	589 (455-619)

Legend: n: number; Kg: kilogram; cm: centimetre; BMI: body mass index; m: meter; BMR: basal metabolic rate; m: male; f: female; HR: peak heart rate; CPET: cardiopulmonary exercise test; bpm: beats per minute; 10m-SRT: ten meters shuttle ride test; predicted of HRpeak: 208 x 0.7 (age); ΔHR: delta heart rate; s: seconds; * missing four patients.

Criterion validity

Of the 18 patients who achieved maximal effort, 11 completed the three tests, two only completed the CPET and the short-time continuous push-test, and one completed the

10m-SRT and the short-time continuous push-test. The other three patients only performed the CPET (Table 2). Table 2 shows the comparison of tests. No significant difference was observed for HR_{peak}, O_2/HR_{peak}, absolute and relative VO_{2peak}, VE_{peak}, and BF_{peak}. The RER_{peak} was significantly smaller in the short-time continuous push-test when compared with the CPET ($X(2)=6.20$, $p<0.015$). The distance achieved in the short-time continuous push-test was significantly smaller when compared with the 10m-SRT ($X(2)=8.22$, $p<0.009$).

Table 2 also shows the short-time continuous push-test, CPET and 10m-SRT correlations. Strong correlations were found between the first test and the last two tests for the variables HR_{peak} (CPET: $r=0.73$ and 10m-SRT: $r=0.77$, $p<0.01$, respectively) and VE_{peak} ($r=0.81$ and $r=0.72$, $p<0.01$). The short-time continuous push-test's relative VO_{2peak} ($r=0.90$ $p<0.01$) and absolute VO_{2peak} ($r=0.84$ $p<0.01$) strongly correlated with the same variables from the 10m-SRT. Similar findings were obtained for RER_{peak} ($r=0.89$, $p<0.001$), VE_{peak} ($r=0.81$, $p<0.01$), and distance ($r=0.72$, $p<0.01$) from CPET with the short-time continuous push-test (Table 2.). A moderate correlation was found for $O2/HR_{peak,}$ BF_{peak} and distance from the 10m-SRT and absolute and relative VO_{2peak} from the CPET with the same variables from the short-time continuous push-test. Differently, the $O2/HR_{peak}$ and BF_{peak} from the CPET and the RER_{peak} from the 10m-SRT showed a weak and non-significant correlation with the short-time continuous push-test (Table 2.)

Table 2. Validity of Continuous push-test: comparison and correlation of cardiac, pulmonary and performance variables among tests.

	Comparison Median (25%-75%)			Correlation r (95%CI)	
Variables	**CPET (n=16)**	**10m-SRT (n=12)**	**Continuous push-test (n=14)**	**CPET *vs.* Continuous push-test (n=13)**	**10m-SRT *vs.* Continuous push-test (n=11)**
HR_{peak} (bpm)	176 (151-188)[a]	186 (166-192)	187 (179-191)	0.73 (0.30-0.91)[a]	0.77 (0.31-0.94)
O_2/HR_{peak} (bpm/ml)	4.1 (3.7-4.7)[b]	5.1 (3.7-6.3)[c]	4.7 (4.0-6.2)[c]	0.48 (-0.21-0.90)[b]	0.65 (-0.25-0.92)[c]
Relative VO_{2peak} (ml/kg/min)	17.0 (14.7-22.0)	17.2 (14.0-25.0)	18.2 (16.4-26.0)	0.55 (-0.00-0.84)	0.90 (0.65-0.97)
Absolute VO_{2peak} (ml/min)	690.0 (520.0-886.1)	687.6 (609.1-1140.2)	782.1 (625.4-1097.8)	0.66 (0.20-0.90)	0.84 (0.48-0.96)
RER_{peak}	1.08 (1.03-1.28)	1.03 (1.00-1.13)	1.08* (0.93-1.15)	0.89 (0.70-0.96)	0.49 (-0.15-0.84)
VE_{peak} (L/min)	31.7 (22.0-36.3)[d]	34.4 (29.5-54.0)	39.1 (27.9-50.0)	0.81 (0.37-0.95)[b]	0.72 (0.21-0.92)
BF_{peak} (breath/min)	47 (34-53)[d]	54 (48-66)	58 (46-64)	0.42 (-0.28-0.83)[b]	0.66 (0.10-0.90)
Distance (m)	569.0 (211.0-855.0)	570.0 (357.5-725.0)	396.0[#] (300.0-448.0)[c]	0.72 (0.25-0.91)[a]	0.61 (-0.03-0.90)[a]

Legend: Median (25%-75%); CPET: cardiopulmonary exercise test; HR: heart rate; bpm: beats per minute; Δ: delta; VO_2: oxygen uptake; ml: millilitre; Kg: kilogram; min: minute; RER: respiratory exchange ratio; VE: minute ventilation; l: liter; BF: breath frequency; O_2/HR: oxygen pulse; m meter; *p<0.016 when compared CPET *vs* Continuous push-test;; [#]p<0.016 when compared 10m-SRT *vs.* Continuous push-test.

[a]missing one patient;

[b]missing three patients;

[c]missing two patients;

[d]missing four patients.

Test retest reliability and agreement

Eight of the 12 patients who completed the test-retest study achieved maximal effort (Table 3). Excellent reliability was found for absolute VO_{2peak}, VE_{peak}, distance and total average speed (ICC>0.90, low SEM and CV values) (Table 3). Good reliability was observed for HR_{peak}, O_2/HR_{peak}, relative VO_{2peak}, BF_{peak}, and average speed obtained in the first, fifth, and sixth minutes (ICC>0.80, low SEM and CV values). Moderate reliability was found for the average speed obtained in the second, third and fourth minute (ICC>0.60, low SEM and CV values) and poor reliability for the RER_{peak} (ICC=0.11), despite great absolute reliability (SEM=0.06 [SDD=0.72]) (Table 3). The Bland Altman plots (Figures 1) demonstrate great agreement for all the variables, indicated by the small limits of agreements (LOA), from test and retest (Table 3 and Figures 1a-d and 2a-d).

Table 3. Reliability of continuous push-test

Variables Median (n=8)	**Continuous push-test 1**	**Continuous push-test 2**	**ICC (95%CI)**	**SEM**	**SDC**	**CV**	**Bias (SD)**	**LOA**
HR_{peak} (bpm)	187 (181-192)	184 (173-191)	0.82 (0.10-0.96)	1.6	3.5	_	4.9 (5.9)	-6.78-16.51
O_2/HR_{peak} (bpm/ml)	4.3 (3.8-5.6)	5.1 (4.3-6.1)	0.88 (0.14-0.98)	0.14	1.04	_	-0.52 (0.5)	-1.51-0.46
Relative VO_{2peak} (ml/kg/min)	18.7 (15.7-25.6)	21.3 (17.2-28.5)	0.89 (0.49-0.98)	0.67	2.28	_	-2.2 (3.6)	-11.68-4.84
Absolute VO_{2peak} (ml/min)	782.1 (680.5-1047.7)	931.5 (752.7-1118.8)	0.92 (0.57-0.98)	_	_	8.25% (4.12-12.62)	0.08* (0.12)	29%below-19%above
RER_{peak}	0.95 (0.90-1.1)	1.02 (0.94-1.1)	0.11 (-7.3-0.84)	0.06	0.72	_	-0.01 (0.14)	-0.51-0.25
VE_{peak} (L/min)	39.1 (28.1-61.4)	36.7 (29.9-59.5)	0.97 (0.84-0.99)	0.68	2.29	_	0.03 (6.9)	-13.52-13.59
BF_{peak} (breath/min)	60 (51-65)	58 (50-63)	0.88 (0.46-0.98)	1.05	2.84	_	1.4 (5.4)	-9.23-12.00

Distance (m)*	396.0 (331.0-459.0)	410.0 (338.0-472.1)	0.97 (0.87-0.99)	1.80	3.72	_	-10.1 (20.5)	-50.28-30.00
Average speed 1º min (m/s)*	1.2 (0.9-1.3)	1.0 (0.7-1.4)	0.89 (0.46-0.98)	0.02	0.43	_	0.06 (0.18)	-0.30-0.41
Average speed 2º min (m/s)*	1.2 (1.0-1.3)	1.0 (0.8-1.3)	0.62 (-0.89-0.93)	0.06	0.66	_	0.08 (0.21)	-0.33-0.50
Average speed 3º min (m/s)*	1.2 (0.9-1.2)	1.2 (0.9-1.3)	0.73 (-0.98-0.95)	0.05	0.64	_	-0.01 (0.19)	-0.38-0.36
Average speed 4º min (m/s)*	1.0 (0.9-1.2)	1.0 (0.8-1.2)	0.63 (0.10-0.97)	0.06	0.67	_	0.04 (0.15)	-0.25-0.34
Average speed 5º min (m/s)*	1.0 (0.9-1.2)	1.0 (0.8-1.3)	0.84 (-0.1-0.97)	0.04	0.57	_	0.00 (0.17)	-0.34-0.33
Average speed 6º min (m/s)*	1.2 (0.9-1.3)	1.0 (0.8-1.3)	0.84 (0.21-0.97)	0.06	0.45	_	0.11 (0.16)	-0.20-0.43
Total average speed (m/s)*	1.1 (0.9-1.3)	1.1 (0.9-1.3)	0.97 (0.88-0.99)	0.01	0.19	_	-0.3 (0.05)	-0.14-0.08

ICC: intra-class correlation coefficient; SEM: standard error of measurement; SDC: smallest detectable change; CV: coefficient of variation; bias: the mean of difference; SD: standard deviation; LOA: limits of agreement; HR: heart rate; bpm: beats per minute; VO_2: oxygen uptake; ml: millilitre; Kg: kilogram; min: minute; RER: respiratory exchange ratio; VE: minute ventilation; l: liter; BF: breath frequency; O_2/HR: oxygen pulse; m meter; min: minute; s: second; *logarithm normal transformed data .

Prediction model of absolute *VO_{2pico} from short-time continuous push-test*

The data of 13 patients who completed the first short-time continuous push-test were used to analyze the absolute VO_{2pico} predicted model. The distance was the main independent variable to predict the absolute VO_{2pico} (R^2 =0.52, p<0.01) (Table 4). The stepwise multiple regression analysis included only variables strongly and significantly correlated with the absolute VO_{2pico}. Moreover, due to multicollinearity, only distance, body mass and heart rate were included. The distance and body mass could explain 72% of the variance in absolute VO_{2pico} (adjusted R^2 =0.72, p<0.01). The resulting equation is

absolute VO_{2peak}=-217.116+1.721 x distance + 9.704 x body mass (standard error of the estimate [SEE]=176.7 ml/min).

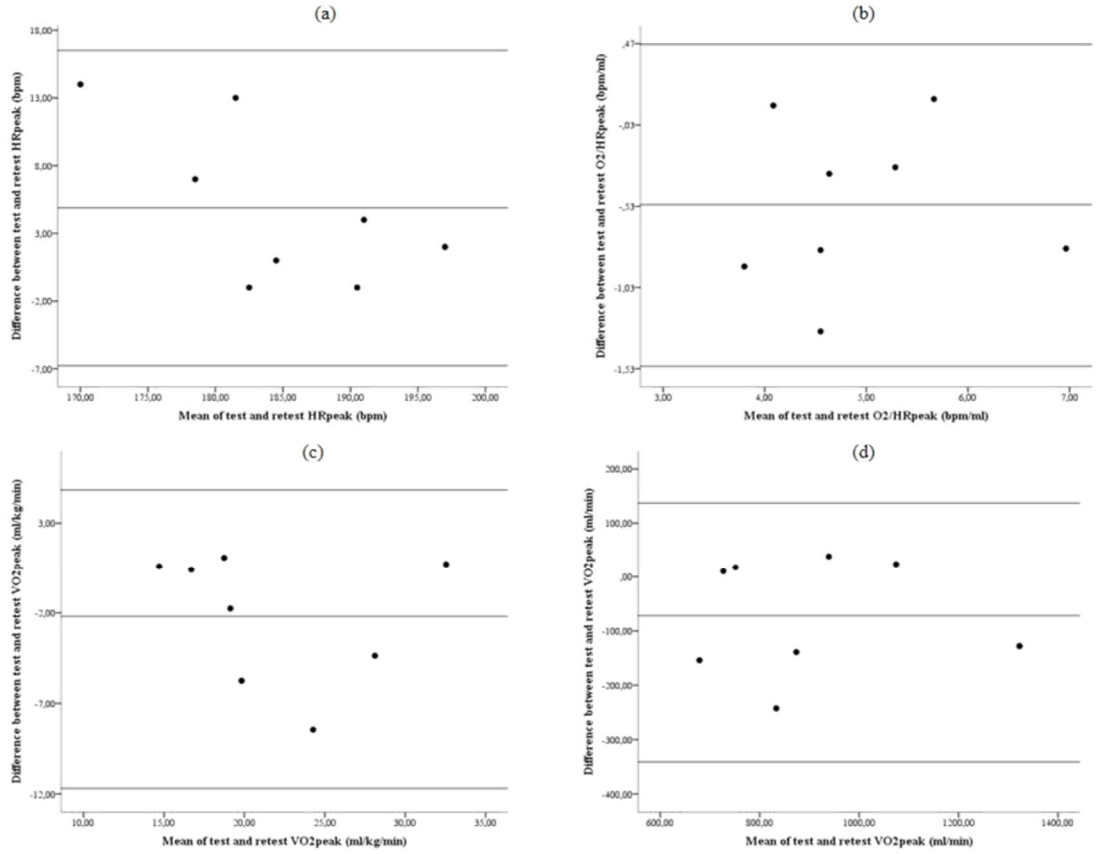

Figure 1 (a-c). Bland–Altman plot of the HR_{peak}, O_2/HR_{peak} and relative and absolute VO_{2peak} during the shot-time continuous push test and retest

Validation of the prediction model of absolute *VO_{2pico}*

The absolute VO_{2peak} data from the eight patients who performed the second short-time continuous push-test (retest) were used to validate the model. There was no difference between the measured (Median: 931.5 ml/min [95%CI: 752.8-1118.8]) and predicted (Median: 817.2 ml/min [95%CI: 715.1-1049.9]) values of absolute VO_{2pico} (p=0.21). Excellent reliability (ICC=0.92 [95%CI: 0.63-0.98]; CV: 7.4% [95%CI: 3.4-11.1%) was obtained between the measured and predicted values of absolute VO_{2pico} (p<0.001). The Bland Altman plots (Figure 3) demonstrate substantial agreement for

measured and predicted values of absolute VO_{2pico} with an average bias of 0.05 (0.13) and LOA of -0.19-0.29.

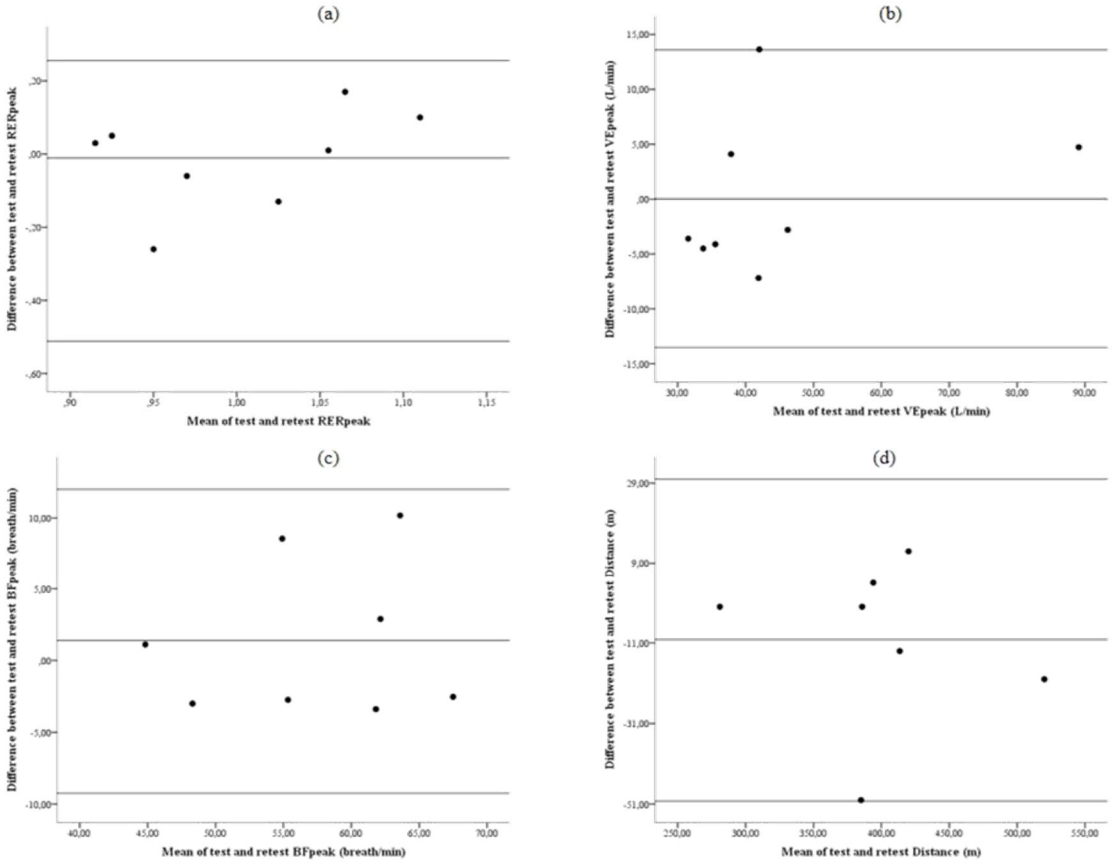

Figure 2 (a-c). Bland–Altman plot of the RER_{peak}, VE_{peak}, $BR_{peak,}$ and distance during the shot-time continuous push test and retest

Table 4. Results of the Linear Regression Analysis: Correlation of independent variables with VO_{2peak}

Test variables	**R^2**	***B***	**P-value**	**95%CI**
Distance	0.52	0.72	0.006	0.8-3.6
Distance olecranon- fist' styloid process	0.50	0.71	0.004	25.5-108.9
Body mass (Kg)	0.47	0.68	0.007	4.2-21.2
Arm span (cm)	0.46	0.68	0.007	3.9-20.3
Age (years)	0.39	0.62	0.020	11.8-110.1
Heart rate	0.36	0.60	0.024	1.7-19.8
Distance acromion-oleocranon	0.31	0.55	0.040	3.3-122.1
Average speed 1º min (m/s)*	0.30	0.55	0.080	-85.0-1298.0
Arm-circumference	0.21	0.46	0.102	-6.2-60.2
Total average speed	0.15	0.38	0.245	-447.5-1541.0
Sex	0.10	0.32	0.26	-177.1-596.8
BMI (Kg/m^2)	0.09	0.31	0.285	-6.5-41.9
Fat-free mass (%)	0.04	0.21	0.523	-11.0-20.3
Fat mass (%)	0.04	-0.21	0.512	-21.0-11.0
Level of lesion	0.02	0.15	0.618	-423.6-684.1

Legend: Kg: kilogram; cm: centimetre; m: meter; R^2: coefficient of determination; *B:* unstandardized beta; 95%CI: confidence interval of 95%.

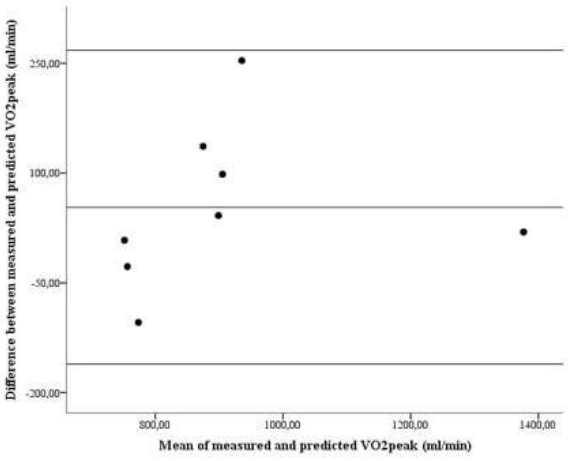

Figure 3 (a-c). Bland–Altman plot of the measured and predicted absolute VO_{2peak} during the shot-time continuous push test and retest.

Discussion

The main object of the present study was to validate and test the reliability of the short-time continuous push-test to assess the aerobic fitness of wheelchair users with SB. Our results showed that the short-time continuous push-test is a valid and reliable test to assess the aerobic fitness of this group. Moreover, using the distance covered in this test and the patient body mass we could develop a valid and reliable prediction model able to estimate 72% of the absolute VO_{2peak}.

Maximal field tests are great alternatives to follow aerobic fitness and prescribe aerobic rehabilitation training for wheelchair-user youth in clinical practice. Its advantages are using a functional activity, wheelchair propulsion[6,20], and no need for sophisticated equipment[9,17]. Even tough, there was limited evidence about maximal field tests for wheelchair-user children and adolescents with SB. The 10m-SRT that require great anaerobic performance and agility[8,9,16], and the "12-minute wheelchair propulsion test," proposed by Franklin et al.[17] which require a total perimeter of 75.32 meters, not feasible for most clinical facilities[5], and has a highly intense duration[5]. Because of that, in the present study, we adapted the "12-minute wheelchair propulsion test," [17], here entitled short-time continuous push-test and tested its psychometric properties.

Regarding the validity of the short-time continuous push-test tested in the presented study, the RER_{peak} was the only variable diverging between this test and the gold standard, arm-crank CPET. This difference might have occurred because six patients of the 14, presented RER_{peak} <1.0 in the continuous push-test, despite achieving objective criteria of maximal effort for HR_{peak}> 180 bpm, subjective signals of maximal effort and a large median of delta HR (HR_{peak}-HR_{rest}) = 83 bpm. Conversely, in the arm-crank CPET most patients achieved RER_{peak}>1.0.

The type of exercise performed and the protocol used may influence the RER_{peak} value[10,11]. Rowland et al.[28] found higher RER_{peak} values resulting from a CPET performed in a lower limb cycle ergometer than at a treadmill[29]. Armstrong et al.[11] obtained different values of RER_{peak} in two different CPET protocols performed on a treadmill without having different values of absolute and relative VO_{2peak}. In the present study, we used two different protocols, incremental (arm-crank CPET) and continuous (short-time continuous push test), and two types of exercise arm-crank (arm-crank CPET) and wheelchair propulsion (short-time continuous push test). This difference in protocols and type of exercise may justify the slight difference in the RER_{peak} between the arm-crank CPET and the short-time continuous push test, despite obtaining a strong and significant correlation ($r=0.89$, $p<0.001$).

Considering 10m-SRT and the short-time continuous push test, the distance covered in these tests was the only variable with a significant difference. In the 10m-SRT, the patient had no time limit to propel their wheelchair as far as possible, while in the short-time continuous push test, they only had 6 minutes. This duration difference can be observed by the 10m-SRT lasting two times more than the short-time continuous push test. In addition, the weak correlation found between O_2/HR_{peak} and BF_{peak} from arm-crank CPET and the short-time continuous push, and between the RER_{peak} from 10m-SRT and the short-time continuous push may be explained by a difference in test performance[8] and problems with the gas analysis measurement. Of the 14 patients who performed a maximal short-time continuous push test, only ten had O_2/HR_{peak} and BF_{peak} values from the CPET, and only 11 had RER_{peak} in the 10m-SRT.

Concerning the short-time continuous push test and retest reliability, measured by the intraclass correlation (ICC), we found poor reliability for the RER_{peak}. Nevertheless, the standard error of measurement (SEM), indicates great agreement between the test and

retest. Atikson et al.[27] reported that the ICC is affected by sample heterogeneity. Therefore, these authors have suggested avoiding the sole use of ICC in reliability studies. The SEM is a complementary measure of reliability to the ICC, because in its calculation the use of standard deviation (SD) party cancels the inter-individual variation present in ICC[27].

VO_{2peak} prediction models allow aerobic fitness assessment without the need for gas analysis and expensive equipment. Recently, Tuijtelaars et al.[18] developed an absolute VO_{2peak} prediction model using the CPET workload peak (W_{peak}) for pediatric patients with SB. The designed model included the W_{peak} and the sex and could predict 93% of the absolute VO_{2peak} obtained from arm-crank CPET. Regarding wheelchair field tests, Bloemen et al.[8] tried to develop a model to predict the absolute VO_{2peak} from the number of achieved shuttles in the 10m-SRT. Three independent variables (number of achieved shuttles, height and body mass) compose the model and could predict 77% of the absolute VO_{2peak} variance. Nevertheless, the large individual errors observed when using the predicted model lead those authors not to indicate its application in clinical practice.

In the present study, the distance covered in the short-time continuous push test could explain 52% of the absolute VO_{2peak} variance. The model, including the distance and body mass, explained 72% of the absolute VO_{2peak} obtained in the short-time continuous push test. Nevertheless, its standard error of the estimate (SEE) indicating the difference between the values predicted and measured by the model was 176.7 ml/min. Smaller SEEs were found in the model of Tuijtelaars et al.[18] (SEE: 96.0 ml/min) and Decker et al.[30] (SEE_{boys}: 120.0 ml/min, SEE_{girls}: 95 ml/min). This higher SEE may be related to the sample size used to develop the predicted model (n=13), despite 26 patients in the study of Tuijtelaars et al.[18] and 124 typical children and adolescents in the study of Decker et al.[30]. Besides that, the developed model was valid and presented excellent

reliability and agreement. Even though professionals using the developed prediction model should be attentive to this difference (SEE).

This study provides a valid and reliable test to assess the aerobic fitness of wheelchair-user children and adolescents with SB. This test has a short duration, is continuous and dynamic, making it more enjoyable and less demanding for deconditioned children and adolescents with SB who self-propel their wheelchairs. Moreover, its valid and reliable prediction model for oxygen uptake (VO_{2peak}) dismisses using gas analysis to follow changes in aerobic fitness, favoring the short-time continuous push test use in the clinical practice as a routine.

Limitations of the study

This study has several limitations. First, not all patients completed the three tests, and 25% of those who performed at least two tests did not achieve maximal effort and were excluded from the statistical analysis. The patient's motivation to attend many assessments on different days might have influenced their failure to reach at least one of the objective criteria of maximal effort. Therefore, the data obtained here are not necessarily transferable to another population. Another limitation was using arm-crank CPET as a gold standard method, despite some studies reporting that the wheelchair ergometer CPET is a better option. Nevertheless, this equipment is not available in our institution.

Conclusion

The short-time continuous push-test is a valid and reliable maximal field test to assess aerobic fitness in wheelchair-users children and adolescents with SB. This limited duration and continuous characteristic make this test tolerable for deconditioned wheelchair individuals. Moreover, when the gas analysis is unavailable, the distance

achieved in this test and the patient body mass may be used to estimate the aerobic fitness of the patient.

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3

Cardiopulmonary Exercise Testing in Neuromuscular Disease (NMD): A Systematic Review.

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Abstract

Introduction: Cardiopulmonary exercise testing (CPET) is increasingly used to determine aerobic fitness in health and disability conditions. Patients with neuromuscular diseases (NMDs) often present with symptoms of cardiac and/or skeletal muscle dysfunction and fatigue that might impede the ability to deliver maximal cardiopulmonary effort. Although an increasing number of studies report on NMDs' physical fitness, the applicability of CPET remains largely unknown.

Areas covered: This systematic review synthesised evidence about the quality and feasibility of CPET in NMDs and patient's aerobic fitness. The review followed the PRISMA guidelines (PROSPERO number CRD42020211068). Between September and October 2020 one independent reviewer searched the PubMed/MEDLINE, EMBASE, SCOPUS, and Web of Science databases. Except for reviews and protocol description articles without baseline data, all study designs using CPET to assess adult or paediatric patients with NMDs were included. The methodological quality was assessed according to the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) recommendations.

Expert opinion: CPET is feasible for ambulatory patients with NMDs when their functional level and the exercise modality are considered. However, there is still a vast potential for standardizing and designing disease-specific CPET protocols for patients with NMDs. Moreover, future studies are urged to follow the ATS/ACCP recommendations.

Keywords: exercise test, exercise modality, feasibility, muscle disease, rehabilitation, aerobic fitness.

Introduction

Neuromuscular diseases (NMDs) are a heterogeneous and complex group of inherited or acquired disorders involving one or more components of the motor unit (motor neuron, peripheral nerve, neuromuscular junction, and skeletal muscle)[1,2]. Because of the disease-specific muscle weakness and fatigue, these patients present limited physical activity, contributing to deconditioning and creating a "vicious cycle" of activity discouragement and worsening overall conditioning[3]. In addition to that, some subtypes of NMDs, such as muscular dystrophy patients, also suffer from cardiomyopathy and disturbances of conduction, which also prevent them from fully engaging in exercise[3,4].

Cardiopulmonary exercise testing (CPET) is an incremental test with gas exchange measurement and performed to the limit of tolerance or until indications for termination[5]. It provides the investigator information on the integrative exercise response of multiple physiological systems (cardiovascular, pulmonary, hematopoietic, neuropsychologic, and skeletal muscle) to meet the increased metabolic demand of oxygen consumption and carbon dioxide production of the active muscles during exercise[5,6]. This is possible because the pattern of oxygen uptake (VO_2), ventilation (VE), and carbon dioxide output (VCO_2) measured breath by breath reflects the efficiency of the heart, lungs, circulation blood, pulmonary blood flow, and peripheral oxygen[5]. Therefore, using CPET is possible to distinguish the dominant physiological system limiting exercising (cardiac, pulmonary, muscle metabolism, or deconditioning), optimizing the therapeutic decision-making process[7].

The non-invasive characteristic of CPET and its usefulness lead to an increased interest in using it to assess exercise limiting factors and the efficacy of interventions in patients with NMDs. For example, Rapin et al.[8] were able to identify peripheral factors

as the main limitation to exercise in adults with muscular dystrophies, metabolic myopathies, and hereditary peripheral neuropathies. Crescimanno et al.[9] observed a slight increase in the aerobic fitness of patients with glycogen storage disease type II in 36 months of enzyme replacement therapy, and Wiesinger et al.[10] prescribed and assessed the efficacy of a six-week aerobic training for adults with inflammatory myopathy.

Despite those interesting findings using CPET in NMDs, no previous study has assessed the safety, quality, and applicability of CPET for this group. The study of such aspects is important because CPET is an intense stress test first developed to assess patients with cardiovascular and pulmonary diseases[6]. Most patients with NMDs have high levels of fatigue and present weaker muscles, more susceptible to contraction-induced muscle fiber injury, than patients with cardiac or pulmonary disease. Therefore, an intense test as the CPET could be detrimental for some NMDs.

Regarding that, this review has four aims: (1) to identify and synthesise evidence about the available CPET protocols for NMDs, (2) to evaluate the quality and feasibility of these protocols, (3) to assess the aerobic fitness of patients with NMDs and (4) to provide recommendations about the use of CPET for this group. We are investigating these properties because the technical quality and delivered effort's quality guarantee the appropriate interpretation of CPET outcomes in clinical practice and research. Moreover, information about completion rate and adverse events can address whether the CPET protocols are practical and suitable for this group or if adaptations are needed. We hypothesize that the available CPET protocols are feasible for patients with high functional levels, such as ambulatory patients, and innovations and adaptations are needed to use this test in less functional patients.

Methods

This systematic review of the literature is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[11], and it was registered in the International Prospective Registry of Systematic Reviews (PROSPERO) under the number CRD42020211068.

Data source and search strategy

Following the approach of Bramer et al.[12], we created a systematic search strategy for the databases MEDLINE using the MESH thesaurus terms for 'NMD' and 'CPET'. NMDs included muscular dystrophies, congenital myopathies, spinal muscular atrophies, amyotrophic lateral sclerosis, post-poliomyelitis, polyneuropathies, Guillain-Barre syndrome and myasthenia gravis. Consecutively, this search strategy was adapted to the databases EMBASE, SCOPUS, and Web of Science. An example of this search strategy is shown in Supplementary Material A.

Between September and October 2020, one reviewer (GD) independently searched all databases and selected the relevant articles based on titles and abstracts. Subsequently, the full-text articles of selected studies were checked for compliance with the selection criteria described below. If there was doubt, a second reviewer (TT) was consulted for the decision on the included articles. Relevant reference lists were also hand-searched to identify additional records. The selection process was supported by an online version of Endnote software (Endnote Clarivate Analytics®).

Selection criteria for eligible articles

Study design and language

Cross-sectional observational studies, cohort-studies, case-reports or control studies, randomised or quasi-randomised clinical trials, and protocol descriptions of clinical trials with baseline data written in English, Portuguese, Spanish, Dutch, German, or French, were included. Narrative literature reviews, systematic reviews, protocol

descriptions of clinical trials without baseline results, or studies of which the full text was not available, were excluded.

Participants

Patients with NMD, without restriction to sex and age, were included.

Studies that evaluated patients with diabetic or compression neuropathies, chronic fatigue syndrome or fibromyalgia, radiculopathy, spinal cord injuries, complex regional pain syndrome, or additional diagnoses to the NMD reported on the study's inclusion and exclusion criteria were excluded.

Methodology

Studies that performed a CPET on patients with NMDs to assess aerobic fitness or intervention effects on aerobic fitness (e.g. training programme, diet or medication), or studies that assessed the psychometric properties of CPET in this group, were included. Studies that did not describe the exercise modality, the interval and/or workload increments, or the velocity and/or grade increments of the CPET protocol, were excluded; likewise, studies, reporting submaximal exercise tests, field tests, electronically assisted tests, or anaerobic tests, were excluded.

Data extraction

Using a standard form, one reviewer (GD) extracted data from the included studies about (1) characteristics of the population (Tables 1a and b), (2) characteristics of the CPET (Tables 2a and b), (3) the quality and feasibility of CPET (Tables 3a and b) and (4) aerobic fitness of the patients (Tables 4a and b). If there was doubt, a second reviewer (TT), was consulted. The percentages of the predicted peak oxygen uptake (VO_{2peak}) and peak heart rate (HR_{peak}) were calculated following reference values for exercise modality and age[7,13–15].

Methodological Quality

The recommendations of the American Thoracic Society/American College of Chest Physicians (ATS/ACCP) for CPET methodology, which include standard information about equipment, modality, protocol, conduct of the test, monitoring, safety and personal issues, were used to determine the methodological quality of included studies[6].

We created an adapted list (Supplementary Material B) and scored all included articles on 18 different criteria. The required information was collected and double-checked by a reviewer (GD), and for each criterion met, an article was attributed one point score. Additionally, a sum score was calculated and the studies were classified as low quality (≤7 points), sufficient quality (>7 points), moderate quality (11 to 14 points) and high quality (≥14 points of the maximum score of 18).

Analysis and Data Synthesis

The information on CPET protocols and outcome parameters obtained from the included studies were qualitatively summarised in overview tables and text. To facilitate the interpretation, the data were grouped based on the sub-classifications of the NMDs and the exercise modality. The NMDs were grouped as: (1) Glycogen storage disorders (GSD), (2) Mitochondrial disorders (MitoD), (3) Inherited myopathies (IHM), (4) Inflammatory myopathies (IM), (5) Motor neuron disorders (MND), (6) Peripheral nerve disorders (PND) and (7) Neuromuscular junction disorders (NMJD) [16]. The exercise modality was classified as upright (UPC), recumbent (RC), semi-recumbent (SRC), and supine (SC) cycle ergometer, arm-crank (AC), and treadmill (T).

The quality of the CPET performance was based on the minimum test duration recommended for age range, and the number of patients that achieved the criteria of maximal effort (Tables 3a and b). The feasibility of CPET was determined based on the

percentage of patients that completed the tests and the number of adverse events reported (Tables 3a and b), and the number of patients who achieved at least 80% of the predicted VO_{2peak} considering age and exercise modality (Tables 4a and b). The studies with sufficient quality scores on the ATS/ACCP adapted list (>7 points), and which met the quality and feasibility criteria that supported the recommendations on how to test patients with NMDs, were included (Tables 3 and 5a and b).

Results

A total of 3618 articles were identified from the databases search after removing the duplicates, and another 26 articles were identified from additional sources (Figure 1). After the initial screening, 227 articles were included and assessed for eligibility. Ninety-two studies were included in the quantitative analysis, of which 74 articles assessed adults, and 18 articles assessed children and adolescents.

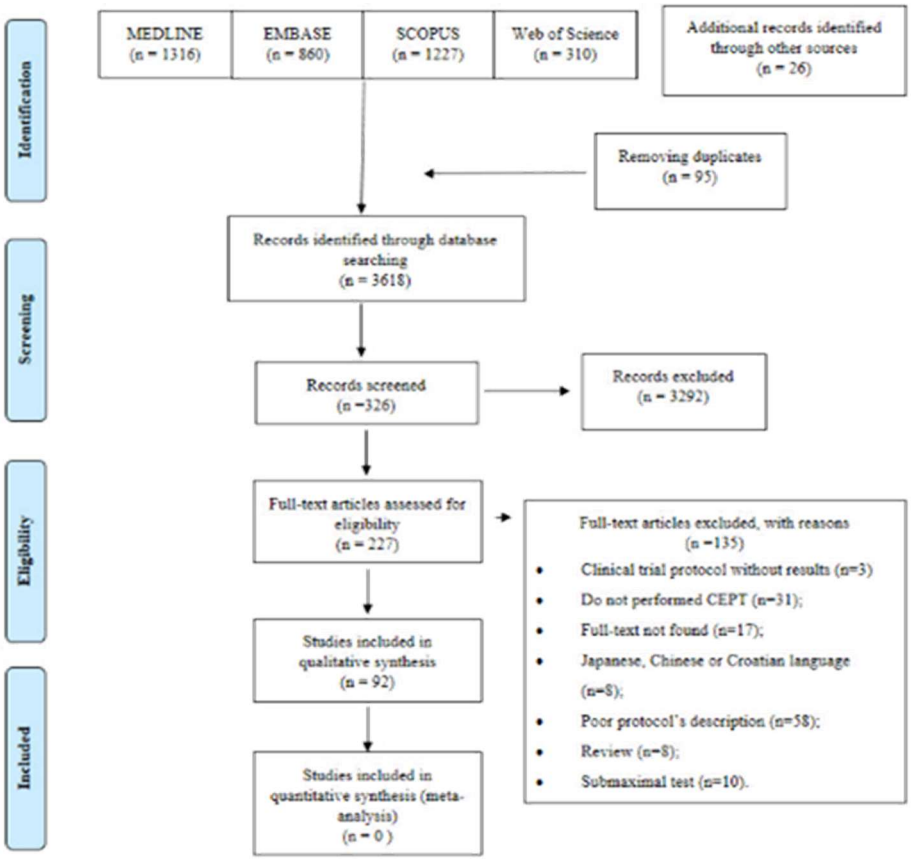

Figure 1. Flowchart

Study design

Most studies in adults (59%, n=44)[8,9,17-58] and paediatric populations (61%, n=11) used a cross-sectional design[59,60-69] (Supplementary Material C). Forty-six percent of the adult studies (n=34) used CPET outcomes to determine the metabolic and exercise response of patients with NMDs[8,17,18,20,21,23–28,30,31,32,34–41,43–46,48,54,56–58,70–72], and 38% of studies (n=28) to prescribe exercise intensity and assess the efficacy of an intervention, medication or diet supplement[9,10,55,73–97]. In the paediatric population, most of the studies (61%, n=11) used CPET outcomes to understand the metabolic and exercise response[60,64–69,98–101], and only 17% of the studies (n=3) aimed to prescribe exercise intensity and assess the efficacy of an intervention or another therapy[102,103,104].

Characteristics of the population

A total of 1237 adults (m, 625; f, 513) and 210 children and adolescents (m, 96; f, 88) with NMD were assessed in the included studies (n=92) (Tables 1a and 1b). Three articles did not report the gender of patients[61,62,73]. An overview of the included studies is shown in the Supplementary Material C. In general, the adult patients were ambulatory (with or without assistive devices) or able to cycle[8,9,17–23,70,73–81,105]. They were inactive, with moderate exercise intolerance[9,22,24–28,71,72–85], and did not have symptomatic cardiac or pulmonary disease[20,23,28,29,30,31,32,33,70,72,74,86,87,88,89]. The paediatric patients were also ambulatory with or without using assistive devices[60,63,98] and sedentary[102,103]. Specifically, for patients with inflammatory myopathy, three studies assessed patients with active and inactive myositis[64,65,99], and two other studies only assessed patients with active[66] and inactive myositis[67].

Table 1a. Study population characteristics – Adults.

Exercise Modality	NMD sub classification	Disease	Studies (n)	Patients (n)	Sex (M/F)	Age (Mean-SD)	Range
UPC	**GSD**	GSD II, V, VII	19	114	65/49	39.0 (11.4)	16-70
	MitoD	MELAS, PEO, RRFD, CPTD	29	325	139/186	38.0 (10.7)	13-96
	GSD, MitoD	_*	1	9	6/3	49.0	28-66
	IHM	MD, FSHD, LGMD, CMyo, CCD, NM, HMM Dystrophies, Myopathies	8	111	74/37	35.1 (8.6)	21-65
	IM	DM, PM	6	78	23/55	51.2 (11.6)	37-78
	MND	ALS, PPS	8	200	86/39	47.5 (7.4)	22-70
	PND	HMSN	3	27	20/7	44.0 (8.5)	20-69
	NMJD	MG	2	16	8/8	54.5 (17.2)	_
	Mix*	HMSN , Dystrophies, Myopathies	2	17	15/2	29.0 (10.4)	16-49
RC	**MND**	SMA	1	14	11/3	27.0 (16.0)	10-48
SRC	**IHM**	LGMD, MD	1	6	4/2	34.0 (5.1)	_
	PND	HMSN	1	2	0/2	44.5	_
T	**GSD**	GSD II, V	3	17	12/5	45.2 (15.2)	16-72
	MitoD	_*	4	70	32/38	33.2 (11.7)	13-60

	IM	DM	1	45	17/28	29.0 (12.0)	10-51
	MND	ALS, PPS	2	76	44/32	54.0 (10.5)	54-76
	PND	HMSN	1	1	1/0	51.0	–
AC	**MND**	PPS	2	39	8/7	34.2 (4.5)	–

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; T: treadmill; AC: arm-crank ergometer; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; PND: peripheral nerve disorders; NMJD: neuromuscular junction disorders; CM: cardiomyopathy; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; PEO: progressive external ophthalmoplegia; RRFD: ragged red fiber disease; CPTD: carnitine palmityl transferase deficiency; MD: myotonic dystrophy; FSHD: facioscapulohumeral muscular dystrophy; LGMD: limb-girdle muscular Dystrophy; CMyo: congenital myopathy; CCD: central core disease; NM: nemaline myophathy; HMM: hereditary myosin myopathy; MS: multiple sclerosis; DM: dermatomyositis; PM: polymyositis; ALS: amyotrophic lateral sclerosis; PPS: post-polio syndrome; SMA: spinal muscular atrophy; MG: myasthenia gravis; HMSN: hereditary motor and sensory neuropathy; n: number; m: male; f: female; SD: standard deviation; -: not reported; * not specified.

Table 1b. Study population characteristics – Paediatric.

Exercise Modality	NMD sub classification	Disease	Studies (n)	Patients (n)	Sex (M/F)	Age (Mean-SD)	Range
UPC	**GSD**	GSD Ia, III, VII	1	3	2/1	12.2 (1.0)	12-13
	MitoD	MCAD, SCAD,MADD	3	13	9/4	11.0 (6.0)	8-20
	IHM	DMD, BMD	3	23	23/0	9.4 (2.7)	5-20
	IM	JDM	7	114	43/55	11.0 (3.6)	6-27
SC	**IM**	JDM	1	4	3/1	15.7 (3.5)	_
T	**GSD**	GSD V	1	1	1/0	8.0	_
	IM	JDM	4	52	15/27	11.0 (2.6)	5-18

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency; DMD: duchenne muscular dystrophy; BMD: becker muscular dystrophy; JDM: juvenil dermatomyositis; n: number; m: male; f: female; SD: standard deviation; -: not reported.

Characteristics of the CPET

Information about the CPET protocol is presented in Tables 2a and b. The upright cycle ergometer exercise modality for CPET was used in 84% (n=62) of the adult studies[8,10,17,18,19–28,31,33,35–41,43–53,56,58,63,70–77,80,82–85,87–89,91–97,105], evaluating most adult patients with mitochondrial disease (n=325) and 67% (n=12) of paediatric studies[60,62,63,64–66,69,98–100,103] and young patients with inflammatory myopathy (n=114). Most upright cycle ergometers were electromagnetically braked (48%, n=30/62 and 83%, n=10/12, adults and children, respectively)[19,20,22–26,28,31,32,35,36,38,41,49–51,55,56,60,62,63–66,76,80,82,85 ,87,92 –95,99,100,103,105]. The treadmill was only used in 13% (n=10/74) of the adult[9,30,34,39,42,54,57,79,86,90] and 28% (n=5/18) of paediatric studies[59,61,101,102,104], and most studies assessed adult patients with motor neuron disease (n=76) and young patients with inflammatory myopathy (n=52). In both adult and paediatric populations, few studies adopted other exercise modalities. The recumbent cycle ergometer (n=1)[78], semi-recumbent cycle ergometer (n=1)[43], and arm-crank ergometer (n=2) were only reported in adults[29,71], and one study assessing children/adolescents used a supine recumbent cycle ergometer[67]. Moreover, two studies used more than one device: the upright cycle ergometer and treadmill, and the upright cycle ergometer and arm-crank[39,71].

In the adult population, 42% of studies with the upright cycle ergometer (n=26)[8,19,20,25,31,32,33,37,40,41,53,65,70,72,73,76,77,83,87,88,91,92,93,94,96,97], three studies with the treadmill[34,86,90] and one with the recumbent cycle ergometer[78] and arm-crank[29] reported the warm-up as part of the CPET protocol. For the paediatric population, more than half of the studies using the upright cycle ergometer (67%, n=8/12)[60,63,64,66,69,99,100,103], and one study using the treadmill[101] and supine cycle ergometer[67] reported the warm-up period (Tables 2a and b). This initial phase of the protocol was most often performed by adults

with motor neuron disease (n=172) and by children with inflammatory myopathies (n=76).

Concerning the exercise protocol and work increment for cycle ergometers, most studies in the adult population used step protocols (73%, n=48/66)[10,17,19,21,22,23,26,27,28,31,35,36,38–41,43–50,52–56,58,71,72,74,75,77,80,81,83,84,85,87,88,89,91,92,93,95,96,105] and individualised workload increments (62%, n=41/66)[8,16,19,20,21,22,24,25,27,28,29,33,37,39,41,43,44,46–49,51–53,55,56,58,71,73,77,78,81,82,85,89,93,94,95,97]. Some exceptions were the studies with inflammatory myopathies at the upright cycle ergometer that used set workload increments (83%, n= 5) (Table 2a)[10,18,40,45,84]. This protocol selection for the upright cycle ergometer differs from the one observed in the studies that assessed children and adolescents, where most upright cycle ergometer studies use a ramp protocol, which is characterized by the continuous, second by second, increase of work rate, and individualised increments of workload (75%, n=9/12)[63,64,66,68,69,98,99,100,103]. Only for the supine cycle ergometer was a step protocol used (Table 2b)[64]. For treadmills, the protocol that was used varied between the studies. The Naughton (speed increment in 0.8 km/h and grade in 3.5% each 3 minutes) and the Bruce protocols (speed increment in 1.3-1.5 Km/h and grade in 2% each 3 minutes) were selected for the adult population with glycogen storage disorders (n=2)[9,39,54], and a protocol developed by Ortega[106] (constant self-selected speed and grade increment in 5% each 3 minutes) was selected for patients with mitochondrial disorders (n=2)[30,38]. In children and adolescents, the Bruce protocol was used to assess patients with inflammatory myopathy in 75% (n=3) of articles[59,61,104] (Table 2b).

Table 2a. Characteristics of CPET – Adults.

Exercise Modality	NMD sub classification	Disease	Studies (n)	Warm-up (n)	Ergometer (n)		Exercise protocol (n)		Work increment (n)	
					M	E	R	S	Set	Ind.
UPC	**GSD**	GSD II, V, VII	19	5	1	9	2	17	5	14
	MitoD	MELAS, PEO, RRFD, CPTD	29	8	2	21	7	22	9	20
	GSD, MitoD	_*	1	1	_	_	1	1	_	1
	IHM	MD, FSHD, LGMD, CMyo, CCD, NM, HMM Dystrophies, Myopathies	8	4	1	2	2	7	1	7
	IM	DM, PM	6	1	_	2	1	5	5	1
	MND	ALS, PPS	8	5	1	2	3	5	3	5
	PND	HMSN	3	3	1	2	2	2	1	2
	NMJD	MG	2	2	1	1	_	2	_	2
	Mix*	HMSN, Dystrophies, Myopathies	2	1	2	1	_	2	1	1
RC	**MND**	SMA	1	1	_	1	1	_	_	1
SRC	**IHM**	LGMD, MD	1	_	_	_	_	1	_	1
	PND	HMSN	1	_	_	_	_	1	_	1
AC	**MND**	PPS	2	1	_	_	1	_	1	1

					Exercise protocol				
Exercise Modality	**NMD sub classification**	**Disease**	**Studies (n)**	**Warm-up (n)**	**Naughton [120]**	**Bruce [121]**	**Balke [122]**	**Ortega [106]**	**Other [86]**

	GSD	GSD II, VPompe, McArdle	3	_	2[&]	2	_	_	_
	MitoD	_*	4	_	1	1	_	2	_
T	**IM**	DM	1	1	_	_	1	_	_
	MND	ALS, PPS	2	1	1	_	_	_	1
	PND	HMSN	1	1	_	_	1	_	_

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; T: treadmill; AC: arm-crank ergometer; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; DAMC: disorders of activation of muscle contraction; IM: inflammatory Myopathies; PND: peripheral nerve disorders; NMJD: neuromuscular junction disorders; CM: cardiomyopathy; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; PEO: progressive external ophthalmoplegia; RRFD: ragged red fiber disease; CPTD: carnitine palmityl transferase deficiency; MD: myotonic dystrophy; FSHD: facioscapulohumeral muscular dystrophy; LGMD: limb-girdle muscular Dystrophy; CMyo: congenital myopathy; CCD: central core disease; NM: nemaline myophathy; HMM: hereditary myosin myopathy; DM: dermatomyositis; PM: polymyositis; ALS: amyotrophic lateral sclerosis; PPS: post-polio syndrome; SMA: spinal muscular atrophy; ; MG: myasthenia gravis; CM: cardiomyopathy; FA: friedreich's ataxia; BTHS: barth syndrome; HMSN: hereditary motor and sensory neuropathy; n: number of studies; m: mechanically; E: electrically; R: ramp; S: step; Ind.: individualized; min: minute. SD: standard deviation; -: not reported; * not specified ; [&] One study used two different protocols.

Table 2b. Characteristics of CPET – Paediatric.

Exercise Modality	NMD sub classification	Disease	Studies (n)	Warm-up (n)	Ergometer (n)		Exercise protocol (n)		Work increment (n)	
					M	E	R	S	Set	Ind.
UPC	GSD	GSDIa, III, VII	1	_	_	_	1	_	_	1
	MitoD	MCAD, SCAD,MADD	3	1	_	1	3	_	1	2
	IHM	DMD, BMD	3	1	_	2	2	1	1	2
	IM	JDM	7	5	_	7	5	2	1	6
SC	IM	JDM	1	1	1	_	_	1	_	1

Exercise Modality	NMD sub classification	Disease	Studies (n)	Warm-up (n)	Exercise protocol			
					Dubowy [15]	Bruce [121]	Balke [122]	Pérez [101]
T	GSD	GDS V	1	1	_	_	_	1
	IM	JDM	4	_	1	3	_	_

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; CM: cardiomyopathy; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency;DMD: duchenne muscular dystrophy; BMD: becker muscular dystrophy; JDM: juvenil dermatomyositis; BTHS: barth syndrome; FA: friedreich's ataxia; n: number of studies; m: mechanically; E: electrically; R: ramp; S: step; Ind.: individualized; min: minute; -: not reported; * not specified .

Quality of test performance

Test duration

Seventeen studies (23%) in adults and eight (44%) studies in children reported the duration of the CPET. From those, four studies assessing adult patients with inflammatory myopathies (n=1 study, 11 patients), mitochondrial disorders (n=2 studies, 16 patients) and glycogen storage disorders (n=1 study, 1 patient) in the upright cycle ergometer presented a mean duration below eight minutes[18,17,83,105] (Table 3a). All paediatric studies reported a mean duration of the CPET above eight minutes[59,60,61,63,65,67,102,104].

3.4.2 Criteria of maximal effort

Concerning maximal effort during the CPET, 22 studies with adult NMDs[8,9,22,26,28,29,34,40,41,44,55,56,58,73,78,85,86,89,91,95,97,105] and seven studies with paediatric NMDs presented criteria for maximal effort[34,59,61,63,65,98,100] (Tables 3a and b). From these studies, adult patients with glycogen storage disorders and motor neuron disease most often performed a maximal CPET in the upright cycle ergometer (98%, n=42, and n=94 patients)[20,21,33,38,73,93,97]. More than 60% of the adults with mitochondrial disorders (n=24), inherited myopathies (n=11), inflammatory myopathies (n=13), and peripheral nerve disorders (n=12) met the criteria of maximal CPET in the upright cycle ergometer[8,20,25,38,40,41,45], and 64% of patients with motor neuron disease (n=9) and 75% of patients with inflammatory myopathies (n=6) achieved the criteria in the recumbent cycle ergometer and treadmill[9,78].

In the paediatric studies, more than 90% of patients with mitochondrial myopathy (n=2) and inflammatory myopathy (n=10 and n=4) met the maximal criteria in the upright cycle ergometer and supine cycle ergometer[64,67,98] (Table 3b). This percentage was lower in inflammatory myopathies (67%, n=10) on the treadmill[59] and in inherited myopathies (11%, n=1) on the upright cycle ergometer[63] (Table 3b).

Feasibility of CPET

Measurement completion

From most of the included studies, it was possible to extract the number of patients who completed the CPET (Tables 3a and b). In a few articles that used the upright cycle ergometer to assess adult patients with glycogen storage disorders (26%, n=5)[19,27,32,39,50], mitochondrial disorders (27%, n=8)[19,20,39,47,48,82,87,92], inherited myopathies (37%, n=3)[19,47,96], motor neuron disease (75%, n=6)[20,21,23,70,71,72], peripheral nerve disorders (67%, n=2)[17,74], myasthenia gravis (n=2)[19,76] and a mix of diseases (n=2)[19,88], the information on feasibility was missing (Table 3a). In contrast, studies assessing children/adolescents clearly present this information (Table 3b).

Considering only the studies that reported the completion rate, three adults with mitochondrial disorders were unable to finish the CPET in the upright cycle ergometer, one due to syncope[17,31] and the other two because of an inability to cycle. Difficulties in cycling were also reported in another six patients with motor neuron disease[45]. In treadmill tests, only one adult with glycogen storage disorders discontinued the CPET, because of dizziness[54] (Table 3a). Moreover, in the test performed at the upright cycle ergometer, three paediatric patients (with glycogen storage disorder, mitochondrial disorder, and inherited myopathy) did not complete the CPET. For the patient with glycogen storage disorder, the reason was an intense myalgia episode; for the other two patients, no explanation was given[69] (Table 3b).

Adverse events

In general, few studies reported on the occurrence of complications or adverse events during the CPET in the adult group (upright cycle ergometer=13, recumbent cycle ergometer=1, treadmill=1)[10,20,22,27,31,51,54,73,74,78,84,93,97] and in the paediatric group (upright cycle ergometer=6, treadmill=3)[59,61,63,64,66,69,99,100,104]. Adverse events occurred in three of the adult studies (upright cycle ergometer=2, treadmill=1)[31,54,74]. Each was an isolated event (one patient in each study), and most of the time connected to an interruption of the test (Table 3a). One paediatric study reported an isolated adverse event with a patient with glycogen storage disorder, and one complication with a patient with an inherited myopathy (Table 3b)[69].

Specific parameters for measuring the safety of CPET were only reported by two studies assessing adult patients[74,76], and by another two comprised of paediatric patients[63,69]. Most of the studies used the comparison of creatine phosphokinase (CPK) levels before and after the test, and values >150 U/L were considered elevated[76]. One study also used the rating of muscle hurt (RMH), where a score >6 indicates severe muscle pain[63].

Table 3a. Quality and Feasibility of CPET – Adults.

			Quality of test performance						Feasibility							
Exercise Modality	**NMD sub classification**	**Disease**	**Studies (n)**	**Duration**		**Criteria of maximal effort**			**Completion rate**		**Adverse events**		**Perception exertion**		**Safety**	
				Studies (n)	**Time**	**Studies (n)**	**Criteria**	**Patients (%)**	**Studies (n)**	**Patients (%)**	**Studies (n)**	**Event (n)**	**Studies (n)**	**Scale/ grade**	**Studies (n)**	**Parameters**
UPC	**GSD**	GSD II, V, VII	19	4	11.0	4	HR_{peak} ≥85% of predicted* RER_{peak}>1.1 Borg≥7	98	14	100	4	0	3	Borg 0-20/19.0	–	–
	MitoD	MELAS, PEO, RRFD, CPTD	29	4	9.4 (2.7)	6	HR_{peak}= & or >80% of predicted&; RER_{peak} ≥1.1-1.2 Borg≥7	63	21	99	2	0/1 (Syncope)	5	Borg 0-10/8.0 (1.3)	–	–
	GSD, MitoD	_*	1	1	10.8	1	HR_{peak}>85% of predicted#, RER_{peak} >1.1, Borg≥7	44	1	100	–	–	–	–	–	–
	IHM	MD, FSHD, LGMD, CMyo, CCD, NM, HMM Dystrophies, Myopathies	8	3	13.0	3	HR_{peak}>85% of predicted& or #; RER_{peak} >1.0-1.1, Borg≥7 or 17-19.	65	5	100	1	1 (CPK>1000) [FSHD]	2	Borg 0-20/ 19.0 (1.0) Borg 0-10/10.0	1	CPK
	IM	DM, PM	6	1	5.8 (2.4)	2	HR_{peak}>90% of predicted&; RER_{peak} ≥1.2;BL>6.0 mmol; Δph>0.04; VR<20%; EqO2>40	62	6	100	3	0	2	Borg 0-20/ 19.0 (0.4)	–	–
	MND	ALS, PPS	8	2	15.3 (-)	1	RER_{peak} >1.1	98	2	100	2	0	2	Borg 0-20/ 18.0 Borg 0-	–	–

														10/7.0 (4.0)		
	PND	HMSN	3	1	10.8	1	HR_{peak}>85% of predicted#, RER_{peak} >1.1, Borg≥7	67	1	100	_	_	1	Borg 0-10/10.0	1	CPK<170 IU·L
	NMJD	MG	2	_	_	1	HR_{peak}≥ predicted**, RER_{peak} ≥1.2, BL>8.0 mmol/L, Borg≥17	_	_	_	_	_	_	_	_	_
	Mix*	HMSN , Dystrophies, Myopathies	2	_	_	_	_	_	_	_	_	_	_	_	_	_
RC	**MND**	SMA	1	_	_	1	OMNI Scale≥8 RER_{peak} >1.0	64	1	100	1	0	_	_	_	_
SRC	**IHM**	LGMD, MD	1	_	_	_	_	_	1	100	_	_	1	Borg0-20/16.0 (1.0)	_	_
	PND	HMSN	1	_	_	_	_	_	1	100	_	_	1	Borg0-20/16.0 (1.0)	_	_
T	**GSD**	GSD II, V	3	3	9.4 (5.5)	1	HR_{peak} ≥85% of predicted& RER_{peak}≥1.1	_	3	94	1	1 (Dizziness)	2	Borg 0-10/8.5 (1.1)	_	_
	MitoD	_*	4	3	12.1 (3.4)	1		_	4	100	_	_	2	Borg 0-10/8.4 (2.1)	_	_
	IM	DM	1	_	_	1	HR_{peak}>80% of predicted&	75	1	100	_	_	1	Borg 0-20/ 18.0 (17.0-19.0)	_	_
	MND	ALS, PPS	2	_	_	1	HR_{peak}>75% of predicted&, 55-65% of	_	2	100	_	_	_	_	_	_

							predicted VO_2									
	PND	HMSN	1	1	25.0	1	_	_	1	100	_	_	_	_	_	_
AC	**MND**	PPS	2	1	9.6 (1.9)	1	BL>8.0 mmol	–	2	100	_	_	2	Borg 0-20/ 18.2 (0.21)	–	–

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; T: treadmill; AC: arm-crank ergometer; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; DAMC: disorders of activation of muscle contraction; IM: inflammatory Myopathies; PND: peripheral nerve disorders; NMJD: neuromuscular junction disorders; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; PEO: progressive external ophthalmoplegia; RRFD: ragged red fiber disease; CPTD: carnitine palmityl transferase deficiency; MD: myotonic dystrophy; FSHD: facioscapulohumeral muscular dystrophy; LGMD: limb-girdle muscular dystrophy; CMyo: congenital myopathy; CCD: central core disease; NM: nemaline myophathy; HMM: hereditary myosin myopathy; MS: multiple sclerosis; DM: dermatomyositis; PM: polymyositis; ALS: amyotrophic lateral sclerosis; PPS: post-polio syndrome; SMA: spinal muscular atrophy; ; MG: myasthenia gravis; HMSN: hereditary motor and sensory neuropathy; n: number of studies; %: percentage of patients; HR_{peak}: heart rate peak; RER_{peak}: respiratory exchange ratio; OMNI/Borg: scale of perception exertion; PE: perception exertion BL: blood lactate level; Δph: delta of blood ph; VR: ventilatory reserve; EqO_2: ventilatory equivalent for oxygen; SD: standard deviation; CPK: creatine phosphokinase; mmol: millimol; U.L: units per liter -: not reported; * not specified.*210-0.65 x age; [&]220-age; [#](210-0.65 x age); **(208-0.7xage)-10.

Table 3b. Quality and Feasibility of CPET – Paediatric.

				Quality of test performance					Feasibility							
Exercise Modality	**NMD sub classification**	**Disease**	**Studies (n)**	**Duration**		**Criteria of maximal effort**			**Completion rate**		**Adverse events**		**Perception exertion**		**Safety**	
				Studies (n)	**Time**	**Studies (n)**	**Criteria**	**Patients (%)**	**Studies (n)**	**Patients (%)**	**Studies (n)**	**Event (n)**	**Studies (n)**	**Scale/ grade**	**Studies (n)**	**parameters**
UPC	**GSD**	GSD Ia, III, VII	1	–	–	–	–	–	1	67	1	1 Myalgia	–	–	–	–
	MitoD	MCAD, SCAD,MADD	3	–	–	1	HR_{peak}>180bpm; RER_{peak} ≥1.0	100	3	92	–	–	–	–	–	–
	IHM	DMD, BMD	3	2	8.1 (1.4)	1	HR_{peak}>180bpm; RER_{peak} ≥1.0	11	3	96	2	1 Elevated CPK [BMD]	1	Borg 0-10/ 7.0 (1.8)	2	RMH >6 RPE 2-5 days after visit CPK
	IM	JDM	7	1	8.1	2	HR_{peak} >95% of predicted; RER_{peak}>1.0-1.1	91	7	100	4	0	_	_	1	–
SC	**IM**	JDM	1	1	10.0 (2.0)	1	HR_{peak}>180bpm; RER_{peak} ≥1.0	100	1	100	–	–	–	–	–	–
T	**GSD**	GSD V	1	–	–	–	–	–	1	100	–	–	–	–	–	–
	IM	JDM	4	4	9.0 (2.1)	2	HR_{peak}>180bpm; RER_{peak} ≥1.0	67	4	100	3	0	_	_	–	–

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency;; DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; JDM: juvenile dermatomyositis; n: numbers of studies; %: percentage of patients; HR_{peak}: heart rate peak; bpm: beats per minute; RER_{peak}: respiratory exchange ratio; Borg: rating of perceived exertion; PE: perception exertion; RMH: the rating of muscle hurt; SD: standard deviation; CPK: creatine phosphokinase; mmol: millimol; U.L: units per liter -: not reported; * not specified, &220-age.

Aerobic fitness of NMD patients

All NMD subgroups presented a reduced aerobic fitness (<80% of the predicted VO_{2peak}), except adults with inflammatory myopathies using the treadmill, and paediatric patients with glycogen storage disorders using the upright cycle ergometer (81% and 82% of the predicted $VO_{2peak,}$ respectively). The lowest aerobic fitness levels were observed in adult patients with motor neuron disease (32% of the predicted VO_{2peak}) on the recumbent cycle ergometer (Table 4a), and in paediatric patients with glycogen storage disorders (38% of the predicted VO_{2peak}) on the treadmill (Table 4b). Moreover, adult patients with glycogen storage disorders, mitochondrial disorders and inflammatory myopathies, and paediatric patients with inherited myopathies and inflammatory myopathies showed a VO_{2peak} below 50% of the predicted value in the upright cycle ergometer (Tables 4a and b).

The highest percentages of the HR_{peak} (≥90% of the predicted value) for the adult population were found using the treadmill for patients with glycogen storage disorders, mitochondrial disorders and inflammatory myopathies. For cycle ergometry, normal values of the HR_{peak} were observed in patients with inherited myopathies on the semi-recumbent cycle ergometer, and in patients with a mix of diseases on the upright cycle ergometer (93% of the predicted value). In children/adolescents, only patients with glycogen storage disorders on the upright cycle ergometer showed an HR_{peak} ≥95% of the predicted value. As expected, low values of the respiratory exchange ratio (RER_{peak} <1.1 in adults and <1.0 in children/adolescents) were observed in patients with glycogen storage disorders using the upright cycle ergometer (RER_{peak}=1.0) and treadmill (RER_{peak}=0.9–0.8) (Tables 4a and b). Adult patients with motor neuron disease using the recumbent cycle ergometer and the arm-crank also showed low RER_{peak} values (Table 4a).

Table 4a. Aerobic fitness of patients - Adults

Exercise Modality	NMD sub classification	Disease	Studies (n)	VO_{2peak} (ml/kg/min)	VO_{2peak} (% of predicted)	Studies (n)	HR_{peak} (bpm)	HR_{peak} (%)	Studies (n)	RER_{peak}	Studies (n)	W_{peak} (watts)
UPC	**GSD**	GSD II, V, VII	18	20.1	44	16	165	88	11	1.0	11	79.4
	MitoD	MELAS, PEO, RRFD, CPTD	26	20.8	47	20	146	80	15	1.2	22	86.0
	GSD, MitoD	_*	_	_	_	1	134	_	_	_	1	67.0
	IHM	MD, FSHD, LGMD, CMyo, CCD, NM, HMM Dystrophies, Myopathies	6	26.0	58	6	161	88	3	1.2	8	122.2
	IM	DM, PM	5	19.0	40	3	146	85	2	1.1	2	107.6
	MND	ALS, PPS	6	21.2	54	5	156	89	4	1.1	7	75.2
	PND	HMSN	2	34.0	74	1	149	84	_	_	2	128.2
	NMJD	MG	2	25.0	64	_	_	_	_	_	1	163.6
	Mix*	HMSN, Dystrophies, Myopathies	2	24.0	50	1	174	93	_	_	1	88.0
RC	**MND**	SMA	1	15.2	32	_	_	_	1	1.0	_	_
SRC	**IHM**	LGMD, MD	1	18.2	43	1	164	93	_	_	1	94.0
	PND	HMSN	1	17.1	50	1	152	82	_	_	1	102.0
T	**GSD**	GSD II, V	3	20.2	48	3	158	90	3	0.9	_	_
	MitoD	_*	4	24.0	52	2	170	91	2	1.2	2	143.0

	IM	DM	1	40.4	81	1	190	101	1	1.1	_	_
	MND	ALS, PPS	1	28.0	61	1	92	56	_	_	_	_
	PND	CMT	1	30.0	71	_	_	_	_	_	_	_
AC	**MND**	PPS	1	21.5	47	1	160	87	1	1.0	1	74.5

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; T: treadmill; AC: arm-crank ergometer; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; DAMC: disorders of activation of muscle contraction; IM: inflammatory Myopathies; PND: peripheral nerve disorders; NMJD: neuromuscular junction disorders; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; PEO: progressive external ophthalmoplegia; RRFD: ragged red fiber disease; CPTD: carnitine palmityl transferase deficiency; MD: myotonic dystrophy; FSHD: facioscapulohumeral muscular dystrophy; LGMD: limb-girdle muscular Dystrophy; CMyo: congenital myopathy; CCD: central core disease; NM: nemaline myophathy; HMM: hereditary myosin myopathy; MS: multiple sclerosis; DM: dermatomyositis; PM: polymyositis; ALS: amyotrophic lateral sclerosis; PPS: post-polio syndrome; SMA: spinal muscular atrophy; MG: myasthenia gravis; HMSN: hereditary motor and sensory neuropathy; n: number; m: male; f: female; SD: standard deviation; VO_{2peak}: oxygen uptake at peak of CPET; ml: milliliter; Kg: kilogram; min: minute;%: percentage; HR_{peak}: peak heart rate during CPET; bpm: beats per minute; W_{peak}: peak workload during CPET; RER_{peak}: peak respiratory exchange ratio; -: not reported; * not specified.

Table 4b. Aerobic fitness of patients – Paediatric.

Exercise Modality	NMD sub classification	Disease	Studies (n)	VO_2peak (ml/kg/min)	VO_{2peak} (% of predicted)	Studies (n)	HR_{peak} (bpm)	HR_{peak} (%)	Studies (n)	RER_{peak}	Studies (n)	Wpeak (watts)
UPC	GSD	GSD Ia, III, VII	1	40.5	82	1	190	97	1	1.0	_	_
	MitoD	MCAD, SCAD,MADD	2	36.2	79	3	182	93	3	1.2	2	134.1
	IHM	DMD, BMD	3	21.0	44	3	147	75	2	1.1	1	55.6
	IM	JDM	4	24.0	48	6	175	89	5	1.2	4	81.0
SC	IM	JDM	1	36.0	71	1	182	93	1	1.1	1	30.0
T	GSD	GSD V	1	19.0	38	1	166	83	1	0.8	_	_
	IM	JDM	4	34.0	67	2	174	87	2	1.0	_	_

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency;; DMD: Duchenne Muscular Dystrophy; BMD: Becker Muscular Dystrophy; JDM: juvenil dermatomyositis; n: number; m: male; f: female; SD: standard deviation; VO_{2peak}: oxygen uptake at peak of CPET; ml: millilitre; Kg: kilogram; min: minute;%: percentage; HR_{peak}: heart rate at peak of CPET; bpm: beats per minute; W_{peak}: peak workload during CPET; RER_{peak}: peak respiratory exchange ratio; -: not reported.

Methodological quality

The methodological quality of the included studies varied. In the adult population, 17 studies (28%) that used the upright cycle ergometer and four studies (40%) that used the treadmill demonstrated respectively sufficient (upright cycle ergometer=16; treadmill=3)[8,23,25,27,31,39,41,42,49,55,56,70,72,77,80,90,94,95] and moderate (upright cycle ergometer=1; treadmill=1)[9,22] methodological quality. One study using the semi-recumbent cycle ergometer had moderate methodological quality[43]. However, no studies achieved a high methodological quality. In children and adolescents, seven studies (50%) using the upright cycle ergometer[63,64,65,66,98,99,103] and two studies (40%) using the treadmill[59,102] demonstrated sufficient methodological quality (Supplementary Material D).

Data syntheses

Tables 5a and b summarise the protocol, quality of test performance, feasibility, and aerobic fitness from the 30 studies (one study used two exercise modalities) with sufficient-to-moderate methodological quality. From these studies, 10 presented information about the quality of the test performance, including test duration[8,9,31,39,59,63,65,72,90,102] and the number of patients reaching maximal effort[8,9,22,41,55,56,59,63,65,95]; over 25 studies reported feasibility or aerobic fitness details (n=27 and 26 studies, respectively)[8,9,22,23,25,27,31,39,41,42,49,55,56,59,62,63–66,72,70,77,80,81,90,94,95,99,102,103].

Excellent feasibility with a completion rate of 100% and low aerobic fitness (<80% of the predicted VO_{2peak}) were found in those studies. However, a high quality of test performance, mainly for maximal effort, was only observed in six studies[8,9,41,59,63,65]. The best evidence of CPET protocols was based on these studies with a higher quality of test performance and feasibility. In this regard, the upright cycle ergometer is

recommended to assess most subtypes of ambulatory adults with NMDs and some ambulatory paediatric patients. The ramp protocol and individualised work increments are advisable for both populations, but different workloads are suggested for adults and paediatric patients. The level of functional capacity[8] or physical fitness of the adult patients[41] can guide the rater to select the best workload from 5 to 25 W/min. For paediatric patients, the distance covered during the six-minute walking test (6MWT) might help to select workloads from 5 to 15 W/min[63]. The treadmill can be used to assess ambulatory adults using the Naughton protocol[9], and children and adolescents with the Bruce protocol[59].

Table 5a. Characteristic of the studies with sufficient score in the ATS/ACCP adapted quality list – Adults.

									Quality of test			Feasibility of test		Aerobic fitness			
Exercise Modality	**NMD sub classification**	**Disease**	**Studies (n)**	**Patients (n) [M/F]**	**Age (Mean-SD)**	**Protocol**	**Studies (n)**	**Duration (min)**	**Studies (n)**	**Criteria of maximal effort Patients (%)**	**Studies (n)**	**Completion rate Patients (%)**	**Adverse events (n)**	**Studies (n)**	**VO_{2peak} (%pred)**	**HR_{peak} (%pred)**	**RER_{peak}**
UPC	**GSD**	GSD V, VII	5	18 [10/8]	35.7 (4.8)	Start: 10-40W Effort: 5-10 W/min (Step) or 20-30W/2min or 40W/3min	2	11.5	1	HR_{peak} ≥85% of predicted	3	100%	–	5	19.0 (42%)	172 (94%)	0.87
						Start: 20-50W (6min) Effort: 10-25W/min (Ramp) Cadence: 60-80-rpm				–							
	MitoD	MitoD, RRFD	9	86 [24/34]	35.5 (9.0)	Warm-up: submaximal W/3-4min or 20W/1min Effort: 5-20W/min or 20-30W/2min (Step) Cadence: 50 rpm	2	10.7	1	HR_{peak}>180 bpm or 85% of predicted; RER_{peak}>1.00	8	86%	(n=1) Syncope	9	23.0 (51%)	140.0 (77%)	1.30
						Warm-up: 0W/4min Effort: 5-25W/min (Ramp) Cadence: 60-80 rpm				100%							
	GSD, MitoD	–*	1	9 [6/3]	49.0	Warm-up: 0W/1-3min Effort: 5-10W/min (Ramp) Cadence: >50rpm	1	10.8	1	HR_{peak}>85% of predicted* RER_{peak}>1.10, Borg≥7	1	100%	–	1	–	134 (77%)	–
										44%							

	IHM	MD, FSHD, LGMD, CMyo, CCD, NM, HMM Dystrophies, Myopathies	2	42 [25/17]	33.2 (2.3)	Warm-up: 0W/1-3min Start:20-50W/6min Effort: 5-25W/min (Ramp), Cadence: 60-80 rpm	2	13.9	1	HR_{peak}>180 bpm or 85% of predicted; RER_{peak}>1.00 65%	2	100%	–	1	31.1 (68%)	158 (85%)	1.10
	IM	DM, PM	1	9 [2/7]	42.0 (3.0)	Effort: 5-15 W/min (Step) Cadence: 60 rpm		–	–	–	1	100%	–	1	14.2 (33%)	127 (71%)	1.10
	MND	ALS, PPS	4	66 [42/24]	51.1 (8.3)	Warm-up: 0W/2min Effort: 3-20W/min (Step) Cadence: 50-80 rpm	1	8.0	–	–	2	100%	–	4	19.6 (49%)	138 (79%)	1.10
	PND	HMSN	1	18 [12/6]	49.0	Warm-up: 0W/2min Effort: 3-20W/min (Ramp) Warm-up: 0W/1-3min Effort: 5-10W/min (Ramp), Cadence:>50rpm	1	10.8	1	HR_{peak}>85% of predicted* RER_{peak}>1.10, Borg≥7 67%	1	100%	–	1	–	133 (76%)	–
SRC	**IHM**	LGMD, MD	1	6 [4/2]	34.0 (5.0)	Start: 25W/2 min Effort:12.5-25W/2min (Step) Cadence: 50-60 rpm	–	–	–	–	1	100%	–	1	17.1 (50%)	152 (82%)	–
	PND	HMSN	1	2 [0/2]	44.5	Start: 25W/2 min	–	–	–	–	1	100%	–	1	18.2 (43%)	164 (93%)	–

						Effort: 12.5-25W/2min (step) Cadence: 50-60 rpm											
T	**GSD**	GSD II, V	2	10 [7/3]	51.7 (10.1)	Naughton protocol (speed increment: 0.8 Km/h/3min grade increment: 3.5%/3min) Bruce protocol (speed increment: 2.7 Km/h, grade increment: 2%/3min)	2	9.2	1	HR_{peak} ≥85% of predicted; RER_{peak}≥1.1 1 75%	2	100%	–	2	19.6 (49%)	142 (82%)	0.97
	MitoD	_*	2	27 [13/14]	30.8 (11.3)	Ortega protocol (constant self selected speed, grade increment: 0-5%/3min) Bruce protocol (speed increment: 2.7 Km/h, grade increment: 2%/3min)	1	10.0	–	–	2	100%	–	2	23.6 (50%)	167 (88%)	1.40
	PND	HMSN	1	1 [1/0]	51.0	Modified Balke protocol (constant speed: 4.8 Km/h, grade increase: 1%/min)	1	25.0	–	–	1	100%	–	1	30.0 (71%)	–	–

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; T: treadmill; AC: arm-crank ergometer; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; PND: peripheral nerve disorders; NMJD: neuromuscular junction disorders; CM: cardiomyopathy; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; PEO: progressive external ophthalmoplegia; RRFD: ragged red fiber disease; CPTD: carnitine palmityl transferase deficiency; MD: myotonic dystrophy; FSHD: facioscapulohumeral muscular dystrophy; LGMD: limb-girdle muscular Dystrophy; CMyo: congenital myopathy; CCD: central core disease; NM: nemaline myophathy; HMM: hereditary myosin myopathy; MS: multiple sclerosis; DM: dermatomyositis; PM: polymyositis; ALS: amyotrophic lateral sclerosis; PPS: post-polio syndrome; SMA: spinal muscular atrophy; ; MG: myasthenia gravis; HMSN: hereditary motor and sensory neuropathy; n: number; m: male; f: female; SD: standard deviation; -: not reported; * not specified.

Table 5b. Characteristic of the studies with sufficient score in the ATS/ACCP adapted quality list – Paediatric.

						Quality				Feasibility			Aerobic fitness			
Exercise Modality	**NMD sub classification**	**Disease**	**Studies (n)**	**Patients (n)**	**Age (Mean-SD)**	**Protocol**	**Studies (n)**	**Duration (min)**	**Studies (n)**	**Criteria of maximal effort**	**Studies (n)**	**Completion rate**	**Adverse event**	**Studies (n)**	**VO_{2pico} (%pred)**	**HR_{pico} (%pred)**	**RER_{pico}**
UPC	**MitoD**	MCAD, SCAD,MADD	1	4 [2/2]	15 (5.1)	Warm-up: 0W/2min Effort: 5-20W/min (Ramp)	–	–	–	–	1	100%	–	1	–	185 (94%)	1.16
	IHM	DMD, BMD	1	9 [9/0]	10.3 (4.7)	Warm-up: 0W/1-2min Effort: 5-10W/min (Ramp)	1	8.3	1	HR_{peak}>180bpm $RER_{peak}\geq1.00$ 1%	1	100%	–	1	25.2 (51%)	156 (79%)	1.10
	IM	JDM	5	66 [24/26]	12.3 (3.7)	Warm-up: 0W/1-3min Effort: 10-20W/min (Ramp) Cadence:>60-80rpm	1	8.1	1	RER_{peak}>1.10 91%	5	100%	–	3	25.3 (51%)	176 (90%)	1.14
T	**IM**	JDM	2	41 [15/26]	11.2 (1.0)	Modified Dubowy (speed increment: 0.5 Km/h, grade increment: 3%/1.5min) Bruce protocol (speed incremente: 1.3-1.5 Km/h, grade increment: 2%/3min)	2	9.3	1	HR_{peak}>180bpm $RER_{peak}\geq1.00$ 67%	–	–	–	2	34.1 (68%)	184 (92%)	1.05

Legend: UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency; DMD: duchenne muscular dystrophy; BMD: becker muscular dystrophy; JDM: juvenil dermatomyositis; n: number; m: male; f: female; SD: standard deviation; -: not reported; n: number of articles; m: male; f: female; SD: standard deviation; -: not reported.

Discussion

Ninety-two studies (A, 74; P, 18) using CPET to assess patients with NMDs were included in this systematic review and evaluated on the quality of test performance, feasibility and methodological quality. Only 30 studies (A, 21; P, 9) met sufficient-to-moderate methodological quality according to the ATS/ACCP recommendations. However, from those, only six studies (A, 3; P, 3) were included in the best evidence synthesis of CPET protocols for patients with NMDs regarding excellent feasibility and quality of test performance.

Methodological quality

The main reasons for the low scores of the studies in the methodological quality checklist regard failure in reporting methodological information, such as calibration, monitoring measurements, for example, blood pressure and oxygen saturation, and performing pretest procedures such as pulmonary function tests. Not following the ATS/ACCP recommendations [6] in performing and reporting CPET may compromise the study's reproducibility and creditability, as well as the patients' safety and performance during the test. Problems in calibration may generate unreliable CPET outcomes, while abnormalities in blood pressure and saturation are primary relative and absolute indications for terminating the test[21,26]. Moreover, since some patients with NMDs present with respiratory muscle weakness, a pulmonary function test can help to identify a pulmonary limitation during exercise[8,107]. Therefore, to increase the body of evidence for the applicability of CPET in NMD, future studies should apply the methodological quality checklist of the ATS/ACCP in the design and report of CPET in NMDs.

Characteristics of the CPET

The upright cycle ergometer was the most frequently used device for assessing various adult and paediatric patients with NMD. Our findings agree with those of other systematic reviews of CPET for healthy, oncologic, and neurologic patients[108–110]. For clinical situations, the upright cycle ergometers are recommended over treadmills, due to their safety, less need for coordination and balance, easy measurement, and better quality of monitoring physiological variables[5,6]. A step protocol with individualised increments was the most frequently used for assessing adults patients with NMDs in the studies with sufficient methodological quality. However, a ramp protocol was selected in the included studies that presented the best evidence synthesis for the adult population, regarding excellent feasibility and quality of test performance[8,41]. A ramp protocol is advisable for use in patients with NMDs, because it has a linear increase of workload allowing slight metabolic changes and neuromuscular recruitment through the CPET[111]. In agreement with this, most studies assessing paediatric patients used a ramp protocol.

For this type of protocol, workload increments from 5 to 25 W/min were prevalent in the studies, with sufficient-to-moderate methodological quality and the best evidence synthesis assessing adult patients. In the paediatric population, the workload steps varied from 5 to 20 W/min. Earlier fatigue will occur in more intense workload steps. Therefore, the workload steps should be selected carefully, using the level of functional capacity[8] or aerobic fitness of the patients[41]. The six-minute walking test (6MWT) for example, might be a good option for screening the functional capacity of the patient before the CPET[63].

When an upright cycle ergometer is not available, a treadmill might be an alternative option for assessing aerobic fitness in some subtypes of NMDs. It was the second most frequently used device in the included studies and in those included in the best evidence synthesis[9,59]. The Naughton and the Bruce protocols offered the best

evidence for respectively assessing adults and paediatric patients[9,59]. The Bruce protocol is a frequently used protocol[112]; however, it has some disadvantages when assessing children and adolescents with reduced functional capacity. The primary disadvantage is posed by the large and unequal increments that impact the obtained exercise response[112], and a secondary disadvantage is the high metabolic demand in the first stages, requiring an oxygen cost of 17.5 ml/kg/min (5 METS), which represents more than 60% of the mean VO_{2peak} achieved by the young NMD patients on the treadmill. Therefore, the Dubowy protocol, with small and even increments (speed increment in 0.5 Km/h and grade in 3% each 1 minute and 30 seconds), is more advisable for assessing aerobic fitness in children and adolescents with NMDs.

Quality of test performance

The recommended test duration was met in the CPET protocols used in all studies with sufficient methodological quality, suggesting that the work rate selected might be appropriated for terminating the CPET in 8–12 minutes, without early termination due to localised muscular fatigue, and low stress of the cardiopulmonary system[5]. However, even in the studies with high methodological quality, few patients performed a maximal CPET regarding the objective criteria (HR_{peak} and RER_{peak}), applied when a plateau in the VO_{2peak} is not observed[113].

In general, we found a reduced HR_{peak} and RER_{peak} within the minimum established limits (1.0 and 1.1) for most subtypes of NMDs. When muscle metabolism is the primary limiting factor of the CPET, a low HR_{peak} is expected, because exercise ends before maximally stressing the cardiovascular system[7,113]. Involvement of the components of the motor unit (one or more) and some structures related to energy production cause changes in the muscle structure and metabolism of patients with NMDs[15,114]. This impacts the oxygen conduction and use by the active muscles[7,41], and

helps to explain the observed low HR_{peak}. Moreover, this suggests that the HR_{peak} may not be a good quality criterion by which to assess maximal performance in patients with NMDs.

Feasibility of CPET

A high completion rate and few adverse events and complications were found in the studies with sufficient methodological quality, indicating excellent feasibility of the CPET protocols for ambulatory patients with NMDs. The feasibility of CPET was also evaluated for other clinical groups, such as adults with multiple sclerosis, advanced cancer[110,115], and children with pulmonary hypertension[116]. In these studies, as observed in the present review, the feasibility of CPET was limited to patients with high physical abilities. Therefore, in order to make CPET part of the daily clinical evaluation of patients with NMDs, the clinician must consider the functional level of patients when selecting the exercise modality. Moreover, less commonly used devices, such as arm-crank ergometers and treadmills with body weight support, can be alternatives by which to assess patients with reduced physical abilities[117-119]. Despite not being explored in this review, another relevant aspect for CPET feasibility when working with NMDs is the patient's ability to follow the rater instructions because. Some NMDs may present cognitive impairments, and a reduced understanding of commands during the CPET may compromise the patient's motivation and, consequently, his performance.

Aerobic fitness in NMD patients

In general, patients with NMDs assessed in the included studies presented with reduced aerobic fitness. The low VO_{2peak} may result from anything that changes the pathway of oxygen uptake, extraction or use by the active muscle. If the assessed patients from the included studies presented any heart involvement, the exercise intolerance could be addressed to the cardiovascular system. However, because they did not present with

associated symptomatic cardiac or pulmonary diseases, the low VO_{2peak} can primarily be justified by the limited capacity of the muscles to extract and use oxygen during exercise (muscle metabolism limitation), associated with the deconditioning effect of the sedentary lifestyle[8,41].

Additionally, heterogenous percentages of the predicted VO_{2peak} were observed in patients with the same subtypes of NMDs who performed the CPET in diverse exercise modalities. Most adults, for example, had a higher predicted VO_{2peak} on the treadmill as compared to the upright cycle ergometer; with the exception of patients with motor neuron diseases. This finding agrees with the observation in healthy subjects that shows a 5–10% higher VO_{2peak} on the treadmill[6]. Indeed, walking on a treadmill activates a higher muscle mass and requires a higher metabolic cost to support the body weight against gravity than does the cycling ergometer[6]. The opposite finding for patients with motor neuron diseases in these devices can be justified by the functional level of the assessed patients who are able to walk on the treadmill with or without hand support[79]. Holding the treadmill handrail while walking affects the metabolic demand of the task, reducing the VO_{2peak}[6].

Paediatric patients also showed a different predicted VO_{2peak} between the diverse devices. In glycogen storage disorders, for example, a higher VO_{2peak} was found using the upright cycle ergometer as compared to the treadmill. However, it is important to notice that the CPET results in the upright cycle ergometer were only based on one patient[101]. Surprisingly, young patients with inflammatory myopathies had a higher predicted VO_{2peak} in the CPET using a supine cycle ergometer as compared to the treadmill. Nevertheless, only patients in disease remission composed the study using the supine cycle ergometer, while younger patients and patients with both active disease and disease

remission composed the studies using the treadmill[59,101,102,104]. Submaximal CPET outcomes were shown for younger inflammatory myopathy patients with active disease[59].

General recommendations for CPET in NMDs

Considering our main finds, we advise physicians and health professionals to use the upright cycle ergometer as the primary exercise modality to assess ambulatory patients with NMDs. The ramp-wise protocol with workload selection based on the patient's functional capacity and aerobic fitness (5 to 25W/min for adults and 5 to 20W/min for young NMDs) is also suggested for this device. Before CPET, we recommend that the patient visit a cardiologist, and during the test, the use of additional measurements to the gas exchange, for example, pulmonary function test, electrocardiogram, and blood pressure. Most NMDs might have asymptomatic cardiac diseases, and these precautionary measures help to guarantee the patient's safety for CPET. Moreover, when assessing the quality of CPET performed, we suggest the professionals and researchers consider the RER_{peak} the principal physiological variable to classify the patient's delivered effort.

Conclusion

The knowledge about exercise limiting factors and aerobic fitness in NMDs is increasing and brings the need to understand the applicability and safety of the gold-standard method, CPET, in assessing these variables for this specific group. Our results direct that CPET is feasible for adult and young patients with NMDs when the patient’s functional level and the exercise modality of CPET are considered. However, to safety favour the implementation of CPET in the routine assessment of patients with NMDs, future researchers are urged to follow the ATS/ACCP recommendations for performing and reporting CPET. Furthermore, there is vast potential for standardisation and design of disease-specific CPET protocols for patients with NMDs.

Expert opinion

From the results of this systematic review, we provide information about the best evidence synthesis of CPET protocols and their feasibility for ambulatory patients with NMDs. Understanding the best evidence for incremental protocols' design and work rate dosage is fundamental for clinicians willing to assess these patients' metabolic and exercise responses without generating early-localised peripheral fatigue. Nevertheless, future studies should assess the applicability of timed tests, such as the six-minute walking test, in screening the functional capacity of the patients and in the guidance of workload selection.

Even though our results suggest that CPET is feasible for ambulatory patients, the low adherence of the included studies to the ATS/ACCP recommendations and the wide variety of available protocols indicate a need for standardisation in performing and reporting CPET for this group. However, it is important to highlight that the reports of the included studies limited our assumptions. Perhaps more patients did not complete the CPET and thereby were excluded from the final sample and not reported in the studies, or perhaps more authors followed the recommended procedures from the ATS/ACCP guidelines but did not mention this in the publications. Moreover, there is no information available regarding the feasibility of CPET for non-ambulatory patients. Researchers of future studies are urged to fill this gap. Emerging devices, such as the lower body positive support treadmills, might offer an alternative and should be explored in the design of specific CPET protocols for less functional patients.

For many years, any exercise has been a stigma for patients with NMDs, due to the theoretical concept that weak muscles work near their maximal limit, and hence leading health professionals did not recommend exercise for this group. Despite this uncertainty, safety was an under-investigated aspect for CPET in this group. The analysis

of safety (bio)markers, such as the CPK level and the rating of perceived muscle hurt before and after CPETs, would encourage CPET use for some progressive NMDs and NMDs associated with cardiomyopathy.

The reduced aerobic fitness of patients with NMDs is another alarming observation from this systematic review. For the healthy and chronically impaired populations, a low aerobic fitness indicates a high risk of morbidity and mortality. Exercise training programmes have great potential in dealing with the harmful effects of reduced physical activity and low aerobic fitness. Most clinical trials included in this review used CPET to prescribe training intensity and to assess its efficacy. Therefore, improvements in CPET protocols for ambulatory and non-ambulatory patients might favour the implementation of this test in the routine assessment of patients with NMDs, for prescribing individual exercise training intensity and assessing the efficacy of an intervention, thereby boosting the development of the first exercise training guidelines for this group.

Finally, our results also suggest that the existing objective criteria of maximal effort should be revised for patients with NMDs, because their muscle metabolism limits the achievement of the $HR_{peak.}$ Others CPET variables, such as anaerobic threshold (AT), oxygen uptake efficiency slope (OUES), and the relation between oxygen uptake and work rate (ΔVO2/ΔWR), should be better explored in futures studies.

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Supplementary Table C. Summary of included studies

Autor	Reference	Design	Population	NMD sub classification	Disease	n	Sex		Age (mean-SD or range)	Disease characterisct	Device	Measurements and equipaments	Protocol	Tets duration (min)	RPE fatigue	Maximal effort criteria
							M	F								
AL-RAHAMNEH et al.	**29**	CS	A	MND	PPS	15	8	7	34.2 (4.5)	Flaccid paralysis of lower limbs, no cardiovascular disease	AC	Facemask, Chest strap Borg 6-20 RPE Blood pressure	Warm-up: 0W/4min Effort: 6-9W/min (Ramp) Workload selection: sex cadence: 50rpm	9.6 (1.9)	19.0 (1.1)	BL>8.0 mmol
BANKOLÉ et al.	**74**	CT	A	IHM	FSHD	16	12	4	40.5 (11.0)	FSHD Type 1, ability to cycle, 6MWT = 539m, no cardiovascular disease	UPC	ECG	Effort: 10-30W/2min (Step)	–	–	–
BARTELS et al.	**63**	CS	P	IHM	Dystrophies	9	9	0	10.3 (4.7)	Able to walk > 20m without assistive device	UPC	Facemask 12-lead ECG Pulse oximeter - SPO_2, OMNI RPE, Blood Pressure/2min	Warm-up: 0W/1-2min Effort: 5-10W/min (Ramp) Workload selection: 6MWT	8.3 (3.6)	7.0 (1.8)	HR_{peak}>180bpm; RER_{peak}≥1.0
BEAN et al.	**90**	CR	A	PND	HMSN	1	1	0	51.0	Strength in the MRC scale=3 (ankle dorsiflexor)	T	Borg 0-10 RPE/3min, Blood pressure/3min	Warm-up: 0W/3min Effort: modified Balke protocol (constant speed: 4.8 Km/h, grade increase: 1%/min)	25.0	–	–
BENDAHAN et al.	**91**	CR	A	MitoD	RRFD	2	2	2	21.0	Bilateral ptosis, ophthalmoparesis, and moderate muscular atrophy	UPC	Hans-Rudolph valve, 3-lead ECG	Warm-up: 0W/3min Effort: 20W/2min (Step)	–	–	HR_{peak}=220-age
BERNTSEN et al.	**34**	CS	A	IM	JDM	45	17	28	28.9 (12.0)	Disease duration = 249.6 months	T	Facemask 12-lead ECG Pulse oximeter-SPO_2, Borg 6-20 RPE	Warm-up: 0W/3min Effort: modified Balke protocol (constant speed: 4.8 Km/h, grade increase: 1%/min)	–	18.0 (17.0-19.0)	HR_{peak}>80% of predicted (220-age)
BLOM et al.	**100**	R	P	IM	JDM	36	17	19	7.8 (5.8-9.9)	Disease duration = 11.5 months	UPC	–	Workload selection: PA Warm-up: 0W/2min	–	–	HR_{peak} >95% of predicted

													Effort: 10,15, or 20W/min (Ramp) Workload selection: height Cadence:>60rpm			for gender and age; RER_{peak}>1.0
BOGAARD et al.	**35**	CS	A	MitoD	NADH-CoQ reductase.	6	2	4	24.3 (13.3)	Mitochondrial enzyme deficiency	UPC	Blood pressure	Effort: 30W/4min (Step)	–	–	–
BOGAARD et al.	**36**	CS	A	MitoD	NADH-CoQ reductase.	6	3	3	20.7 (7.7)	NADH-CoQ reductase deficiency	UPC	Blood pressure	Effort: 30W/4min (Step)	–	–	–
BRAGA et al.	**86**	CT	A	MND	ALS	48	32	16	62.6 (11.6)	ALSFRS-R scale ≥ 30; FVC (% predicted) ≥ 70%	T	3-lead ECG Pulse oximeter – SPO_2	Warm-up: 0W/3-4min Effort: 5-15W/min (Ramp) Workload selection: 6MWT	–	–	HR_{peak}>75% of predicted (220-age); 55-65% of predicted VO_{2peak}
BRAVO et al.	**37**	CS	A	MM	RRFD	12	5	7	35.1 (11.8)	Progressive external ophthalmoplegia	UPC	12-lead ECG Borg 0-10 RPE	Warm-up: 0W/3min Effort: 5-10W/min (Ramp) Cadence: 60rpm	–	6.0 (3-0-9.5)	–
CARROLL et al.	**19**	CS	A	GSD, MitoD IHM, PND, NMJD, Mix	Dystrophies GSD V, II, RRFD CPTD Dystrophies CMyo Myopathies, HMSN	29	19	10	27.3 (10.0)	Functional grade at Vignos scale: 1-2 Walk and climb stairs with or without assistance	UPC	–	Warm-up: 0-33W/4min Effort: 12-16W/min (Step) Workload selection: type of ergometer and strength	–	–	–
CARROLL et al.	**44**	CR	A	MitoD	CPTD	1	1	0	21.0	6 months after rnyoglobinuria episode Standardized meat-free diet	UPC	Mouthpiece	Warm-up: 33W/4min Effort: 16W/min (Step) Workload selection: type of ergometer and strength	–	–	–
CEJUDO et al.	**87**	CT	A	MitoD	PEO	18	10	8	44.0 (11.0)	VO_2max <83% of predicted FEV1/FVC≥70	UPC	ECG Pulse oximeter - SPO_2	Warm-up: 0W/3min Effort: 20W/2min (Step) Cadence - 60rpm	–	8.1	–

									%, no join/bone deformities		Borg 0-10 RPE					
CRESCIMANNO et al.	**9**	CS	A	GSD	GSD II	8	5	3	49.1 (12.6)	Ambulatory, Sedentary, Enzyme replacement therapy	T	Mouthpiece ECG Pulse oximeter - SPO_2 Borg 0-10 RPE, Blood pressure/3min,	Effort: Naughton protocol (speed increment: 0.8 Km/h, grade increment: 3.5%/3min)	9.0 (6.5-12.0)	9.0 (6.0-10)	HR_{peak} ≥85% of predicted (220-age); RER_{peak}≥1.1
DANDURAND et al.	**17**	CS	A	MitoD	PEO	15	4	11	44.3 (19.8)	Able to cycle	UPC	ECG Pulse oximeter - SPO_2	Start : 5W 1ºst min, 25W 2ºnd min Effort: 5,10, 15, or 20W/min (Step)	7.0 (5.0-9.0)	–	–
DELANEY et al.	**38**	CS	A	MitoD GSD	GSD V, MitoD	12, 21	7, 6	5, 15	35.0 (19.0) 44.0 (9.0)	–	UPC	12-lead ECG	Effort: 5-10W/1.5min (Step)	–	–	–
DRINKARD et al.	**68**	CS	P	IM	JDM	12	2	10	11.6 (3.6)	Disease stable = 3 months No cardiopulmonary abnormalities	UPC	12-lead ECG	Effort: 5-15W/min (Ramp) Cadence: 60rpm	–	–	–
EL MHANDI et al.	**76**	CT	A	PND	HMSN	9	8	0	33.0 (8.5)	CMT1A, CMT2, Walk independently with or without AFO and canes, Strength at MRC scale≥3	UPC	12-lead ECG	Warm-up: 20W/6min Effort: 10W/min (Ramp), Cadence: 70rpm	–	–	–
ELLIOT et al.	**39**	CS	A	MitoD GSD	RRFD, CPTD GSD V	11, 2	5, 2	6, 0	62.0, 26.2 (11.7)	–	T+UPC	Facemask or mouthpiece, 12-lead ECG, Borg 0-10 RPE Blood pressure/min	Effort: Bruce protocol (speed incremente:1.3-1.5 Km/h, grade increment 2%/3min) or 20-30W/2min (Step)	10.0	–	–
FERNÁNDEZ et al.	**42**	CS	A	MitoD	-	16	8	8	34.0 (11.0)	–	T	Facemask 3-lead ECG Borg 0-10 RPE	Effort: Ortega protocol (constant self selected speed, grade increment: 0-5%/3min)	–	8.1 (2.2)	–
FERNÁNDEZ et al.	**30**	CS	A	MitoD	-	26	15	11	34.0 (11.0)	TLC > 80% of predicted, FEV1/FVC>65 %	T	ECG Pulse oximeter - SPO_2 Borg 0-10 RPE	Effort: Ortega protocol (constant self selected speed, grade increment: 0-5%/3min)	14.0 (3.0)	–	–
FERRI et al.	**77**	CT	A	MND	ALS	16	12	4	53.1 (4.6)	Able to cycle, diagnosis<48 months	UPC	12-lead ECG, Pulse oximeter - SPO_2 Borg RPE	Warm-up: 0W/2min Effort: 3,5 or 15W/min (Step)	–	–	–

												Blood pressure	Workload selection: physical fitness level Cadence: 60 rpm			
FLAHERTY et al.	**31**	CS	A	MitoD	-	26	6	22	36.0 (9.0)	FEV1/FVC>70 %	UPC	12-lead ECG, Pulse oximeter - SPO_2 Blood pressure	Warm-up 20W/1min Effort: 20W/min (Step)	–	–	–
FLORENCE et al.	**88**	CT	A	IHM, PND	FSHD, LGMD, CCD, NM, CMyo Myophaties HMSN	12	11	1	30.0 (9.7)	No cardiovascular abnormality Non-progressive or slow-progressive muscle weakness	UPC	ECG	Warm-up: 33W/4min Effort: 16W/min (Step)	–	–	–
GIMENES et al.	**82**	CT	A	MitoD	CPEO	12	8	4	34.5 (11.2)	Chronic progressive external ophthalmoplegia Sedentary	UPC	12-lead ECG	Effort: 5-15W/min (Ramp)	–	7.0	–
GIMENES et al.	**24**	CS	A	MitoD	_	14	7	7	35.4 (10.8)	Chronic progressive external ophthalmoplegia, ptosis Sedentary	UPC	12-lead ECG	Effort: 5-15W/min (Ramp)	–	–	–
GOURCEROL et al.	**40**	CS	A	IM	DM,PM	10	4	6	52.0 (12.0)	Interstitial lung disease	UPC	12-lead ECG	Warm-up: 20W/3min Effort: 10W/min (Step)	–	–	HR_{peak}>90% ;RER_{peak}≥1.1; BL>6.00 mmol; Δph>0.04; VR<20%; EqO2>40
GRASSI et al.	**41**	CS	A	MitoD, GSD, IHM	GSDV, RRFD, Myopathies	6, 6, 25	5, 3, 20	1, 3 5,	26.0 (3.0) 37.8 (6.0) 32.0 (3.0)	_	UPC	12-lead ECG, Pulse oximeter - SPO_2 Borg RPE	Warm-up: 0W/few min Start: 20-50W/6min, Effort: 10-25W/min (Ramp) Workload selection: physical fitness level Cadence: 60-80rpm	McA+ MitM: 12.0/ Myo: 15.0-17.0	–	HR_{peak}≥85% of predicted
GROEN et al.	**66**	CS	P	IM	JDM	12	7	5	10.8 (2.1)	Active myositis	UPC	Facemask, 2-lead ECG Pulse oximeter - SPO_2	Warm-up: 0W/1min Effort: 10,15, or 20W/min (Ramp) Workload selection: height Cadence>60-80rpm	–	–	–

HABERS et al.	**64**	CS	P	IM	JDM	11	7	4	14.0	Active and inactive myositis	UPC	Facemask, 12 lead-ECG, Pulse oximeter-SPO2 Blood pressure/2min	Warm-up: 0W/3min Effort: 10,15, or 20W/min (Ramp) Workload selection: height Cadence>60-80rpm	–	–	–
HABERS et al.	**102**	CT	P	IM	JDM	26	10	16	12.1	Disease duration= 38.4 months,sedentary	T	–	Effort: Modified Dubowy (speed increment: 0.5 Km/h, grade increment: 3%/1.5min)	11.0 (1.8)	–	–
HAGBERG et al.	**43**	CS	A	GSD	GSD V	5	3	2	28.0 (11.0)	–	UPC	ECG	Start: 30-40% of VO_{2max}/4min, Effort: 5-10W/min (Step) Cadence: 60-70rpm	–	–	–
HAGBERG et al.	**44**	CS	A	GSD	GSD V	4	2	2	30.0 (3.0)	–	UPC	–	Start: 30-40% of VO_{2max}/4min, Effort: 5-10W/min (Step) Cadence: 60-70rpm	–	–	RER_{peak}>1.0
HEBERT et al.	**45**	CS	A	IM	PM	11	4	7	46.0 (16.0)	Active and inactive myositis Disease duration = 42 months	UPC	ECG Pulse oximeter - SPO_2	Effort: 10W/2min (Step)	–	–	–
HEINICKE et al.	**25**	CS	A	MitoD	_	5	3	2	42.0 (17.0)	Severe exercise intolerance	UPC	12-lead ECG Borg 0-10 RPE Blood pressure	Warm-up: submaximal W/3-4min Effort: 5-10W/min (Step) Cadence: 50 rpm	–	10.0	–
HICKS et al.	**65**	CS	P	IM	JDM	14	3	11	11.2 (3.0)	Inactive or moderate active myositis Disease duration = 41 months Stable medications ≥3months	UPC	12-lead ECG Blood pressure	Effort: 5-15W/min (Step) Cadence: 60 rpm	8.1	_	RER_{peak}>1.1
HOOPER et al.	**26**	CS	A	MitoD	_	3	1	2	41.3 (6.3)	Dyspnea and fatigue with minimal activity	UPC	ECG Blood pressure	Start: 5W Effort: 5W/15s (Step)	–	–	HR_{peak}>80% of predicted [220-(0.65xage)]
JEPPESEN et al.	**46**	CS	A	MitoD	_	16	9	7	43.0 (2.0)	–	UPC	–	Effort: 5-10W/2min (step)	–	–	–

JEPPESEN et al.	**47**	CS	A	MitoD, IHM	- Dystrophies	15, 10	7, 7	8, 3	38.0 (4.0) 36.0 (4.0)	–	UPC	–	MitM: Effort: 5-10W/2min (Step) MyoD: Effort: 15-20W/2min (Step)	–	–	–
JEPPESEN b et al.	**48**	CS	A	MitoD	PEO	24	10	14	40.0 (3.0)	Chronic progressive external ophthalmoplegia	UPC	–	Effort: 5-10W/2min (Step)	–	–	–
JONES et al.	**73**	CT	A	MND	PPS	37	_	_	40.0	Strength at MRC≥3 (quadriceps and hip flexor)	UPC	ECG Blood pressure	Warm-up (0W/1min) Effort: 20W/min (Ramp) Cadence: 50-70rpm	15.5	_	RER_{peak}>1.1
KNOBIL et al.	**72**	CR	A	MND	PPS	1	0	1	51.0	FEV1/FVC=85 %	UPC	ECG Pulse oximeter – SPO_2 Borg 0-10 RPE Blood pressure	Warm-up: 0W Effort: 20W/min (Step), Cadence: 50-70rpm	8.0	_	_
LANFRANCONI et al.	**70**	CT	A	MND	ALS	17	14	3	52.2 (9.7)	Able to cycle None or stable cardiac disease	UPC	12-lead ECG Pulse oximeter SPO_2 Borg RPE	Warm-up: 0W/2min Effort: 3, 5, 10 or 15-20W/min (Ramp) Workload selection: habitual activities	–	–	–
LEE et al.	**103**	CT	P	MitoD	CPTD	4	2	2	15.0 (5.1)	MET(hours/week)= 20.2	UPC	ECG	Warm-up: 0W/2min Effort: 5-20W/min Workload selection: exercise capabilities	–	–	–
LINDHOLM et al.	**49**	CS	A	MitoD GSD	PEO, MELAS GSD V	16, 4	8, 2	8, 2	35.5 (8.1) 43.8 (8.9)	–	UPC	Facemask 12-lead ECG Pulse oximeter - SPO_2 Borg 0-20 RPE Lactate	Start: 10W Effort: 20W/2min (step)	–	–	–
LUCIA et al.	**83**	CR	A	GSD	GSD V	1	1	0	29.0	BMI>30 Kg/m^2 Sedentary	UPC	ECG Borg 0-10 RPE Lactate Glucose	Warm-up: 0W/10min Start :10W Effort: 10W/min (Step)	5.0	17.0	_
MARZORATI et al.	**93**	CT	A	GSD	GSD II	4	2	2	45.0 (6.0)	–	UPC	ECG	Warm-up: 15-30W/5min Effort: 5-10W/min (Step)	–	–	–
MEZZANI et al.	**20**	CS	A	MND, MitoD	ALS _	24, 6	12, 2	12, 4	63.0(12.0)56.0 (7.0)	Normal resting ECG Able to perform CPET	UPC	–	Warm-up: 0W/1min Effort: 5,7, 10, or 15W/min (Ramp) Workload selection: habitual activities level Cadence: 60 rpm	–	–	–

MONTES et al.	**78**	CT	A	MND	SMA	14	11	3	27.0 (16.0)	SMA type 3 a/b Able to walk≥25m without assistance	RC	OMNI RPE	Warm-up: 5W/2min Effort: 5-10W/min (Ramp) Cadence: 50-80 rpm	–	–	OMNI Scale≥8.0; RER_{peak}>1.0
MOUSSON et al.	**105**	CR	A	MitoD	-	1	1	0	29.0	Difficult climbing 3 stair steps	UPC	Facemask ECG Lactate	Start: 20W Effort: 10W/min (Step)	7.0	–	RER_{peak}>1.1
ALEMO M et al.	**84**	CT	A	IM	DM/PM	23	6	17	58.0	Disease duration = 84.0 months, Exercise≤1x/week, Stable medication≥1.0 month	UPC	ECG Borg6-20 RPE	Start: 30-40W Effort: 10W/min (Step)	–	19.0	–
NABBEN et al.	**94**	CT	A	MitoD	MELAS,PEO	10	3	7	40.0	–	UPC	12-leag ECG Borg6-20 RPE Blood pressure	Warm-up: 0W/4min Effort: 5-20W/min (Ramp) Workload selection: functional capacity Cadence: 70 rpm	–	–	–
NOURY et al.	**50**	CS	A	GSD	GSD V	6	3	3	34.5 (26.9)	–	UPC	ECG	Start: 20%of predicted maximal power/2min Effort: 10%of predicted maximal power/min, Cadence: 60rpm	10.0	–	–
O'DOCHARTAIGH et al.	**27**	CS	A	GSD	GSD V	5	2	3	32.6 (4.21)	Reduced exercise capacity Leg pain	UPC	12-lead ECG, BP	Start: 25W/1 min Effort: 5-10W/min (Step) Workload selection: familiarization test	–	–	–
OMORI et al.	**104**	CR	P	IM	JDM	1	0	1	7	Disease duration = 60 months, Strength: handgrip 5.5 Kg 1 maximal repettion at leg press: 13 Kg	T	–	Effort: Bruce protocol (speed incremente: 1.3-1.5 Km/h, grade increment: 2%/3min)	12.3	–	–
ONCU et al.	**79**	CT	A	MND	PPS	28	12	16	42.1 (8.7)	Able to walk 30m≤60s, Paralytic polio 30-40 years ago New symptoms	T	12-lead ECG Blood pressure	Effort: Naughton protocol (speed increment: 0.8 Km/h, grade increment: 3.5%/3min)	–	–	–
ONG et al.	**51**	CS	A	GSD	GSD V, VII	6	3	3	34.0 (5.9)	–	UPC	–	Effort: 5W/min (Ramp)	–	–	–

PATERSON et al.	**32**	CS	A	GSD	GSD V	4	4	0	34.5 (6.8)	No history of respiratory or cardiovascular disease	UPC	Mouthpiece, 2-lead ECG	Warm-up: 0W/10min Effort: 5W/min (Ramp) Cadence: >60rpm	–	–	–
PÉREZ et al.	**101**	CR	P	GSD	GSD V	1	1	0	8.0	Muscle weakness and myalgia	T	Facemask 12-lead ECG	Warm-up: 0W/15min Start: 2.5 Km/h + 1% Effort: speed increment: 0.1 Km/h, grade increment: 0.5%/10 s	–	–	–
PIIRILÄ et al.	**52**	CS	A	GSD	GSD V, VII	4	3	1	43.0 (18.9)	–	UPC	Borg 6-20 RPE	Start: 40W, step – Effort: 20W/2min or 40W/3min (Step) Workload selection: exercise habits and performance	–	19	–
RAHBEK et al.	**89**	CT	A	NMJD	MG	15	7	8	55.6 (17.2)	No cardiorespiratory, or orthopedic problems Time since diagnosis = 91.2 months	UPC	Chest strap Borg 6-20 RPE	Warm-up: 0W/5min Start: 25.5-80W, Effort: 11.25-42.5W/1.5min (Step) Workload selection: age, gender, disability level Cadence: 55-80 rpm	–	–	HR_{peak}≥ (208-0.7xage); RER_{peak}≥1.1; BL>8.0 mmol/L; Borg≥17
RANNOU et al.	**53**	CS	A	GSD	GSD V, VII	3	0	3	33.6 (15.2)	–	UPC	ECG	Start: 20%of predicted maximal power/2min Effort: 10%of predicted maximal power/min, Cadence: 60rpm	–	–	–
RAPIN et al.	**8**	CS	A	MitoD, GSD IHM, PND	CPTD, GSD, Distrophies, FSHD, LGMD, HMSN	9, 17, 18	6, 5, 12	3, 12, 6	49.0 35.0 49.0	Ambulatory Able to perform CPET	UPC	12-lead ECG Borg 0-10 RPE Blood pressure	Warm-up (0W/1-3min), Effort: 5-10W/min (ramp) Workload selection: functional capacity Cadence: >50 rpm	10.8	10	HR_{peak}>85% of predicted (210-0.65xage); RER_{peak}>1.1, Borg≥7
RILEY et al.	**54**	CS	A	GSD	GSD V	7	5	2	36.1 (18.1)	–	T	ECG Borg 0-10 RPE Blood pressure	Effort: Bruce protocol (speed incremente: 1.3-1.5 Km/h, grade increment: 2%/3min) or Naughton protocol (speed increment: 0.8 Km/h, grade increment: 3.5%/3min)	9.7 (2.8)	8.0	–

ROEF et al.	**95**	CT	A	MitoD	CID	4	0	4	20.5 (4.2)	Mild muscle weakness	UPC	Mouthpiece Clip nose 3-lead ECG Pulse oximeter – SPO_2	Effort: 10-15W/min (Step) Workload selection: FEV1	–	–	HR_{peak}>180 bpm; RER_{peak}>1.0
ROEF a et al.	**55**	CS	A	MitoD	CID	3	0	3	23.0 (6.0)	Mild muscle weakness	UPC	Mouthpiece Clip nose 3-lead ECG Pulse oximeter – SPO_2	Effort: 10-15W/min (Step)	–	–	HR_{peak}>180 bpm; RER_{peak}>1.1
ROEF b et al.	**56**	CS	A	MitoD	CID	3	0	3	22.3 (2.5)	Mild muscle weakness	UPC	Mouthpiece Clip nose 3-lead ECG Pulse oximeter – SPO_2	Workload selection: FEV1	–	–	HR_{peak}>180 bpm; RER_{peak}>1.2
SANJAK et al.	**21**	CS	A	MND	ALS	35	32	3	38.0 (3.0)	Ambulatory with or without cane Disease duration= 26.1 months	UPC	–	Start: 0.5 kp, Effort: 0.5kp/5min (Step) Cadence: 50-60rpm	–	–	–
SCALCO et al.	**75**	CT	A	GSD	GSD II	17	12	5	46.2	Ambulatory	UPC	–	Start: 0-20W/1 min Effort: 5W/2min (Step)	–	–	–
SILVA et al.	**33**	CS	A	GSD, MitoD IHM	GSD, MitoD, Dystrophies, Myopathies	4, 12, 11	2, 6, 11	2, 6, 0	32.0 (12.2) 40.0 (14.6) 24.0 (13.0)	No cardiac or pulmonary disease	UPC	Mouthpiece Clip nose ECG Blood pressure	Warm-up: 0W/3min Effort: 7.5, 10 or 15W/min (Ramp) Workload selection: functional level	–	–	–
SIMILÄ et al.	**80**	CR	A	GSD	GSD VII	1	1	0	59.0	Strength at MRC scale = 4, Able to walk 10 Km/h at slow pace	UPC	12-lead ECG Pulse oximeter – SPO_2 Borg 0-20 RPE Blood pressure	Start : 40W Effort: 40W/3min (Step)	_	19.0	_
SOCKOLOV et al.	**60**	CS	P	IHM	Distrophyes	13	13	0	8.4 (1.3)	Ambulatory	UPC	ECG	Warm-up: 0W/1min Start: 26 W Effort: 13W/2min (Step)	8.0	–	–
SPERFELD et al.	**57**	CS	A	MitoD	MELAS, PEO	17	4	13	36.0 (13.4)	–	T	Facemask Heart rate monitor Blood pressure	Effort: Naughton protocol (speed increment: 0.8 km/h, grade increment: 3.5%/3min)	–	–	–
STANGHELLE et al.	**71**	L	A	NMD	PPS	62	20	43	51.0 (10.0)	Prior episode of paralytic polio At least 20 years of stability	UPC+ AC	ECG Borg 0-20 RPE	AC: Start: 10W Effort: 20W/3min (Step) UPC: Start: 25W Effort: 25-50W/3min (Step)	–	18.0	–

SUNNERHAGEN et al.	**96**	CT	A	IHM	HMM	8	4	4	42.1 (11.9)	New weakness and fatigue symptoms Proximal muscle weakness and atrophy	UPC	Borg0-10 RPE Blood pressure	Warm-up: 0W/3min Effort: 10W/min (Step)	–	–	–
TAIVASSALO et al.	**29**	CT	A	MitoD	PEO	8	3	5	40.3 (9.1)	Mild to severe exercise intolerance	UPC	12-lead ECG, Borg 0-10 RPE	Effort: 5-10W/1-2min (Step)	–	–	HR_{peak}(220-age)
TAIVASSALO et al.	**28**	CS	A	MitoD	MELAS, PEO	40	18	22	37.0 (12.0)	Diversified level of exercise intolerance No cardiomyopathy	UPC	12-lead ECG, Borg 0-10 RPE	Effort: 5-10W/1-2min (Step)	–	–	HR_{peak}(220-age)
TAKKEN a et al.	**59**	CS	P	IM	JDM	15	5	10	9.56 (2.7)	Disease duration = 34.42 months	T	Facemask 2-lead ECG (bipolar)	Effort: Bruce protocol (speed incremente:1.3-1.5 Km/h, grade increment	6.4 (2.3)	–	HR_{peak}>180 bpm; RER_{peak}≥1.0
TAKKEN b et al.	**61**	CS	P	IM	JMD	10	_	_	10.6 (2.5)	Disease duration = 53.6 months	T	Facemask 2-lead ECG (bipolar)	Effort: Bruce protocol (speed incremente:1.3-1.5 Km/h, grade increment	7.4 (2.6)	–	HR_{peak}>180bpm; RER_{peak}≥1.0
TAKKEN a et al.	**62**	CS	P	IM	JDM	16	_	_	13.8 (6.4)	Disease duration = 72 months	UPC	Facemask 2-lead ECG (bipolar)	Effort: 20W/min (Step)	–	–	–
TAKKEN et al.	**69**	CS	P	GSD MitoD, IHM	GSD Ia, III, VII MCAD, SCAD, MADD, Dystrophies	3, 7, 1	2, 5, 1	1, 2, 0	12.2 (0.6) 9.0 (3.0) 14.8	_	UPC	Facemask 2-lead ECG (bipolar)	Warm-up: 0W/2min Effort: 10,15 or 20W/min (Ramp) Workload selection: height and functional level Cadence: >60rpm	–	–	–
TAKKEN a et al.	**98**	CR	P	MitoD	MADD	2	2	0	9.4	Quadriceps strength = 181.5 newtons	UPC	Facemask 2-lead ECG (bipolar)	Effort: 10W/min (Ramp)	–	–	HR_{peak}>180bpm; RER_{peak}≥1.0
TAKKEN et al.	**99**	L	P	IM	JDM	13	7	6	11.2 (2.6)	Active and inactivity myositis	UPC	Facemask 3-lead ECG Pulse oxymeter – SPO_2	Warm-up: 0W/1min Effort: 10,15, or 20W/min Workload selection: height and disease activity Cadence: 60-80rpm	–	–	–

VAN BRUSSEL et al.	**67**	CS	P	IM	JDM	4	3	1	15.7 (3.5)	Inactivity myositis	SC	Facemask	Warm-up: 0W/1min Effort: 0.2-0.4 Kg/min (Step) Workload selection: gender Cadence: 70rpm	10.0 (2.0)	–	HR_{peak}>180bpm; RER_{peak}≥1.0
VAN DEN BERG et al.	**97**	CT	A	GSD	GSD II	23	12	11	46.0	Treatment with enzyme replacement therapy≥52weeks Disease duration = 82.8 months	UPC	Borg 6-20 RPE	Warm-up: 0W/4min Effort: 5-20W/min (Ramp) Workload selection: functional capacity	–	–	HR_{peak} >90% of predicted; RER_{peak}>1.1
WEINSTEIN et al.	**22**	CS	A	IM	PM	9	2	7	42.0 (3.0)	Ambulatory Fatigue interfering with the routine for 6 months Sable regime of medication	UPC	12-lead ECG, Borg 6-20 RPE Blood pressure	Effort: 5-15 W/min (Step) Cadence: 60 rpm	–	18.7 (1.5)	HR_{peak}=220-age
WIESINGER et al.	**18**	CS	A	IM	PM/DM	11	2	9	49.0 (14.0)	Inactive or mildly active myositis, Strength at MRC scale ≥3 (knee extensor and hip flexor)	UPC	Mouthpiece 12-lead ECG Blood pressure	Start - 25W/2 min Effort: 25W/2min (Step) Cadence: 50-60 rpm	5.8 (2.4)	–	–
WIESINGER et al.	**10**	CT	A	IM	PM/DM	14	5	9	51.0	Proximal muscle weakness Stable medication ≥3 months	UPC	Mouthpiece 12-lead ECG Blood pressure	Start: 25W/2 min Effort: 25W/2min (Step) Cadence: 50-60 rpm	–	–	–
WILLÉN et al.	**23**	CS	A	MND	PPS	32	16	16	49.5 (9.5)	Able to cycle Walk with or without assistive device No cardiac disease	UPC	Facemask 3-lead ECG Borg 0-10 RPE Blood pressure	Start: 0W Effort: 10W/min (Step)	–	7.0 (4.0)	–
WRIGHT et al.	**81**	CT	A	PND, IHM	HMSN, Dystrophies, Myopathies	2, 6	0, 4	2, 2	44.5 34.0 (5.0)	Ambulatory without assistive devices	SRC	RPE Blood pressure	Start: 25W/2 min, Effort: 12.5-25W/2min (Step) Cadence: 50-60 rpm	–	16.0 (1.0)	–

YLIKALLIO et al.	**58**	CS	A	IHM	LGMD, Myopathy	12	6	6	56.0 (10.5)	–		UPC	–	Start: 40W/3 min Effort: 20 W/2min or 30, 40, or 50W/3min (Step) Workload selection: gender and physical condition Cadence: 50-60 rpm	10.0	18.8 (0.8)	Borg = 17-19; RER_{peak}>1.0

Legend: CT: cross sectional; CT: clinical trial; CR: case report; R: restrospctive; L: longitudinal; A: adults; P: paediatric GSD: glycogen storage disorders; MitoD: mitochondrial disorders; IHM: inherited myopathies; IM: inflammatory Myopathies; MCAD: medium-Chain Acyl CoA; SCAD: short-Chain AcylCoA dehydrogenase deficiency; MADD: Multiple AcylCoA dehydrogenase deficiency; JDM: juvenil dermatomyositis; n: number; m: male; f: female; SD: standard deviation; 6MWT : six-minute walking test; m: meter; MRC: Medical research council strength scale; ALSFRS-R: Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; FVC: forced vital capacity; VO_{2max}: maximal oxygen uptake; FEV1/FVC: ratio between the forced expiratory volume in one second and forced vital capacity; CMT: Charcot-Marie-Tooth; TLC: total lung capacity; MET: metabolic equivalent: BMI: body mass index; CPET: cardiopulmonary exercise testing; Km/h: kilometer per hour; UPC: upright cycle ergometer; RC: recumbent cycle ergometer; SRC: semi-recumbent cycle ergometer; SC: supine cycle ergometer; T: treadmill; ECG: electrocardiogram; RPE: respiratory perception exertion; W: watts; min: minute; rpm: rotations per minute; Kg: kilogram; Kp: kilopound -: not reported;

4

Cardiopulmonary Responses of Children/Adolescents with Neuromuscular Disease on Anti-Gravity Treadmill Compared to Arm Ergometer: A Piloty Study

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Status: article in elaboration.

Abstract

Objective: To assess the quality and feasibility of a cardiopulmonary exercise test (CPET) using the lower body positive pressure (anti-gravity) treadmill and the arm-crank ergometer for patients with neuromuscular disease (NMD). Secondarily, to compare the exercise responses of the patients in the two tests.

Design: Pilot study.

Setting: Child Development & Exercise Center, Wilhelmina Children's Hospital (WKZ).

Participants: Patients with spinal muscular atrophy (SMA), type III-IV, and Becker muscular dystrophy (BMD) (n=4). Median (95%CI) age of 16.5 (8.2-29.7) years.

Interventions: Not apply

Outcomes: The arm-crank CPET and the anti-gravity treadmill CPET.

Results: Regarding feasibility, high RER_{peak}, BF_{peak}, and test duration were obtained in the anti-gravity treadmill CPET. Considering exercise response, two patients had similar values of absolute and relative VO_{2peak} in both CPET and two patients presented high absolute and relative VO_{2peak} in the arm-crank CPET. Moreover, 75% of patients preferred the anti-gravity treadmill CPET.

Conclusion: Our results of feasibility (high RER_{peak}, test duration), exercise response (high BF_{peak}), and acceptability (preference of patients) indicate that the anti-gravity treadmill CPET is a good option to assess the exercise response of patients with NMDs. Nevertheless, future studies should explore CPET protocols for this device using individualized body weight support and speed increments. The arm-crank CPET needs some adaptations to be feasible for this group (smaller load increments). However, these results should be interpreted cautiously due to the low sample size.

Key-words: LBPP treadmill, oxygen uptake, chronic disease, children, adolescents,

Introduction

Neuromuscular diseases (NMDs) are a heterogeneous group of disorders characterised by the involvement of one or more motor unit components (motoneuron, peripheral nerve, neuromuscular junction, and skeletal muscle)[1-3]. In general, they present abnormal muscle function leading to a range of symptoms[2]. Duchenne/Becker muscular dystrophy (DMD/BMD) and spinal muscular atrophy (SMA) are among the most common inherited paediatric NMDs, and in which the lower limb muscles are the most affected, impairing the patients' mobility level[2].

Children and adolescents with reduced mobility are less active than their healthy peers[4,5], and the inactivity compromises physical fitness increasing their risk of developing metabolic (obesity and diabetes) and cardiovascular disease later in life[6,7]. Beneficial effects, specifically on aerobic fitness, have been reported with exercise training programs in paediatric patients with chronic disease[8-11]. Nevertheless, less is known about it in paediatric patients with NMDs who are non-ambulatory or has impaired mobility.

For those patients, the election of upper limbs for exercise may be an alternative to improve aerobic fitness. However, the amount of muscle mass active impacts the physiological responses obtained during exercise[7,12]. Studies performed in healthy adults, for example, have shown that the physiological values obtained during an arm-crank CPET are approximately 70% lower than those obtained CPET on a lower limb ergometer[13,14].

Considering these aspects, future aerobic exercise programs in this population should try retain large muscle groups working. The lower body positive pressure (anti-gravity) treadmill is an innovative device that allows unloading the lower extremity,

offering the healthcare professional the opportunity to train patients with really reduced muscle strength in the lower limbs on a standing position[15]. The anti-gravity treadmill, consists of a waist-high-chamber that is inflated with positive air pressure, in relation to external enviroment. This difference between internal and external air pressure creates a lifting force around the mass center of the subject reducing body weight, and, consequently, the ground reaction force[15].

In spite of the advantages of this device, some studies performed with recreational and elite athletes using the anti-gravity treadmill, also found differences in the physiological responses generate in this device due to the increase of the body support, such as decrease values of oxygen comsumption (VO_2) and heart rate (HR)[16,17]. In children and adolecents with disabilities the anti-gravity treadmill was only used to improve balance control and gait pattern in different types of cerebral palsy[18]. In adults with muscular NMD, BMD and limb-girdle´s diseases, Berthelsen et al.[19] used this device to investigate the effects of combined aerobic and strength training. After 10 weeks of training (3 times/week) at 50% body weight. The authors found an 8% increase in walking distance assessed by the six-minute walk test and a reduction in steady-stead heart rate, which could indicate an improvement on aerobic fitness[19].

Although the expanding knowledge of the anti-gravity treadmill on the physiological variables in adults, less is known about this in children and adolescents, specially in those with disabilities. Thereby, it is important to test this device fesiability for paediatric patients with NMDs who are non-ambulatory or has impaired mobility, and to understanding the difference in physiological response of performing exercise on the anti-gravity treadmill and in the arm-crank ergometer for this group. Therefore, the aim of this study was to assess the quality and feasibility of anti-gravity treadmill CPET and an arm-crank CPET for youth with NMD. Secondarily, to compare the exercise responses

of the patients in the two tests. We hypothesis that the exercise performed on the antigravity treadmill produces a higher exercise response than the one at arm-crank ergometer.

Methods

Study design and participants

It is a pilot study performed from April to July of 2021. During this period 13 children and adolescents with SMA, DMD and BMD followed at the Child Development & Exercise Center, Wilhelmina Children's Hospital (WKZ) were invited to participate. Only four of those patients attended the study assessments (Figure 1). The inclusion of patients followed the criteria: genetically confirmed diagnosis of SMA (type II-IV), DMD or BMD, age ≥ 6 years old, already followed at the WKZ, height ≥ 142 and ≤ 193 cm, weight ≤ 181 Kg, able to follow tests instructions and able to walk short (≥5 meters) or medium distances (≥50 meters) with or without assistive device. Patients who had fracture of the lower or upper limbs in the last year, present upper limb strength below 4 in the medical research council (MRC) scale of muscle strenght or any acute medical event during the invitation period were excluded. The study was conducted in accordance with the Declaration of Helsinki. All patients and the parents of patients below 16 years old gave written informed consent before participating.

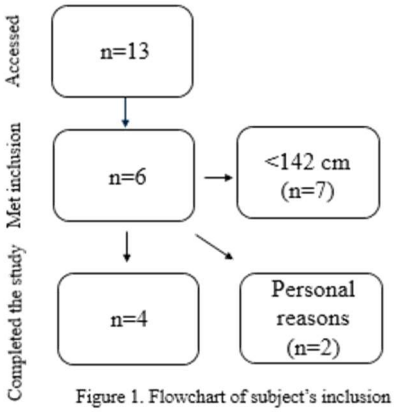

Figure 1. Flowchart of subject's inclusion

Study protocol

The patients attended one extra visit at the "Muscle for Muscles" exercise laboratory of the WKZ. Before the tests, a trained physical therapist (GD) measured their height (cm), weight (electronic scale - Seca, Hamburg, Germany) and asked about their functional mobility (Functional mobility scale – FMS)[20]. The height of the wheelchair-bound patients (n=3) was estimated from their ulna length (the distance between the proximal end of the ulna to the styloid process) using a flexible segmometer[21], and the height of ambulatory patients using a vertical stadiometer (Ulmer Stadiometer, Ulmer,Germany). The body mass index (Kg/m^2) was calculated by dividing the body mass by height squared. Sequentially, the patients performed a CPET on the anti-gravity treadmill (AlterG® Anti-Gravity Treadmill™, California, The United States), and a CPET in the arm-crank ergometer (Lode Angio, Procare BV, Groningen, The Netherlands). An interval time of 30 minutes separated the two measurements. After the tests the trained physical therapist (GD) asked the patients which test (anti-gravity treadmill or arm-crank ergometer) they liked more.

Measures

Anti-gravity treadmill CPET

Incremental exercise test with 50% body weight support (BWS). The protocol was composed by: (1) rest: 2 minutes stand with 50% of BWS; (2) warm-up: 1 minute and 30 seconds walking at 2 Km/h; (3) effort: increase of the speed in 0.5 Km/h each 1 minute and 30 seconds; (4) cool-down: 2 minutes walking at 2 Km/h. After the test, the body weight support was gradually decrease from 50% to 0%.

Arm-crank CPET

Incremental exercise test composed by: (1) rest: 2 minutes sat in front of the arm-crank; (2) warm-up: 1 minute unloaded cycling (0W); (3) effort: increase of the load in 5W/minute; (4) cool-down: 2 minutes unloaded cycling (0W). The patients were encouraged to keep a 60-80 rpm cadence in all stages of the effort.

Before the tests, the patients were instructed to walk and cycle as far as they could, and the tests were immediately interrupted when the patient signalized inability to continue the test because exhaustion even with the encouragement of the evaluator.

During the tests the patients wore a facemask and a heart rate (HR) monitor strap (Polar® V800, USA) to continuously measure and monitor the gas exchange and HR. The gas analysis system (Cortex Metamax B3, Cortex Medical GmbH, Leipzig, Germany) was calibrated before each visit, and measured ventilation, VO_2 and carbon dioxide production (VCO_2), breath-by-breath. Specifically, for the test on the anti-gravity treadmill the patients also wore a neoprene kayak-type short, which allow their attachment to the treadmill chamber. Perception exertion of lower and upper extremities were evaluated before and after the tests, using the perception exertion OMNI scale (0-10)[22].

Data analysis and statistics

The peak of CPETs variables was considered as the mean value obtained in the last 30 seconds, and the HR_{peak} the highest HR obtained in each test. The data did not followed a normal distribution and was presented as median and 95% confidence interval (95%CI). The oxygen uptake efficiency slope (OUES) is a submaximal and alternative measure to the ventilatory threshold when it cannot be determined, it reflects the efficiency of ventilation in relation to the VO_2. The OUES was calculated using the

regression equation $VO_2 = a \cdot \log(VE) + b$, where a = OUES and b is the intercept of y-x axis[23]. A higher slope represents a more efficient VO_2. The feasibility of the CPET protocols used on the anti-gravity treadmill and in the arm-crank ergometer was evaluated based on the test duration, the achievement of objective criteria of maximal effort (HR_{peak} ≥ 90 or 95% of the HR_{peak} predicted and RER_{peak} ≥ 1.1 or 1.0 for adults and children/adolescents, respectively), and subjective criteria of maximal effort $OMNI_{peak}$ ≥ 8. We descriptive compared the individual exercise response of each patient between the CPET on the anti-gravity treadmill and in the arm-crank ergometer.

Results

Table 1 shows the characteristic of the patients. 50% of patients were female, median age of 16.5 (95%CI 8.2-29.7) years old, 52.0 (95%CI 28.4-77.9) Kg, 164.5 (95%CI 145.2-184.2) cm, and body mass index of 18.3 (95%CI 12.2-26.6) Kg /m^2. 75% of patients were able to walk on level surfaces in short distances (5m), 50% use wheelchair for medium distances (50m) and 75% for long distances (500m).

Table 1. Patient characteristics

							FMS		
Patient	**Diagnosis**	**Sex**	**Age (Years)**	**Weight (Kg)**	**Height (cm)**	**BMI (Kg/m^2)**	**5m**	**50m**	**500m**
A	SMA III	M	16.0	41.1	164.0	15.3	5	1_E	1_E
B	SMA III	F	14.0	38.8	150.0	17.2	2	1_E	1_E
C	BMD	M	17.0	62.8	180.0	19.4	5	5	5
D	SMA IV	F	29.0	70.0	165.0	25.7	5	2	1
Median (95%CI and %)	_	F (50%)	16.5 (8.2-29.7)	52.0 (28.4-77.9)	164.5 (145.2-184.2)	18.3 (12.2-26.6)	5 (75%)	1 (50%)	1 (75%)

Legend: Median (95%CI and %)); SMA: spinal muscular atrophy; BMD: Becker muscular dystrophy; M:male; F: female; Kg; kilogram; cm: centimeters; BMI: body mass index; 5: able to walk in level surfaces; 1: wheelchair; 2: walker; E: electric wheelchair

Tests' feasibility

In the CPET using the anti-gravity treadmill, three patients achieved a RER_{peak} ≥ 1.00 and a test duration ≥ 6 minutes. Only patient B achieved a HR_{peak} ≥95% of the predicted HR_{peak}. Patients A, C and D reached respectively 67, 69 and 76% of the predicted HR_{peak}. Only patient B reported a peak perception exertion ≥8 (Table 2). Patient D could not perform the CPET on the anti-gravity treadmill with 50% of BWS. Thus, we gave extra support to her (80%BWS).

In the CPET using the arm-crank ergometer, patients B and C achieved a RER_{peak} ≥ 1.00, but only patient B showed a test duration ≥ 6 minutes. None of the patients achieved an HR_{peak} ≥95% of the predicted HR_{peak} (A:67%; B;93% C:80%, D:88% of the predicted HR_{peak}), and only patient B reported a perception exertion≥8, Another aspect that should be highlighted is that patients A and B were not able to cycle the arm ergometer with a cadence≥60 rpm (Table 2). Moreover, three patients (A, C and D) reported to prefer to perform a CPET on the anti-gravity treadmill than in the arm-crank ergometer.

Table 2. CPETs performance

	Anti-gravity treadmill				**Arm-crank ergometer**			
Patient	**HR_{peak} (bpm)**	**RER_{peak}**	**Duration (min)**	**$OMNI_{peak}$ (0-10)**	**HR_{peak} (bpm)**	**RER_{peak}**	**Duration (min)**	**$OMNI_{peak}$ (0-10)**
A	132	1.04	7.28	2	132	0.85	3.12	7
B	198	1.13	7.10	8	182	1.02	3.30	9
C	150	1.02	13.03	6	157	1.08	9.00	7
D	126	0.91	05.53	7.5	161	0.88	5.23	7.5
Median (95%CI and %)	141 (99-203)	1.03 (0.9-1.2)	7.2 (2.3-13.4)	8-7.5 (50%)	159 (125-190)	0.95 (0.8-1.1)	4.3 (0.8-9.5)	7 (50%)

Legend: HR_{peak}: peak heart rate; bpm: beats per minute; RER_{peak}: peak respiratory exchange ratio; min: minute; $OMNI_{peak}$: perception exertion scale

Comparison of exercise responses between the tests

The exercise response of the patients is presented Table 3. Comparing the individual exercise response between the two tests, patients A, B had a higher absolute and relative VO_{2peak} in the arm-crank ergometer than on the anti-gravity treadmill. Differently, patient B and D showed similar values of absolute and relative VO_{2peak} between the tests. The OUES slope of patients A, C and D was higher on the anti-gravity treadmill than in the arm-crank ergometer (Table 3). The ΔHR (HR_{peak}-HR_{rest}) of patients B and C was higher in the CPET on the anti-gravity treadmill (Table 3).

The VE/VO_{2peak} and VE/VCO_{2peak} were higher in the CPET on the anti-gravity treadmill for patients A, B and D. In the CPET using the arm-crank ergometer patients A, B and D had a higher $VT_{peak.}$ The VE_{peak} varied a lot between the patients and tests (Table 3). Patients A, B and D on anti-gravity treadmill obtained high BF_{peak}. A higher VE/VCO_2 slope was also found for patients A, C and D in the arm-crank ergometer.

Table 3. CPETs exercise response

	Anti-gravity treadmill				Arm-crank-ergometer			
Variables	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Speed $_{peak}$ (Km/h)	3.5	3.0	5.5	3.0				
Load $_{peak}$ (W)					11	10	40	19
ΔHR (HR_{peak}-HR_{rest}) (bpm)	55	80	69	20	58	64	64	63
VO_{2peak} (L/min)	0.60	0.53	1.37	0.61	0.74	0.74	1.33	0.63
$VO_{2\ peak}$ (ml/Kg/min)	14.6	13.6	21.8	9.0	18.1	19.0	21.3	8.8
VO_2/HR $_{peak}$ (ml/beat)	4.8	2.7	9.2	5.0	5.8	4.3	8.6	3.9
$VE/VO_{2\ peak}$	39.8	34.5	26.6	29.7	32.1	29.2	28.9	24.9
$VE/VCO_{2\ peak}$	38.2	30.4	25.9	32.7	37.3	28.7	26.6	28.2
VE $_{peak}$ (L/min)	29.4	25.7	39.8	23.8	27.4	26	42.1	19.9
VT $_{peak}$ (L/min)	0.67	0.44	1.46	0.53	0.93	0.82	1.41	0.59
BF $_{peak}$ (breaths/min)	44.0	60.6	27.2	48.0	30.1	32.3	29.1	33.8
VE/VCO_2 slope	40.9	39.0	26.5	30.7	50.6	33.7	28.8	31.9
OUES slope	900	600	1900	1400	700	900	1600	1200
Distance (m)	380	320	910	260				

Legend: Mean; Km/h: kilometer per hour, W: watts; $VO_{2peak:}$ peak oxygen uptake; L: litter; ml: millilitre; min: minute; Kg: kilogram; VO_2/HR $_{peak:}$ peak oxygen pulse; $VE/VO_{2\ peak:}$ relation between minute ventilation and oxygen uptake; $VE/VCO_{2\ peak:}$ relation between minute ventilation and carbon dioxide production; VE_{peak}: minute ventilation; $VT_{peak:}$ tidal volume; BF_{peak}: breath frequence; AT: anaerobic threshold; RER_{peak}: peak respiratory exchange ratio; OUES: oxygen uptake efficiency slope; m: meters;- not reported.

Discussion

This is the first study to assess the exercise response of patients with BMD and SMA on the anti-gravity treadmill, arm-crank ergometer and test the feasibility of a CPET in the two devices for this group. Our results showed high RER_{peak}, BF_{peak}, and long test duration in the CPET using the anti-gravity treadmill, and high (n=2) and similar (n=2) absolute and relative VO_{2peak} in the arm-crank ergometer. Moreover, 75% of patients preferred the CPET in the anti-gravity treadmill.

A plateau in VO_2 at the end of a CPET is not a common find in the disability and paediatric population[24]. Thus, complementary parameters, such as $RER_{peak} \geq 1.1$ or 1.0, $HR_{peak} \geq 90$ or 95%, or perception exertion≥8, are necessary to confirm that the patient delivered maximal effort. In the present study, we found high RER_{peak} in most of the patients (n=3) performing the test on the anti-gravity treadmill and half (n=2) of the patients performing the test in the arm-crank ergometer, but low HR_{peak} and peak perception exertion. Reduced HR_{peak}, despite high RER_{peak} was reported in others studies assessing paediatric patients with DMD/BMD in the upright cycle ergometer[25], and various adults and paediatric patients with NMDs evaluated in diverse exercise modalities in a recent systematic review of the literature[26].

Although NMDs comprises a heterogeneous group of disorders characterized by the involvement of one or more components of the motor unit[2], in common, these patients present changes in muscle structure and metabolism, impacting the oxygen conduction and use by the active muscles. This malfunction may explain the reduced values of HR_{peak} observed in the present study because exercise ends before maximally stressing the cardiovascular system, for example, due to leg fatigue[24]. In this context, where the peripheral system is the main limiting exercise, low values of peak perception exertion, workload peak, VO_{2peak}, and early anaerobic threshold are also expected[27].

Indeed the assessed patients showed low VO_{2peak} regarding typical and disabled peers in both CPETs. Concerning reference values, the VO_{2peak} of patients was <50% of predicted (range 20-45%) for a CPET performed in an upright cycle ergometer[23,28], and <percentile 3 of reference values from a conventional treadmill[29]. Compared to disable peers with DMD in the upright cycle ergometer and spina bifida - SB on a conventional treadmill, the VO_{2peak} of patients on the anti-gravity treadmill was 50% lower[25,30]. On the arm-crank ergometer, the VO_{2peak} of patients was 70-80% lower than the one obtained by patients with cerebral palsy and SB in the same exercise modality[31,32].

Although both CPETs could detect severely reduced aerobic fitness in the patients with NMDs, we found high and similar VO_{2peak} in the arm-crank ergometer compared to the CPET performed on the anti-gravity treadmill. This find differs from the literature[13,14] and from the results of a previous pilot study we performed in typical youth and adults, where low VO_{2peak} was observed in exercise test performed with upper extremity when compared to exercise with the lower extremity. Because the CPET performed on the anti-gravity treadmill should involve a larger active muscle group than the CPET in the arm-crank ergometer, we expected higher metabolic demand in the first test. It is consensus that the body weigth support (BWS) reduces the metabolic demand of the exercise, and consequently, the physiological response[33-35]. Together with the disease-associated muscle atrophy, this fact might have affected the amount of active muscle mass in the test performed on the anti-gravity treadmill. Despite that, most patients presented extremely higher BF_{peak} in the CPET performed on the anti-gravity treadmill, suggesting that this first CPET was more demanding for the patients because BF_{peak} is responsible for increasing the VE_{peak} at around 60-70% of exercise intensity[23].

Conclusion

Our results of feasibility (high RER_{peak}, enough test duration), exercise response (high BR_{peak}), and acceptability (preference of patients) indicate that a CPET using the anti-gravity treadmill is a good option to assess the exercise response of patients with NMDs. Nevertheless, future studies should explore CPET protocols for this device using individualized BWS (50%-80% BWS) and speed increments (0.25-0.5 km/h) regarding patients' functional levels. The CPET using the arm-crank ergometer need some adaptations, such as smaller load increments (2-3W/min), to be feasible for this group. However, due to the low sample size, these results should be interpreted with caution.

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5

Effects of an Upper Limb Aerobic-Strength Training on Aerobic Fitness And Muscle Strength of Youth With Chronic Disease: A Randomized Before-and-After Trial

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Abstract

Objective: To test the immediate and late effect of an upper limb combined program on aerobic fitness and muscle strength of youth with chronic disease.

Design: Randomized before-and-after trial, registered at the Brazilian clinical trials registry (ReBEC access code: RBR-98cknq).

Settting: Rehabilitation Centre from the Clinical Hospital of Ribeirão Preto Medical School of the University of São Paulo (CER-HC-FMRP-USP) and Integrated Rehabilitation Centre of the Ribeirão Preto State Hospital (CIR-HERP).

Subjects: Youth diagnosed with spina bifida (n=9), Charcot-Marie-Tooth (n=3), and dystrophinopathies (n=2), median age of 12.0 (9.8-12.8) years.

Methods: The patients were randomized to start the combined program with high-intensity-interval training (HIIT) or strength training. Their aerobic fitness was assessed through a cardiopulmonary exercise test (CPET), muscle strength by isometric and isokinetic contractions using a hand-held or isokinetic device, and muscle resistance through a fatigue test on the isokinetic device.

Intervention: 14-week upper limb combined program consisting of HIIT (8 weeks) and strength training (6 weeks) twice a week.

Main outcomes measures: Peak oxygen uptake (VO_{2peak}), peak heart rate (HR_{peak}), peak torque (PT), maximal isometric strength (MIS), and muscle resistance.

Results: The 14-week combined program improved the relative ($t=15$, $p<0.05$, $r=-0.62$) and absolute VO_{2peak} ($t=10$, $p<0.05$, $r=-0.62$) and HR_{peak} ($t=9.5$, $p<0.05$, $r=-0.67$). Similar effects were obtained for the isometric ($t=16.5$, $p<0.05$, $r=-0.54$) and isokinetic ($t=3.5$, $p<0.05$, $r=-0.76$) PT of elbow flexors, peak workload ($t=1.5$, $p<0.05$, $r=-0.83$), distance ($t=1.0$, $p<0.05$, $r=-0.86$), and CPET duration ($t=3.0$, $p<0.05$, $r=-0.83$). No difference was observed for these outcome after six months, except for muscle resistance ($t=10.5$, $p<0.05$, $r=-0.63$).

Conclusion: The upper limbs 14-week combined (aerobic-strength) program improves aerobic fitness, performance, and flexor muscle strength of youth with chronic disease. Adaptations at intensity and duration are needed to increase muscle strength.

Key-words: children, adolescents, aerobic fitness, muscle strength, combined program

Introduction

Children and adolescents with chronic diseases have limitations caused by their underlying disease[1,2]. These limitations discourage activity and reduce patients' participation worsening their overall condition[1,2]. Metabolic and cardiovascular disease are additional problems to hypoactivity for both healthy and disabled youths[3-7], and aerobic fitness is reported as the main factor associated with it[3,6].

Reduced aerobic fitness has been described for ambulatory[5,8-13], and non-ambulatory patients with chronic childhood disease[5,7,8,14,15]. Therefore, therapeutic interventions focused on improving aerobic fitness are a fundamental goal of pediatric rehabilitation to reduce cardiovascular risk in this group[1,2].

Several studies showed beneficial effects of aerobic exercises on aerobic fitness of ambulatory[16-18], non-ambulatory[19], and both[20] youth with cerebral palsy (CP), the most common infant chronic disease (1.5 to 2.5:1,000 live births)[21]. Nevertheless, few studies explored these effects in other childhood chronic diseases[7,22,23,25].

Continuous and high-intensity-interval training (HIIT) are the two types of aerobic training. Nevertheless, HIIT is more efficient than continuous-moderate training in increasing aerobic fitness and reducing cardiovascular disease risk in healthy children and adolescents[26]. Even though only two studies tested HIIT on children and adolescents with a chronic childhood disease[7,18], and these studies' results diverged. One study evaluating eight ambulatory youth with CP found a significant increase in the oxygen uptake (VO_{2peak}) after 24 HIIT sessions[18], and the other, assessing 70 youth with a range of disease and mobility levels (most ambulatory patients), did not observe changes in the VO_{2peak} after an 8-week training[7].

Many factors contribute to aerobic fitness gains after exercise[1,2,27]. The training frequency, for example, was the reason given by Zwinkels et al.[7] for the poor change in

the VO_{2peak} after the HIIT. Nevertheless, the type of exercise and the task's specificity are other factors that might influence it. Regarding the first one, higher levels of muscle strength have been associated with reduced cardiovascular risk[27,28]. Therefore, training programs also targeting muscle strength are important to increase aerobic fitness of youth with chronic diseases which always present primary or secondary changes in the skeletal muscle system, such as muscle weakness and fatigue[1,29,30]. To the best of our knowledge, only two studies tested the effects of combined (aerobic and strength training) on the aerobic fitness of youth with chronic disease[24,31].

Considering the specificity of the task, the lower limbs are, in most cases, the main affected members by the underlying disease in this group[29,30]. Besides, most exercise protocols designed to improve aerobic fitness in this youth group focused on the lower limbs. In this context, it would be interesting to assess the effects of upper limb aerobic and strength exercise on the aerobic fitness of children and adolescents with chronic disease[7,16-18,22-23]. Thus, this study aimed to test the immediate and late effect of an upper limb combined program on aerobic fitness and muscle strength of youth with chronic disease. We hypothesized that the combined program would immediately increase the VO_{2peak} and upper limb muscle strength and that these changes would remain after six months. Moreover, we believed that the training order, randomization (aerobic-strength or strength-aerobic), will not change the training effect, and no differences will be observed between the first period of the two training (aerobic vs. strength).

Methods

This current randomized before-and-after trial is registered at the Brazilian clinical trials registry (ReBEC access code: RBR-98cknq, UTN code: U1111-1240-0996).

Study design

This study is a randomized before and after trial with a 14-week combined aerobic-strength program and follow-up after six months, conducted according to CONSORT guidelines[32]. The aerobic training period corresponded to eight weeks, and the strength training to six weeks. Patients were randomly assigned to aerobic or strength training in the first training period.

Participants

Sixty-nine patients with a chronic childhood disease (spina bifida, Charcot-Marie-Tooth, and dystrophinopathies) followed in the outpatients of the Rehabilitation Centre from the Clinical Hospital of Ribeirão Preto Medical School of the University of São Paulo (CER-HC-FMRP-USP) and living in Ribeirão Preto area were assessed for eligibility in the study. Nevertheless, from those, only 30 met the inclusion criteria and accepted participating (Figure 1).

Inclusion criteria were confirmed diagnosis of spina bifida, Charcot-Marie-Tooth, and dystrophinopathies, age from eight to 16 years old, and with any mobility function level (independent walking, use of assistive device or wheelchair). Patients who answer "no" to all questions from the Physical Activity Readiness Questionnaire - PAR-Q[33], with less than two risk factors for coronary heart disease, and able to develop a cardiopulmonary exercise test (CPET) confirmed by a physician and follow instructions.

Exclusion criteria included comorbidities associated with underlying disease contra-indicating maximal exercise test, bone fractures in the upper limbs within one year before the study, hydrocephaly or neuromuscular disorder causing motor incoordination, or the inability to understand and perform the tests.

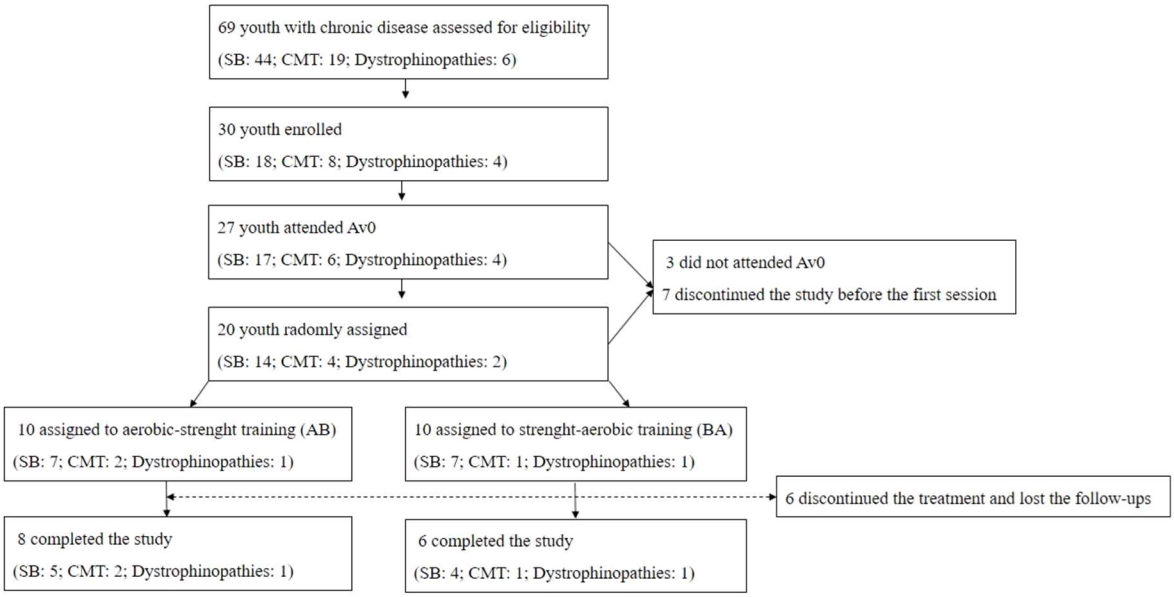

Figure 1. Flowchart

Settings

The evaluations were conducted in the CER-HC-FMRP-USP during the same period (afternoon). The training sessions were performed twice a week at the opposite period of the school schedule in the CER-HC-FMRP-USP (afternoon sessions) or the Integrated Rehabilitation Centre of the Ribeirão Preto State Hospital – CIR-HERP (morning sessions).

The Ethics Committee of the CER-HC-FMRP-USP (CAEE: 16647119.6.0000.5440) approved this study. All legal guardians and patients signed the consent form.

Intervention

High-intensity aerobic interval training

Each exercise session was supervised by a physiotherapist with experience in pediatric neurology and consisted of the following:

1. *Warm-up:* 5 minutes cycling an unloaded electro-magnetically braked arm-cranking ergometer (Lode Angio, Lode BV, Groningen, the Netherlands) at a self-selected speed;
2. *HIT:* 30-second all-out intervals at 85% of workload and heart rate (HR_{peak}) achieved in the CPET (description below), separated by active recovery break cycling the ergometer at a self-selected speed;
3. *Cool-down:* 5 minutes cycling the unloaded arm-ergometer at a self-selected speed.

The first-week training comprises eight 30-second all-out intervals and two-minute recovery breaks. The second week consists of ten 30-second all-out intervals and two minutes recovery breaks, and the last four weeks of 12 30-seconds all-out intervals and one minute and 30 seconds (Supplementary material 1 – Figure S1).

Strength training

The strength training session also was supervised and consisted of the following:

1. *Warm-up:* 5 minutes cycling an unloaded arm-ergometer;
2. *Strength training:* elbow extensor, flexor, and shoulder abductor (Supplementary material 1 - Figure S2 (a-j). The initial load was adjusted to correspond to 20% of the isometric peak torque (PT) obtained in the isokinetic dynamometer test (elbow extensor and flexor) and maximal isometric muscle strength (IMS) in the hand-held test (shoulder abductor), both tests described below. In the third week, the training load was adjusted to 25% of the PT and IMS and, in the fifth week, to 30% of the PT and IMS.
3. *Functional exercises:* two functional exercises that change every two weeks (Supplementary material 1 – Table S1 and Figure S2): Trunk side inclination (first

and second weeks), trunk rotation (first to third week), reaching objects sideways (third and fourth weeks), trunk strength with an elastic band (fourth and fifth weeks) and push-ups (fifth and sixth weeks).

4. *Cool-down:* 2 minutes of deep diaphragmatic breathing.

The strength training started with three sets and four repetitions (first week). Nevertheless, repetitions increased in the second (six repetitions), fourth (eight repetitions), and fifth week (ten repetitions). All training loads for each training session were recorded in an individual training diary for each patient (e.g., exercise adherence, training load, and adverse events)

Outcome variables

All outcome measures were measured at baseline (Av0), in the middle (Av1) of training AB (week eight) or BA (week six), in the end of training (Av2 - week 14), and following six months after intervention (Av3).

Primary

The primary outcomes are aerobic fitness measured by the peak oxygen uptake (VO_{2peak}) and peak heart rate (HR_{peak}) during the CPET, muscle strength measured by peak torque (PT), and maximal isometric strength (MIS), using, respectively, the isokinetic dynamometer (isometric and concentric contractions), and hand-held dynamometer, and the muscle resistance measured by the number of contractions during the fatigue test (with concentric voluntary contractions) using the isokinetic dynamometer.

Secondary

The secondary outcomes are body composition measured by the percentage of fat-free, fat mass, and basal metabolic rate using the bioelectrical impedance, and aerobic performance measured by the workload, distance, and test duration obtained in the CPET.

Recruitment

The patients were recruited from the Infant neuro-rehabilitation (NRI) and Infant neuro-genetic (NGEI) outpatients of HC-FMRP-USP from the second semester of 2019 to the first semester of 2020. After that, the recruitment was interrupted due to the COVID-19 pandemic. The recruitment started again from the second semester of 2021 to the first semester of 2023.

Randomization and blinding

After the baseline assessment and before the first training session, a blind researcher drew one of two folded papers containing the training order AB (aerobic-strength) or BA (strength-aerobic). The researchers who conducted patients' assessments (Av0-Av3) were also blind to the training order. Nevertheless, because of the difference in the exercises from the aerobic and strength training, the patients and the physiotherapist responsible for the training sessions were not blind to it.

Data collection

Height

The height of ambulatory patients was obtained using a standing stadiometer. The height of wheelchair users was estimated from the arm span, the distance from one middle fingertip to the other.

Body mass

A standard electronic scale (WCS, Shanghai, China) and an electronic wheelchair scale (ZTFI LD1050, São Paulo, Brazil) measured the body mass of ambulatory and wheelchair users' patients. The body mass of wheelchair users' patients was calculated by subtracting the total mass of the patient seated in the wheelchair from the mass of the wheelchair only.

Body composition

Body composition was assessed using a bioelectrical impedance (Biodynamics 450, São Paulo, Brazil). Patients were positioned supine, and two pairs of sensors pad were placed on the participant's right side (wrist-hand and ankle-foot), following the manufacturer's recommendations. Fat-free mass (%), fat mass (%), basal metabolic rate (BMR), and body mass index (BMI) in Kg/cm^2, were used in this study.

Maturation

The Brazilian version of the Tanner questionnaire assessed the patient's sexual maturation[34]. The patients and their caregivers received the questionnaire answer sheet in a private room. They were asked to select and mark the auto-explain images which most resemble the children or adolescents about genital development, pubes hair amount, and breast development. Sexual maturation was classified into three stages: I: Infant or pre-pubertal; II, III, IV: pubertal; and V: post-pubertal.

Level of lesion

An expert physical therapist assessed and classified the level of spinal cord lesions in patients with SB. The classification consisted of four levels: I) thoracic: no sensation and muscle strength below the hip; (II) high lumbar: some sensation below the hip and

some strength at the hip adductors, flexor muscles, or knee extensors; (III) low lumbar: muscle strength at the knee flexors, ankle dorsiflexors or hip abductors; (IV) sacral: strength at the plantar flexors of the ankles and hip extensors[35].

Functional mobility

All patients' functional mobility was assessed through a standard patient caregiver interview using the functional mobility scale (FMS), cross-cultural adapted to Brazilian Portuguese[36]. During the interview, the caregivers was questioned about how the children or adolescents move around in the house, school, and community (distances of 5 meters, 50 meters, and 500 meters, respectively).

Level of physical activity

The patient's physical activity was assessed through a formal interview using the Brazilian version of the physical activity questionnaires for children (PAQ-C) and adolescents (PAQ-A)[37]. These questionnaires classify physical activity into five types: 1) extremely sedentary; 2) sedentary; 3) moderate activity level; 4) active; 5) high activity level, based on the last seven days. Slight adaptations were made to apply it to wheelchair users' patients.

Aerobic fitness

The CPET was performed using an electro-magnetically braked arm-cranking ergometer (Lode Angio, Lode BV, Groningen, the Netherlands). The physical therapist (GBQD) positioned the patients in a chair or on their wheelchairs in front of the ergometer at a distance allowing the elbow to extend almost fully during the synchronic cycling and an aligned height among the patients' shoulder and device's pedal axes[15,38]. This position was maintained during all assessment phases: test instructions, preparation, pre, and post-test measurements. The incremental protocol consisted of (a) warm-up: 1 minute of

unloaded cycling (0W) and increase of the cycling pace to achieve 60 rpm; (b) effort: 5W/minute ramp-wise rise in the load and continuous cycling between 60 to 80 rpm; (4) cool-down: 2 minutes unloaded cycling (0W) in a comfortable self-selected pace[15,38]. Patients were instructed to cycle as far as possible for the effort phase. The test was immediately interrupted when the patient signalized inability to continue because of arm pain or exhaustion, even with the encouragement of the evaluator (pace<40rpm), or when cardiac alterations were observed in the electrocardiogram.

During the CPET, patients wore a facemask, a heart rate monitor strap (GARMIN®, EUA), and an electrocardiogram monitoring electrode (3M™ Red Dot™ with foam tape and sticky tape gel, EUA) to continuously measure and monitor the gas exchange and heart rate, respectively. The gas analysis system (K5 COSMED, Rome, Italy) was calibrated before each test.

Peak torque isometric and isokinetic

The PT of elbow flexors and extensors was evaluated using an isokinetic dynamometer (Biodex Mult Joint System 4®, Biodex Medical Systems Inc., New York, USA). Trained physical therapists (EJM, KLTC, and CSBF) positioned the patient on the dynamometer chair with a back angle set at 90° and stabilized with belts on the chest, pelvis, and arms[39]. The mechanical axis of the dynamometer was aligned with the lateral epicondyle of the patient's humerus. The shoulder was positioned at 30° in the scapular plane, 30° of abduction in the frontal plane, 0° of flexion, the forearm in a neutral position, and the elbow at 90º of flexion[41]. First, the patients were familiarized with the equipment, performing three maximal contractions for each muscle. Only the preferential limb's muscles were evaluated. After that and a rest period, the patients performed three maximal isometric contractions of elbow flexors and extensor, sustained for five seconds and 20 seconds intervals between each other. Subsequently, they performed five reciprocally

coupled maximal concentric elbow flexion and extension movements with a range of motion (ROM) of 70º at an angular velocity of 120°s-¹. The rest interval between the isometric and isokinetic test was ten minutes. During the test, the physical therapist verbally encouraged the patient to exert maximum force during contractions. The obtained PT isometric values were used to calculate the patient's training load.

Maximal isometric muscle strength

A Handheld dynamometer (Lafayette Instrument®, Lafayette, UK) evaluated the elbow extensor, flexor, and shoulder abductor's IMS. For the elbow assessment, the patient was positioned supine, with the elbow flexed at 90º, the shoulder in a neutral position, and the forearm supine. For the elbow flexor, the dynamometer was positioned on the anterior wrist face and at the wrist posterior face for the elbow extensor. For the shoulder, the patient was still supine with the elbow flexed 90º. The shoulder was abducted 45º, and the grip neutral[40]. The dynamometer was positioned near the lateral epicondyle of the humerus. The patient performed three maximal contractions in all tests, maintained for 5 seconds. During the test, the evaluator verbally encouraged the patient to perform a maximum contraction without moving the assessed limb, and 30 seconds intervals were ensured between each repetition[40].

Muscle resistance

The fatigue protocol consisted of: (a) baseline: five reciprocal maximal concentric elbow flexion and extension with ROM of 70° and angular velocity of 120°s-¹; (b) fatigue: unlimited reciprocal maximal concentric elbow flexion and extension with a ROM of 50 and 120° of elbow flexion and angular velocity of 120°s-¹. The test was interrupted when the patient reached values below 50% of PT obtained at baseline, in at least three consecutive contractions (elbow flexion/extension/flexion or elbow extension/flexion/extension). During the tests, the patients was verbally encouraged to

perform maximum contractions, and visual feedback provide a real-time display of the dynamometer force output on the computer screen.

Data processing and analysis

The mean value obtained in the last 30 seconds of each test indicated the peak of VO_2. The HR_{peak} was the highest HR obtained in each test. The analysis was performed in the software SPSS® (version 23) and Microsoft Excel. Histogram and box plot checked the normal distribution of the variables. Most variables followed a normal distribution. However, because of the sample size the numerical variables were reported as median and 95% confidence interval (95%CI). Ordinal data were shown as frequency and percentage. The paired Wilcoxon signed-rank test was elected to determine the effect of training, comparing the baseline period (Av0) and the end (Av2) of combined program, and the effect of training after 6-months comparing Av2 and Av3. A p-value<0.05 was considered for statistical significance, and the effect size was calculated using the formula $r = \frac{z}{\sqrt{n}}$ were *z* is the z-score calculated and *n* is the number of observations[41]. Effect size values between 0.50-0.80 were considered medium and >0.80 large effect[41]. The unpaired test u de mann-whitney evaluated the effect of randomization (training order AB *vs.* BA), and difference between the aerobic and strength training. For this last analysis, only the first period of the training order AB (aerobic training) and BA (strength training) was used. A p-value<0.05 was considered for statistical significance.

Results

Participants

From the 30 patients who accepted participate only 27 completed the baseline assessment. Seven patients withdraw before the first training session and other five after

1 month of training due to personal reasons (Figure 1). Thus, 14 patients completed the training, and 12 the assessment after 6-months. Table 1 summarizes the patients' characteristic. The patients presented a median age (95% confidence interval) of 12.0 (9.8-12.8) years old, a body mass index of 22.6 (17.0-27.4) Kg/m^2. Most patients (64%) were male, in the post-pubertal sexual maturation status (57%), wheelchair users for mobility in the community (FMS 500 m: 57%) and had an extremely sedentary level of physical activity (55%) (Table 1).

Table 1. Patients characteristic

Variables	**All patients n=14**	**Training AB n=8**	**Training BA n=6**	**Follow-up n=12**
Diagnostic		**n**		
SB	9	5	4	7
CMT	3	2	1	3
Dystrophinopathies				
CMD	1	1	-	1
MD1	1	-	1	1
		Median (95%CI)		
Age (years)	12.0 (9.8-12.8)	11.5 (8.9-12.5)	14.0 (8.7-15.6)	12.0 (9.8-13.0)
Body mass (Kg)	42.0 (34.4-65.7)	34.2 (23.0-61.2)	57.0 (28.4-93.0)	39.5 (31.3-61.0)
Arm span/ height (cm)	152.5/ 147.2	140.0/ 169.5	140.0 /142.5	152.5/ 172.0
BMI (Kg/m^2)	22.6 (17.0-27.4)	17.8 (12.8-24.7)	24.3 (16.5-37.2)	21.4 (15.4-26.1)
Fat-free mass (%)	66.1 (60.3-75.9)	71.3 (57.4-82.4)	61.3 (53.0-78.5)	69.2 (61.2-79.0)
Fat mass (%)	33.7 (24.2-9.7)	29.0 (17.7-42.4)	39.2 (21.5-47.5)	31.0 (21.3-38.7)
BMR (Kcal)	906.5 (768.4-1253.0)	775.5 (595.2-1190.2)	1196.5 (666.0-1670.0)	906.5 (733.0-1192.0)
Preference limb (D/E)	13/1	7/1	6/0	11/1
		n		
Sex (m/f)	9/5	6/2	3/3	8/4
Sexual maturation				
pre-pubertal	5	3	2	4
pubertal	1	1	-	1
post-pubertal	8	4	4	7
FMS 5m				

wheelchair	5	2	3	3
walker	2	1	1	2
crutches	1	1		1
independent on level surfaces	5	4	1	5
independent on any surfaces	1	_	1	1
FMS 50m				
wheelchair	6	2	4	4
walker	1	1	_	1
crutches	1	1	_	1
independent on level surfaces	5	4	1	5
independent on any surfaces	1	_	1	1
FMS 500m				
wheelchair	8	4	4	6
independent on level surfaces	5	4	1	5
independent on any surfaces	1	_	1	1
Level of lesion*				
thoracic	2	1	1	2
high lumbar	1	1	_	1
low lumbar	5	2	3	4
sacral	1	1	_	1
Level of physical activity[#]				
extremely sedentary	5	4	1	5
sedentary	3	1	2	2
moderately active	1	_	_	1
active	_	_	_	

Legend: n: number; SB: spina bifida; CMT: Charcot-Marie-Tooth disease; CMD: congenital muscular dystrophy; MD1: myotonic dystrophy type 1; Kg: kilogram; cm: centimetre; BMI: body mass index; m: meter; BMR: basal metabolic rate; kcal; kilocalorie; m: male; f: female; *only for patients with SB; [#] missing information from five patients.

Primary outcomes

After a 14-week combined program, there was a positive effect for aerobic fitness on both the relative ($t=15$, $p<0.05$, $r=-0.62$) and absolute VO_{2peak} ($t=10$, $p<0.05$, $r=-0.62$) and HR_{peak} ($t=9.5$, $p<0.05$, $r=-0.67$) (Table 2). Similar effects were also obtained for the muscle strength regarding the isometric ($t=16.5$, $p<0.05$, $r=-0.54$) and isokinetic ($t=3.5$, $p<0.05$, $r=-0.76$) PT of elbow flexors (Table 2).

Secondary outcomes

The aerobic performance of the patients also improved after the 14-week combined program on the peak workload (t=1.5, p<0.05, r=-0.83), distance (t=1.0, p<0.05, r=-0.86), and duration of the CPET (t=3.0, p<0.05, r=-0.83) (Table 2). No significant changes were observed for the percentage of fat-free mass, fat mass, and basal metabolic rate (Table 2).

Table 2. Effect of combined training on the primary and secondary outcome variables.

Primary outcomes	**Av0 (n=14)**	**Av2 (n=14)**	**Z**	**effect size (r)**
Relative VO_{2peak} (ml/kg/min)	18.1 (13.1-22.6)	19.3* (14.8-26.5)	-2.3	-0.62
Absolute VO_{2peak} (ml/min)	712.6 (569.5-1116.4)	877.4* (649.7-1252.2)	-2.7	-0.72
HR_{peak} (bpm)	155 (152-161)	165* (152-177)	-2.5	-0.67
PT isometric elbow Ext (Nm)[a] - PF	14.4 (9.8-27.6)	16.7 (10.4-27.3)	-0.9	_
PT isometric elbow Flex (Nm)[a] - PF	15.1 (9.0-25.3)	17.9* (9.5-30.7)	-2.0	-0.54
PT isokinetic elbow Ext (Nm)[a] - PF	15.6 (9.0-23.8)	16.7 (11.1-26.2)	-1.1	_
PT isokinetic elbow Flex (Nm)[a] - PF	12.3 (8.5-17.4)	14.3* (9.4-22.7)	-2.8	-0.76
IMS shoulder abductor - R/L (KgF)[c]	8.0 (4.2-13.3)/ 8.0 (4.5-11.6)	8.5 (5.1-13.7)/ 8.3 (4.4-12.4)	-1.5/ -0.6	_
IMS elbow Flex - R/L (KgF)[d]	7.6 (5.9-9.9)/ 8.7 (5.8-11.8)	10.0 (5.8-12.9)/ 10.6 (5.5-14.1)	-0.8/ -1.4	_
IMS elbow Ext -R/L (KgF)[d]	7.9 (4.8-11.6)/ 7.8 (3.7-11.3)	8.4 (4.9-11.8)/ 7.8 (5.1-10.5)	-0.3/ -0.6	_
Fatigue test repetitions (n)[a]	50.0 (28.0-103.0)	95 (66.5-115.5)	-1.7	_
Secondary outcomes				
Fat-free mass (%)[b]	66.1 (57.9-78.2)	72.8 (63.8-80.7)	-1.2	_
Fat mass (%)[b]	33.7 (21.7-42.9)	27.0 (22.7-36.1)	-1.0	_
BMR (Kcal)[b]	906.5 (658.7-1271.0)	1124.5 (729.2-1330.7)	-1.8	_

Workload (watts)	32.0 (20.0-45.7)	40.5* (23-5-56)	-3.1	-0.83
Distance (m)	409.0 (157.0-853.0)	656.0* (216.0-1242.7)	-3.2	-0.86
Test duration (s)	431.0 (282.5-595.5)	531* (376.2-730.7)	-3.1	-0.83

Legend: Median (95%CI); HR: heart rate; bpm: beats per minute; VO_2: oxygen uptake; ml: millilitre; Kg: kilogram; min: minute; Ext: extensor; Flex: flexor; Nm: newton-meter; PF: preference limb; IMS: maximal isometric muscle strength; R: right; L: left; KgF: kilogram-force; n: number; %: percentage; BMR: basal metabolic rate; Kcal: kilocalorie; m meter; s: seconds. *p<0.05 when compared Av0 vs. Av2;
[a]missing one patient;
[b]missing four patients;
[c]missing five patients;
[d]missing six patients

Training order and first training period

No difference was observed between the training orders when comparing the Av2 of group AB and BA and training periods when comparing the end of aerobic exercise (AB - Av1) with the end of strength exercise (BA – Av1) (Supplementary material 2 – Table S2).

Follow-up

No difference was observed for the primary and secondary outcome variables after 6-months of the combined training, with an exception for a significant drop (t=10.5, p<0.05, r=-0.63) in the patient's muscle resistance, measured by the number of repetitions reached at the fatigue test (Table 3).

Table 3. Effect of combined training on the primary and secondary outcome variables after a 6-months follow-up.

Primary outcomes	**Av2 (n=12)**	**Av3 (n=12)**	**Z**	**effect size (r)**
Relative VO_{2peak} (ml/kg/min)	23.0 (16.1-27.3)	18.4 (16.5-28.6)	-0.4	_
Absolute VO_{2peak} (ml/min)	877.3 (673.4-1188.2)	915.1 (704.4-1025.0)	-0.1	_
HR_{peak} (bpm)	165 (152-180)	160 (147-173)[b]	-0.8	_
PT isometric elbow Ext (Nm) - PF	15.4 (10.3-26.7)	15.3 (11.1-33.0)	-1.6	_

PT isometric elbow Flex (Nm) - PF	17.3 (9.2-30.3)	19.0 (11.0-33.0)	-0.9	_
PT isokinetic elbow Ext (Nm) - PF	15.4 (10.7-24.6)	16.0 (11.4-28.0)	-0.8	_
PT isokinetic elbow Flex (Nm) - PF	14.0 (9.1-21.1)	14.6 (9.1-24.3)	-1.0	_
Fatigue test repetitions (n)	93.5 (66.0-93.5)	57.5* (48.5-97.2)	-2.2	-0.63
Secondary outcomes				
Fat-free mass (%)	72.8 (64.0-81)[b]	73.0 (67.0-82.0)	-0.4	_
Fat mass (%)	27.0 (23.0-36.1)[b]	27.0 (18.2-33.2)	-0.3	_
BMR (Kcal)	1124.5 (729.2-1331.0)[b]	978.0 (673.0-1310.0)	-1.6	_
Workload (watts)	40.5 (25.5-60.0)	43.0 (28.0-52.0)	-0.3	_
Distance (m)	656.0 (259.0-1424.0)	732.0 (313.5-1067.0)	-0.7	_
Test duration (s)	531.0 (400.0-769.5)	563.5 (371.0-663.0)	-0.4	_

Legend: Median (95%CI); HR: heart rate; bpm: beats per minute; VO_2: oxygen uptake; ml: millilitre; Kg: kilogram; min: minute; Ext: extensor; Flex: flexor; Nm: newton-meter; PF: preference limb; n: number; %: percentage; BMR: basal metabolic rate; Kcal: kilocalorie; m meter; s: seconds. *p<0.05 when compared Av2 vs. Av3;
[a]missing one patient;
[c]missing three patients;

Discussion

The main object of the present study was to test the immediate and late effect of an upper limb combined program on aerobic fitness and muscle strength of youth with chronic disease. Our results showed that the proposed program improves aerobic fitness, aerobic performance, and isometric and isokinetic elbow flexor muscle strength of children and adolescents with chronic disease. Moreover, these effects remain after six months.

To the best of our knowledge, only two studies tested the effect of a combined (aerobic and strength) program on the aerobic fitness of children and adolescents with a chronic disease, specifically CP[24], and dermatomyositis[31]. Unlike the present study, these

authors' protocol most focused on the lower limbs. In the study of Unnithan et al.[24] the aerobic interval training was performed on a 60-m uphill outdoor course with an initial intensity of 65% of predicted HR_{peak}, and the strength exercises included upper limbs and lower limbs muscle strength using weights, in the study of Habers et al[31]. the aerobic interval training was performed on the treadmill with an intensity starting at 60-70% of HR_{peak}, and the strength training included squats and sit-ups. Both programs lasted 12 weeks, two weeks less than the present study. Nevertheless, they had a frequency of three times a week, once more than in our study.

Even though the training protocol from the present study shows some differences regarding duration, frequency, intensity, and type of exercise (upper limbs) from the studies of Unnithan et al.[24] and Habers et al.[31], we observed improvements in the primary outcome, aerobic fitness, and elbow flexor muscle strength, of trained patients. The relative and absolute VO_{2peak} increased by 6% and 19%, respectively (medium effect). A rise of 6% was also obtained for the HR_{peak} and 15% for the isometric and isokinetic PT of elbow flexors (medium effect). The secondary outcome variable, aerobic performance, also increased. A rise of 21% was observed for the peak workload, 38% for the distance covered in the CPET, and 17% for the CPET duration (large effect). These findings agree with the ones from Unnithan et al.[24] that observed an increase of 18% (medium effect, -0.55) in the relative and absolute VO_{2peak} and CPET duration when comparing the training and the control groups.

Unlike the present study, Habers et al.[31] only observed a trend of increase in the relative VO_{2peak} and isometric muscle strength of the knee extensor and hip flexor when comparing the training group and the usual care control.[31] This difference from our results and Habers et al. [31] study, might be justified by the patients' physical activity level. In the present study, most youths were classified as extremely sedentary or sedentary, while in

Habers et al. [31] the trained patients were active. In very deconditioned patients, even low training frequencies (less than two times a week, for example), with adequate training intensity, might increase their aerobic fitness[28]. Therefore, the patients from the present study had much room to improve their aerobic fitness and performance due to their deconditioning, which was different from the patients from Habers et al.[31] study.

Regarding muscle strength, after 14-week training, we only observed gains in one muscle group (elbow flexors). Some factors may justify it. The first one is related to the length of our strength training that had a duration smaller than the recommended in the literature (8 weeks) for healthy children and adolescents[27], and disabled youths[28]. Secondarily our strength training intensity was based on a study designed to strengthen adult patients with a slow progressive neuromuscular disease (CMT)[42]. Thus, our strength training intensity may have been too light for our youths without progressive diseases, such as the patients with SB who represent most of our sample (64%). Even though, gains in the isometric and isokinetic muscle strength of the elbow flexor was obtained, indicating an increase in motor neuron recruitment[27].

Only a trend of increase in the fat-free mass, basal metabolic rate, and reduction in the fat mass was observed in the present study. Combined programs have been reported to have additional beneficial effects than only one training type alone to reduce fat mass and increase fat-free mass in youth[27]. Moreover, a trend of increase in the fat-free mass was also observed by Habers et al.[31]

Interestingly, the patients' training gains remained after six months of the program's end, except for the muscle resistance measured by the fatigue test. Detraining generally occurs after 2 to 3 months without training[27]. The dose/intensity of the exercise is related to the capacity to keep exercise gains[27]. In the present study, a vigorous intensity of aerobic exercise (85% of peak workload) was used and may also have contributed to

maintaining the beneficial effects of training on the aerobic fitness and performance after it ended. Conversely, the low intensity of the strength training (30% of the PT and IMS) could have influenced the muscle resistance loss.

Moreover, despite no standard follow-up using physical activity questionnaires and accelerometer devices to assess physical activity and lifestyle changes, most patients who attended the present training program were referred to adapted sports, gyms, and usual physiotherapy at the end of their protocols. Thus, our training may have changed the patients and their family's habits which may be extrapolated from the training gains maintained after six months.

Few studies in the literature tested the effect of aerobic, strength, or combined training on the aerobic fitness and muscle strength of children and adolescents with chronic disease, mainly using the upper limbs, making it difficult to discuss our results. Nevertheless, the medium to large effects observed on aerobic fitness and performance in this study are extremely relevant for reducing cardiovascular risk in non-ambulatory and mobility-impaired youth with chronic disease, bringing a new perspective to the pediatric rehabilitation area.

Limitations of the study

This study has several limitations. The first is the small sample size that impaired the subdivision of data for analysis regarding the patient's diagnosis, sexual maturation, sex, and mobility level, and the absence of a control group. Therefore, the data obtained here are not necessarily transferable to another population. Another limitation was using a subjective device (questionnaire of physical activity) instead of an objective device (accelerometer) to assess the patient's level of physical activity and did not have information about the patient's level of physical activity from all assessments and follow-

ups. The elected questionnaire is based on school activities, and because of the COVID-19 pandemic, many patients only had online classes until the end of 2021, invalidating the questionnaire's use.

Conclusion

The combined 14-week aerobic-strength program focused on the upper limbs effectively improves aerobic fitness, performance, and flexor muscle strength of youth with SB, CMT, and dystrophinopathies. To increase upper limb muscle strength, we suggested enhancing the strength training intensity, mainly for patients with SB, and the training duration for at least 8 weeks. Future studies are recommended to compare the effect of this combined program to usual care, aerobic or strength exercise alone.

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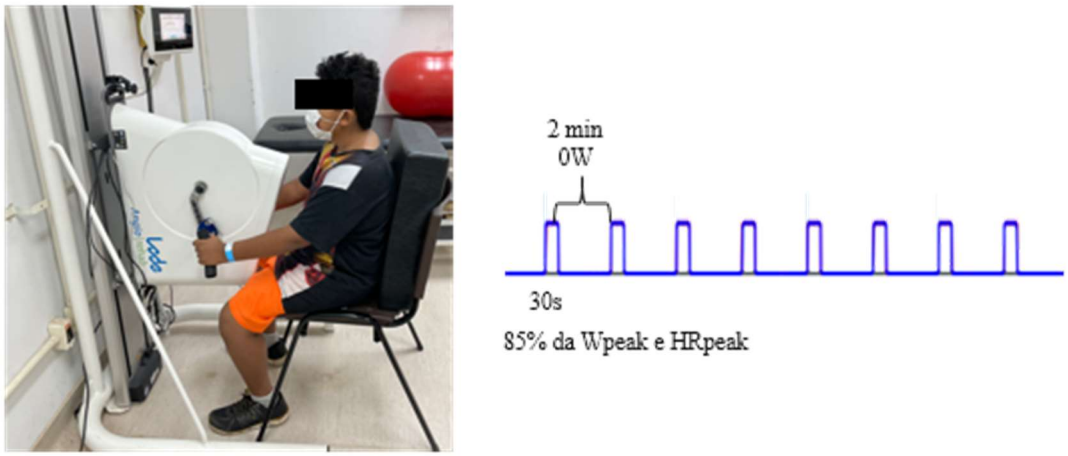
Supplementary material 1 - Table S1. Functional exercises

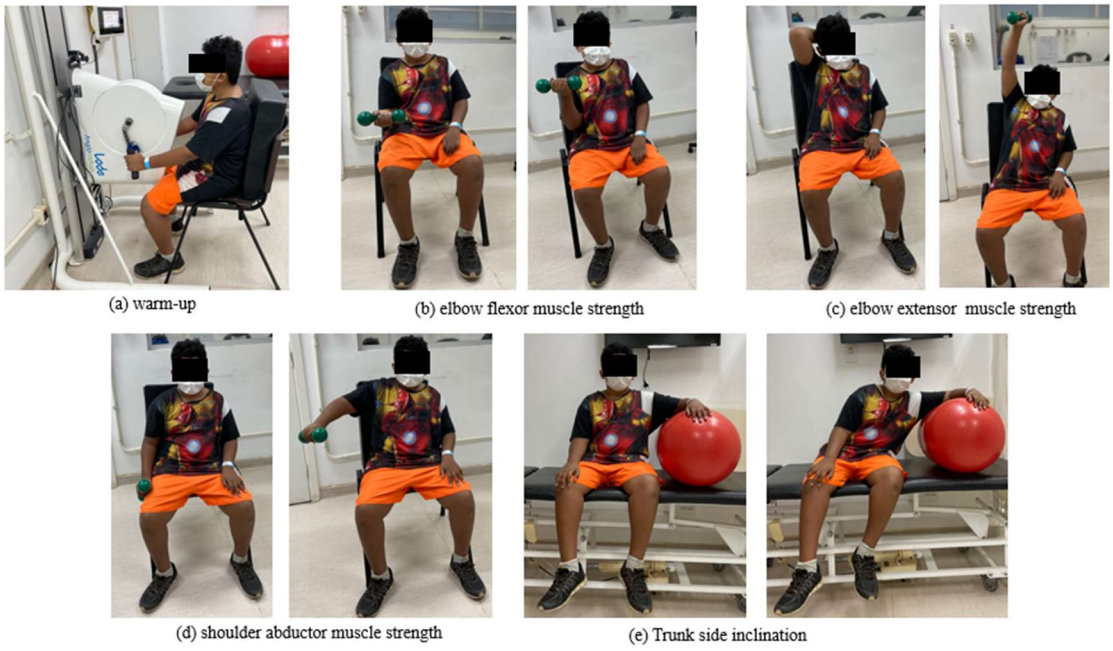
Week	Exercise	Description	Duration
1 and 2	Trunk side inclination	Trunk side inclination (at least 30 cm) as fast as possible. The patient was positioned sitting with the upper limbs on the therapeutic ball.	3 x 60 s
1 to 3	Trunk rotation	Trunk rotation holding a ball with both hands and arms straight at shoulder level, as fast as possible. Patients able to walk were positioned standing, and wheelchair users were placed sitting.	3 x 60 s
3 and 4	Reaching objects sideways	The patient reached ten rings, positioned sideways of his body (at least arm's length), and positioned them in front of him (at least arm's length). Patients able to walk were placed standing, and wheelchair users were placed sitting.	3 x 60 s
4 and 5	Trunk strength with an elastic band	The patient was positioned sitting, holding the edge of an elastic band with each hand, arms straight at the shoulder level. He was instructed to pull and push the elastic band, bending and extending his elbows.	3 x 60 s
5 and 6	Push-ups	The patient was positioned sitting with the arms supported on the stretcher. He was instructed to perform ten push-ups as fast as possible.	3 x 10 rep

Legend: s: seconds; rep: repetitions.



(a) HIIT

Supplementary material 1 - Figure S1 (a)



Supplementary material 1 - Figure S2 (a-j)

Supplementary material 1 - Figure S2 (a-j)

6

General Discussion

GENERAL DISCUSSION

In this thesis, we analyzed the feasibility, quality, and psychometric properties of the cardiopulmonary exercise test (CPET) for children and adolescents with chronic diseases who are non-ambulatory or have a mobility impairments. In addition, we tested the effects of a combined (aerobic-strength) program to reduce cardiovascular disease risk in this group. This last chapter presents the chapters two to four intersection, clinical implications from the obtained results, and directions for future research.

Chapters' intersection and clinical implications

Aerobic fitness assessment and treatment is an important goal in pediatric rehabilitation. In the last decade, the knowledge regarding aerobic fitness test and exercise have been expanded for ambulatory children and adolescents with chronic disease such as cerebral palsy (CP)[1-4], spina bifida (SB)[5-7], Duchenne muscular dystrophy (DMD)[8,9], spinal muscular atrophy (SMA)[10], dermatomyositis[11], among others. Nevertheless, limited evidence is available for non-ambulatory and mobility-impaired youth with chronic diseases[12-17].

The arm-crank CPET and the 10m-SRT are maximal CPETs to assess the aerobic fitness of non-ambulatory and mobility-impaired children and adolescents with chronic diseases, mainly CP[18], SB[12], and osteogenesis imperfect (OI)[19]. However, the arm-crank CPET requires specialized and expensive equipment and professionals, which are only sometimes available in clinical practice[20,21], and the 10m-SRT test dynamic, brings reservations about the measured main variable: aerobic fitness, agility, or anaerobic performance[12,18].

Chapter two shows the short-time continuous push test validity and reliability and its prediction model for oxygen uptake (VO_{2peak}). The prediction model dismisses using gas

analysis to follow changes in aerobic fitness, favoring the use of this newly designed test to assess aerobic fitness in the clinical practice as a routine. Moreover, the new test's short duration and continuous dynamic make it more enjoyable and less demanding for deconditioned children and adolescents with chronic diseases who can self-propel their wheelchairs.

Even though we had provided a new CPET to assess the aerobic fitness of children and adolescents with chronic diseases who can self-propel their wheelchairs, there was still limited evidence regarding the physiology of CPET with upper limbs, and CPET for children and adolescents who have a progressive chronic disease affecting the lower limb strength, resistance, and balance to perform a safe and maximal CPET on a treadmill or lower limbs bicycle ergometer, and also those who not have enough power in the upper limbs to self-propel a wheelchair or win the resistance of the arm-crank ergometer such as patients with severe neuromuscular disease (NMD)[22].

Regarding the physiological gaps, there are no objective criteria of maximal effort for youth performing the CPET with the upper limbs, and the criteria available for children and adolescents performing CPET with the lower limbs (HR_{peak}>95% of the predicted and a RER_{peak}>1.0) do not seem to fit for the upper limbs. The reduced active muscle mass in the upper extremity influences the value of physiological variables obtained in the CPET[23,24]. Studies performed in healthy adults, for example, have shown that the physiological values obtained during an arm-crank CPET are approximately 70% lower than those obtained in a lower limbs bicycle ergometer CPET[25,26].

Thus, **chapters three** and **four** were designed to answer the question about CPET for mobility-impaired youth with NMD and understand more about the physiology of CPET with reduced active muscle mass. In **chapter three**, we systematically reviewed the

literature to learn about the quality and feasibility of available CPET protocols to assess the aerobic fitness of NMD. Nevertheless, because we only found 18 studies that evaluated children and adolescents, we also included studies composed of adults with NMD. In this systematic review, we identify a range of devices and protocols used to assess the aerobic fitness of patients with NMDs. However, most protocols did not have their psychometric properties tested and were designed for patients at the beginning of the disease or with few mobility impairments.

In **chapter four**, from the knowledge obtained with the systematic review, we could design two CPET protocols to assess aerobic fitness in patients with NMD, one in an arm-crank ergometer and the other in a lower body positive pressure treadmill. However, because of the COVID-19 pandemic, fewer patients attended the study developed in **chapter four**. Nevertheless, the preliminary results from the lower body positive pressure treadmill CPET allowed us to understand more the relation between CPET physiology and the amount of active muscle mass. Just as we observed for the arm-crank CPET, the HR_{peak} does not seem a great maximal effort indicator for the lower body positive pressure treadmill CPET. A low amount of active muscle mass due to disease, device assistance (body support), or just because the muscle area is small (such as the muscles biceps and triceps) leads to peripheral fatigue before stressing the cardiovascular system[27,28]. In these cases, the $RER_{peak}>1.0$ seems the best indicator of maximal effort.

Best and gold standard assessment tools are developed in research to allow the development of specific and individualized rehabilitation programs focused on improving disease symptoms and the quality of life of the patients and their families. Thus, **chapter five** compiled all the knowledge from our aerobic fitness assessment studies to design an effective rehabilitation program for non-ambulatory and mobility-impaired youth with chronic diseases. The 14-week upper limb combined aerobic-strength program benefitted

the aerobic fitness, performance, and elbow flexors muscle strength of non-ambulatory and mobility-impaired children and adolescents with chronic disease. Moreover, after six months of training, its gains remained.

Directions for future research

Even though much knowledge and new perspective have been generated from this thesis, there are still many unanswered questions. Future studies should explore maximal criteria for upper limb CPET and CPET with lower active muscle mass in children and adolescents. This information will provide quality parameters for the cardiorespiratory variables obtained and facilitates the CPET interpretation. Developing upper-limb CPET reference values is also fundamental to guide the adequate interpretation of CPET results for non-ambulatory and mobility-impaired children and adolescents with chronic disease. More precision in CPET interpretation also allows the design of specific and individualized interventions for this group. In addition, newly available devices such as the lower body positive pressure treadmill and assistive cycle ergometers should have their properties tested to assess aerobic fitness and be used in therapeutic interventions.

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