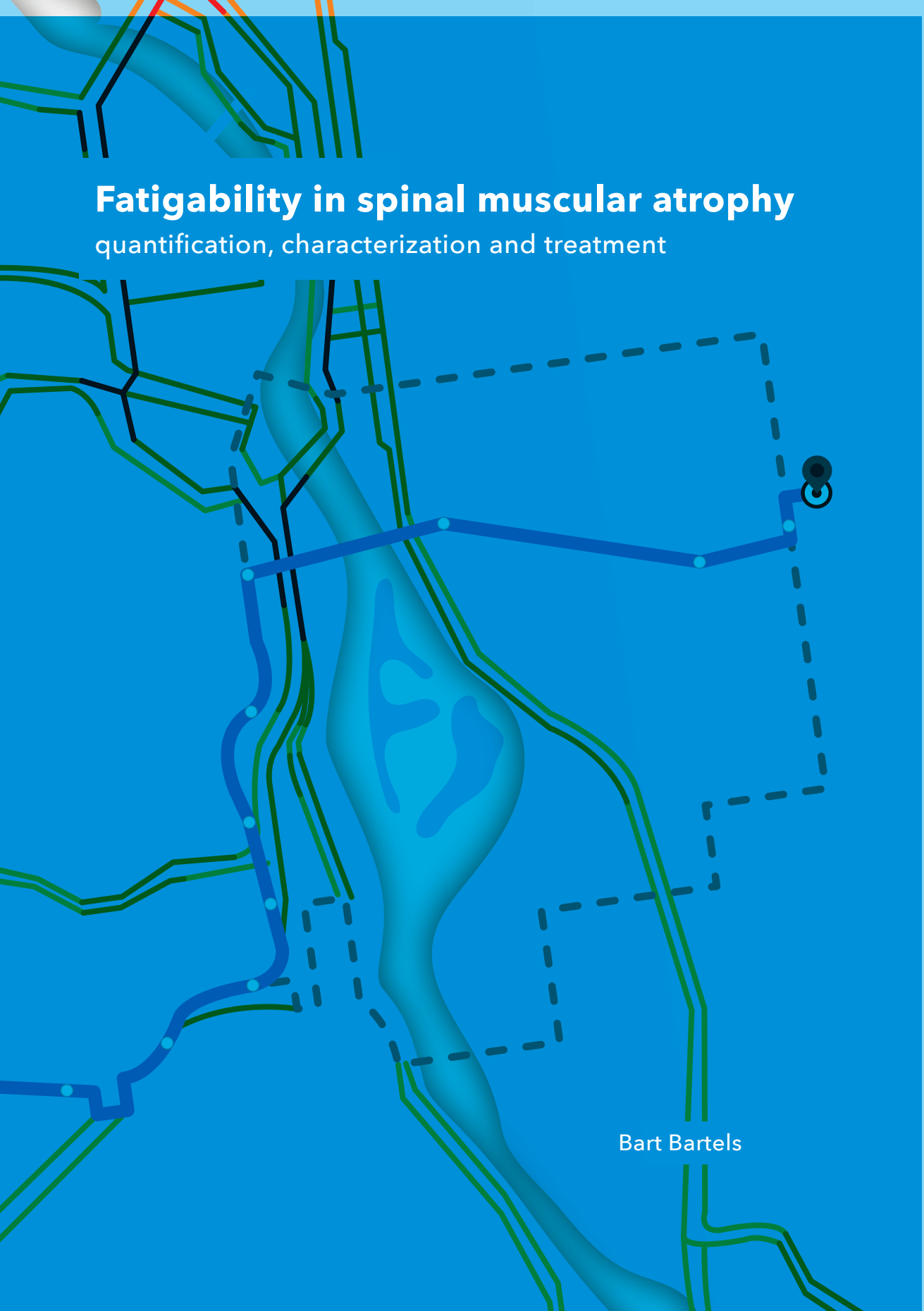
# Fatigability in spinal muscular atrophy

quantification, characterization and treatment



Bart Bartels

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# Fatigability in spinal muscular atrophy

*quantification, characterization and treatment*

Vermoeibaarheid bij spinale musculaire atrofie

(met een samenvatting in het Nederlands)

Proefschrift

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Chapter 1

# General introduction

## Incidence and prevalence

Hereditary proximal Spinal Muscular Atrophy (SMA) is a severe, autosomal recessive neuromuscular disease. With an incidence of 1:6000 -1:12.000 living births it is one of the more common genetic disorders[1]. It is, in fact, one of the most important genetic causes of infant mortality and childhood morbidity[2]. The prevalence is largely unknown. Estimations of the number of patients in the Netherlands range from 450 to over 1000[1]. Each year, approximately 15-20 children are born with SMA in the Netherlands. The UMC Utrecht is the Dutch SMA expertise center and gathers clinical information for the national SMA registry (www.treatnmd.eu/patient_registries). It contains detailed clinical data of more than 400 children and adults with SMA.

SMA is characterized by degeneration of spinal cord α-motor neurons and severe muscle weakness and wasting[3]. These clinical and pathological characteristics were first described at the end of the 19th century by Guido Werdnig and Johan Hoffmann and led to the descriptive name of the disorder[4,5]. Curiously, the eponym Werdnig-Hoffmann disease was until recently used to describe cases with infantile onset (i.e. SMA type 1), whilst their description was of later onset cases, now described as SMA type 2. In the course of the 20th century a number of publications led to the insight that SMA has an impressive range of severity[6-8]. Most patients demonstrate a childhood onset and are characterized by stalled gross motor development followed by progressive muscle weakness. At the time of diagnosis, hypotonia and not acquiring the ability to sit, stand or walk, are often the most important complaints. Severe complications of SMA include scoliosis, feeding difficulties, respiratory complications and severe functional limitations[3,9].

## Pathophysiological background

The genetic background of SMA is relatively simple but the architecture of its locus is highly complex. The cause of SMA was finally elucidated by the group of Judith Melki in 1995. It is caused by deletion or mutation of the survival motor neuron 1 (*SMN1*) gene at the 5q11.2-q13.3 locus which throughout evolution was shaped by duplications, inversions and subsequent mutations[10]. In contrast to other species, humans carry not one, but two homologous yet different *SMN* genes, e.g. *SMN1* gene and *SMN2* gene. Whereas loss of the single *SMN* gene present in other animal species is fatal early in embryonic development, the presence of the nearly identical human *SMN2* gene in humans ensures sufficient levels of *SMN* protein for embryonic development, but does at the same result in SMA. This *SMN2* gene crucially differs from *SMN1* by a C to T transition in exon 7 which leads to an abundant alternative

spliced isoform that excludes exon 7 (*SMNΔ7*) and thereby shortened *SMN* protein in addition to low amounts of full length *SMN* protein. The *SMN2* copy number is the most important known modifier of disease severity, since higher copy numbers are associated with higher levels of *SMN* protein and a milder disease phenotype[11-13]. *SMN* protein is highly expressed during prenatal development and present in high concentrations in spinal cord and the brain stem which implicates an important role in development and maturation of motor neurons[14,15]. *SMN* protein is expressed ubiquitously and has a large number of household functions in cells. Which of these functions is crucial for motor neuron function still remains to be established.

## Phenotype

### *Classification*

SMA has a large spectrum of disease severity ranging from neonatal respiratory insufficiency and death to relatively mild impairments in patients with adult onset disease. SMA was originally classified into three main subtypes according to the age of disease onset and the achievement of the motor milestones unsupported sitting and unsupported walking: the 'severe' type 1 with a disease onset before 6 months and the inability to sit independently, the 'intermediate' type 2 with an onset before 18 months and the ability to sit but not walk and the 'mild' type 3 with the ability to walk independently for at least a short period of time[6,7]. Since then, subclassifications have been introduced to capture variability within these three types. Moreover, at least one additional type characterized by adulthood onset, SMA type 4, has been added (table 1)[8,11,16,17]. Patients in the higher range of the three main types achieve important additional motor milestones such as head control (1C) and standing (2B) and demonstrate better prognosis regarding survival (1C) , scoliosis and pulmonary support (2B) and ambulatory function (3B). Retention of the ability to sit and walk is not considered in the classification which makes it less meaningful in older patients, although the distinction ambulant and non-ambulant is often used to reflect the functional convergence between patients with type 2 and type 3.

**Table 1.** SMA classification

| | **Type 1 (severe) (Werdnig-Hoffman disease)** | **Type 2 (intermediate)** | **Type 3 (mild) (Wohlfahrt-Kugelberg-Welander)** | **Type 4 (mild)** |
|---|---|---|---|---|
| **Incidence** | 50% | 30% | 20% | <1% |
| **Age at onset** | < 6 months<br><br>1A: prenatal onset<br>1B: onset before 3 months<br>1C: onset between 3-6 months | 6-17 months | 3A: 18-36 months<br>3B: 3-30 years | >30 years |
| **Unsupported sitting** | No | Yes | Yes | Yes |
| **Unsupported walking** | No | No | Yes | Yes |
| **Survival (mean)** | 1A: < 1 month<br>1B: 8 months<br>1C: 26 months – adulthood | Majority survive into (early) adulthood. | Normal lifespan | Normal lifespan |
| **Natural history** | 1A: hypotonia and respiratory insufficiency directly after birth, no head control<br>1B: hypotonia after neonatal period, no head control/rolling<br>1C: head control, roll over, no pulmonary complications before 2 years of age | 2A: may lose sitting ability<br>2B: stand with or without support<br><br>Scoliosis: earlier in 2A<br>Respiratory support: earlier in 2A | 3A: early loss of ambulation<br>3B: later loss of ambulation | Ambulant until late in life |
| **Typical SMN2 copy number** | 2-3 | 3 | 3-4 | 4 |

### *Patterns of muscle weakness*

All patients with SMA experience muscle weakness with earlier onset of the disease being associated with more severe weakness[18,19]. Axial- and lower limbs muscles are generally earlier and more affected than upper limb muscles. Weakness is more pronounced in proximal than distal muscle groups[18,20]. Muscle weakness is usually quite symmetrical and more pronounced in specific respiratory (intercostal muscles), proximal limb (deltoid, triceps brachii, iliopsoas and quadriceps) and bulbar muscles (lateral pterygoid) while certain muscle groups are relatively spared (biceps brachii, distal muscles, diaphragm and facial muscles)[18,21-24]. This selective weakness was

ascribed to a possible segmental vulnerability of lower cervical and upper lumbar innervated muscles during early childhood, but this hypothesis has not been confirmed by studies that also included older children and adults[18,20,25]. A relatively new insight is that the axial and proximal muscle weakness is related to vulnerability of specific pools of motor neuron located in the spinal cord[26]. The distinct pattern of muscle weakness in SMA compared to muscular dystrophies can be observed in the walking pattern and getting up from the ground (i.e. the Gowers maneuver) of patients with SMA type 3. A boy with Duchenne muscular dystrophy typically walks tiptoed with significant lumbar lordosis that reflects gluteal weakness. When getting up from the ground, he flexes his knees and trunk and places his hands on his knees, i.e. he pushes himself upward to compensate for hip extensor weakness. A child with SMA walks with legs straightened due to knee extensor weakness. This is also clear when she or he gets up from the ground when knees are locked in an extended position and the child gets up with legs wide apart, a position that is reminiscent of a giraffe drinking. A thorough examination of muscle strength and movement patterns may thus provide key information about the type of neuromuscular disease and guide additional genetic testing.

### *Disease progression*

SMA, irrespective of type, is a progressive disease, with decreasing muscle strength and loss of motor abilities over time[18,27-32]. Patients with SMA type 1 demonstrate a gradual decline in muscle strength and motor function from disease onset[16,18,32,33]. The median survival without disease modifying treatment or the requirement of at least 16 hours per day of ventilation various greatly between type 1a-c[8,11,16,34] (table 1). Patients with SMA type 2 and type 3 develop new motor milestones during early childhood followed by stalled motor development and show a decline in muscle strength and a loss of acquired motor milestones during late childhood and adulthood[18,30-32]. A substantial part of patients with SMA type 2 and SMA type 3 eventually lose the ability to respectively sit and walk unsupported[18,29]. Limited life expectancy in patients with SMA type 2a is mainly caused by respiratory failure[34,35].

## Treatment

Between the start of this study in 2014 and present day, the landscape of SMA has changed dramatically due to the introduction of the first effective disease-modifying treatments[13]. The perspective on management of the disease has shifted towards a pro-active approach, for example regarding the start of mechanical ventilation in patients with SMA type 1 and the intensity

of contracture management and physical therapy in patients with SMA type 2[9]. Treatment is however not curative, and upcoming clinical trials will likely focus on combinatorial interventions of SMN protein enhancing therapies and complementary therapies, tailored to disease type, disease duration and age[36].

### *Upregulation of SMN protein*

The SMA phenotype is caused by low levels of the SMN protein due to a loss-of-function of the *SMN1* gene and the availability of only small amount of functional SMN protein produced by the *SMN2* back up gene[37]. Therapeutic strategies to restore SMN protein concentrations include replacement of the *SMN1*-gene or modulation of the low functioning *SMN2*-gene[36].

In December 2016, a phase 2 open label study in infant-onset SMA was the first to report an improvement in survival, respiratory function and motor function in patients treated with Nusinersen, an antisense oligonucleotide drug that modifies pre-mRNA splicing of SMN2 to promote full length SMN protein, thereby providing a proof of principle that full length SMN increases in general and antisense oligonucleotide therapy in particular are viable therapeutic approaches in patients with SMA[13,38]. In April 2017, the European Medicines Agency (EMA) approved the first medicine, Nusinersen (Spinraza®) to treat patients with SMA. The approval was based on the results of two randomized, double blinded, sham controlled clinical studies[39,40]. During these studies, patients received four intrathecal doses of Nusinersen in the first two months followed by a maintenance dose every 4 months. The study in 121 patients with SMA type 1 demonstrated a 47% decreased risk of death or permanent ventilation and 51% motor milestone response in treated patients[38]. The study in 126 children with SMA type 2 up to 12 years of age showed an improved motor function in treated patients compared to a decline in motor function in controls[41].

In May 2019, the Food and Drug Administration approved the first gene-replacement therapy to treat pediatric patients less than 2 years of age with SMA. A similar approval is expected in the European Union in May-June 2020. The approval in the United States was based on an ongoing phase 3 study and a completed phase 1 study in 15 patients with SMA type 1 with symptoms at the age < 6 months. All patients received a single dose of intravenous adeno-associated virus serotype 9 carrying SMN complementary DNA encoding the missing protein. The phase 1 study demonstrated improvement in survival and the achievement of new motor milestones including sitting without support in 11 of 12 patient treated in the high dose cohort[42].

### *Supportive care*

Before the introduction of Nusinersen (Spinraza®), clinical management consisted of supportive care only[43-45]. A guideline for standards of care was first published in 2007 by an international consortium of experts and has since then been used as benchmark for usual care and as an inclusion criterium for clinical trials[24]. Recommendations primarily focused on the consequences of muscle weakness, management of pulmonary complications, nutritional and gastrointestinal support, orthopedic care, rehabilitation and end-of-life care[24,43]. In 2018, the standards of care were updated following the need to include important advances such as the increased survival of SMA type 1 patients due to the introduction of non-invasive ventilation and enteral feedings and the introduction of treatment options and the changing perspectives of caregivers and patients in the light of the increasingly proactive management of the disease[9,46]. The new standards of care advocate an important role of rehabilitation including regular sessions of physical therapy, the use of braces and orthosis and exercise[9]. However, specific evidence based guidelines for contracture management and exercise are lacking due to insufficient scientific evidence.

### *Combinatorial therapies*

Despite the fact that treatment with Nusinersen (Spinraza®) and gene therapy has led to significant improvements in survival and motor function in mainly young children with SMA, severe residual symptoms of the disease can be anticipated in a large proportion of treated patients. The magnitude of effect of SMN augmenting therapies is closely related to disease duration implicating a limited response in older patients and those with a delay to diagnosis[36,47]. A major issue with intrathecal injection of Nusinersen (Spinraza®) is the concern that it does not directly target peripheral tissues known to be affected in SMA such as the neuromuscular junction and muscle, cardio-vascular system, autonomic nerve system and pancreas and liver[15,48,49]. The prevalence of patients with SMA with newly emerging physical and systemic impairments will likely rise in the future. Other therapeutic approaches that have been explored or are being investigated include systemic delivery of antisense oligonucleotides to target both the central nervous system and non CNS-tissues[50], neuroprotection[51], enhancement of neuromuscular transmission[52] and myo-activation or muscle trophic agents[53,54]. The development of combinatorial therapies of SMN replacement therapy and adjunctive therapies to maintain and improve the motor unit, i.e. motor neuron, neuromuscular junction and muscle, as a whole during different phases of the disease will be the next step in the lifelong treatment of patients with SMA[55,56].

## Outcome measures in SMA

### *Functional scales*

In 2003, Main and colleagues developed the first SMA specific scale, the Hammersmith Functional Motor Scale (HFMS), to evaluate and monitor function of children aged 2,5 years and older with limited mobility[57]. The HFMS is an ordinal scale and consists of 20 upper and lower limb activities such as rolling, sitting, lifting the head from prone and supine, crawling and standing. Each item is scored on a 3 point scoring system and a total score between 0-40 is achieved by summing al scores. Typically, developing children are expected to reach a maximum score at 2 years of age. In 2007, the Hammersmith Functional Scale Expanded (HFMSE), an expanded version of the HFMS, was created to allow assessment of higher functioning patients with SMA type 2 and ambulatory patients with type 3 as well[58]. Thirteen items sensitive to change in ambulatory patients such as walking, stair climbing and running were added from the Gross Motor Function Measure (GMFM), a scale developed for children with cerebral palsy[59], resulting in a scale of 33 items with a maximum score of 66. In 2017, Rasch analysis and input from clinical experts were used to revise the HFSME and develop a psychometrically robust outcome measure for a large spectrum of patients with SMA ranging from weak non-ambulant to strong ambulant patients with SMA[58,60]. The Revised Hammersmith (RHS) consists of 33 items scored on a ordinal scale and two timed tests with a maximum score of 69. Relevant items for extremely weak patients and strong ambulant patients were added from respectively the CHOP INTEND and the North Star Ambulatory Assessment (NSAA) to reduce floor and ceiling effects[61,62]. The HFMS, HFMSE and RHS have been developed to assess motor functions of the trunk and lower extremities rather than changes in upper limb tasks such as being able to lift a glass of water and push a button. The Revised Upper Limb Module (RULM) was developed to capture meaningful upper limb functions and complement motor function assessment in patients with SMA[63,64]. In 2010, Glanzman and colleagues developed the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), a motor function scale for infants with SMA with severe degree of muscle weakness, poor repertoire of motor skills and limited tolerance to the prone position[61,65]. The Six- Minute Walk Test (6MWT), a self-paced walking test used to asses functional capacity, was adopted for SMA to capture specific changes in ambulatory function of SMA type 3 patients[66,67].

This core set of a consensus-based selection of physical outcome measures mainly consists of functional scales tailored to the different SMA types (table 2) (39). There is no functional scale available that covers the whole spectrum

of patients with SMA. The predominant use of functional scales to evaluate disease progression in SMA is based on the historical observation that patients show deterioration in functional abilities despite an apparent stable or only slowly progressive decline in muscle strength over time[19,25,27-29]. In the last decade, these functional scales have been extensively used and updated to optimally characterize natural history and capture efficacy of clinical trials in the large spectrum of patients with SMA. The importance of these efforts is aptly illustrated by the success of recent phase 3 trials that have shown efficacy of antisense oligonucleotides and gene therapy on motor skills in infants and children with SMA by demonstrating improvement on the CHOP INTEND and the HFMSE[38,41,42]. Treatment response was defined as an increase of respectively 4 and 3 points on the CHOP INTEND and HFMSE. The minimal clinical important difference of these scales is largely unknown[61,65,68,69]. Other primary and secondary endpoints on motor milestone achievement, such as the Hammersmith Infant Neurological Examination (HINE), World Health Organization (WHO)-criteria and the Bayley Scales for Infant and Toddler Development, were introduced in these studies to capture clinically meaningful improvements. None of these scales have been properly validated in SMA[70-72]. This clearly shows that selecting both a valid and clinically relevant outcome measure remains a major challenge. Although functional scales and the acquisition of motor milestones have proven to be sensitive to detect changes in younger children, they are expected to be insufficiently sensitive to measure treatment effects in older children, adults and people with milder disease phenotypes or may not assess dimensions of physical function specifically relevant for these patient categories. SMA in older children and adults is more often characterized by a long-present combination of severe weakness and contractures. It is therefore unlikely that efficacy of treatment can be shown by improvement in motor function and motor milestones. To prepare for trials in older patients existing outcome measures need to be adapted or new tests have to be developed to capture meaningful changes in these patients.

**Table 2.** Functional scales in SMA

| Functional scales | SMA type 1 | type 2 and weaker type 3 | Type 3 |
|---|---|---|---|
| CHOP INTEND | + [16,33,42,73] | | - |
| HFMS/HFMSE (>3.5 years) | - | + [41,51,74] | |
| RHS | - | + [75] | + [75] |
| MFM (≥3.5 years) | - | + [51,74,76,77] | + [74,76,77] |
| RULM | - | + [41] | - |
| 6MWT | - | - | + [78,79] |

CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. HFMSE = Hammersmith Functional Motor Scale Expanded. RHS = Revised Hammersmith Scale. MFM = Motor Function Measure. RULM = Revised Upper Limb Module. 6MWT = 6 Minute Walk Test.

### *Fatigability and perceived fatigue*

Fatigue, often used as an umbrella term for both subjective feelings of fatigue and physiological fatigue or fatigability, is one of the most frequently reported complaints in chronic patients and may have a substantial impact on daily life functioning and quality of life[80]. Fatigue can be a temporary condition (state) or a chronic condition (trait)[81]. In the current literature, the terms fatigue and fatigability are often used interchangeably although they are different phenomena that are often independent and certainly measured in different ways[82]. The lack of standard definitions and the inconsistency of terminology hamper the advancement in understanding of the pathophysiological background of fatigability and the development of appropriate outcome measures[82]. The taxonomy of fatigue and fatigability as recently proposed, clarifies the different concepts and means of measurement and can be used as a starting point from which different aspects of fatigue and fatigability can be addressed[80]. To provide a clear understanding it is essential to separately discuss *definitions* of perceived fatigue and fatigability, the choice of *assessment* and possible *causes*. For the purpose of this study, we will focus on fatigability, taking in account the possible interactions with perceived fatigue.

## Definitions

*Perceived fatigue* refers to subjective sensations. It comprises the subjective sensations of weariness, increasing sense of effort, mismatch between effort expended and actual performance or exhaustion and can be subdivided in 'subjective physical fatigue' and subjective mental fatigue[80,81]. 'Subjective physical fatigue is the amount of effort a subject feels to complete certain physical activities, such as performing manual labor, walking, jogging,

running, lifting weights, which require skeletal muscles to generate force. 'Subjective mental fatigue' is the effort the subject feel they must put forth to pay attention to tasks.

*Fatigability* refers to objective changes in performance and reflects the inability to continue exercise at the same intensity with a resultant deterioration in performance[80]. Fatigability includes both 'physical fatigability' and 'mental fatigability'. Physical fatigability concerns the motor domain and is measured by quantifying the decline in peak force, power, speed or accuracy. Mental fatigability concerns the cognitive domain and comprehends a decline in either reaction time or accuracy over time or by using a probe task.

## Assessment

### *Perceived fatigue*

Self-report scales and questionnaires are used to determine perceived fatigue, impact of perceived fatigue on function and related factors such as depression, sleep quality and pain.

### *Fatigability*

The extent of fatigability may vary according to the method of testing. Therefore, it is important to specify in each situation the type of change in performance that is being described as fatigability[83]. There are different protocols that can be used to assess physical fatigability:

1) Continuous performance of a prolonged task
    a. Intermittent submaximal exercise protocol mimicking activities such as walking or cycling and fatigue develops over a longer period
        i. Sub-maximal force protocol[84]
            1. Repetitive contractions at 40-50% MVC
    b. Continuous maximum protocol mimicking activities such as lifting heavy objects or sprinting
        i. Sustained maximal voluntary contraction (MVC)
            1. Maintain maximal force
2) Comparison performance on a probe task before and immediately after prolonged performance of a separate fatigue-inducing task

## Causes of fatigability

From a neurological perspective, causes of fatigability are generally classified based on the anatomical site and a distinction is made between central and peripheral factors (figure 1)[80,81,84,85]. Central fatigability develops at or proximal to the anterior horn cells and includes changes in motor cortex and spinal excitability leading to central drive deficits. Peripheral limitations include alterations or deficits at the level of the neuromuscular junction, peripheral nerve and muscle resulting in loss of muscle force. Peripheral mechanisms can be further specified by distinguishing activating factors (electrical signal transport system elements such as motor nerve conduction, neuromuscular junction function and surface membrane conduction) and contractile factors within the muscle (intracellular energy store, calcium conductance, muscle fiber type, excitation contraction-coupling and cross bridge functioning)[86,87].

In the domain of exercise physiology, fatigability is often related to deficits in the oxygen transport system (cardiac output, lung volume and perfusion, circulation, blood volume, hematocrit and hemoglobin content, hemoglobin dissociation curve, capillary density, mitochondria density) and/or to reduced energy utilization efficiency in disease or aging based on the model of chronic disease, hypoactivity and deconditioning[88,89]. The combination of a decrease in overall fitness and an increase in energy expenditure, due to unstable homeostasis and biomechanical efficiency, reduces the available amount of energy for physical and cognitive activities (figure 2). As a consequence, physical activities need near maximum energy available which generates fatigue adaptive behavior, eventually leading to hypoactivity. Different from fatigability defined from a neurological perspective, a decrease in performance occurs because of an increased relative intensity at which activities of daily life have to be performed, rather than an absolute endurance intolerance.

Endurance performance is regulated by an interaction between fatigability, perceived fatigue and homeostatic factors (cerebral glycogen level, inflammatory cytokines, etc. ) and psychological factors (perceptions of effort, expectations, motivation, etc.) may play a role as well[85,90-92]. Therefore, the question to be asked here is therefore not so much what factors are possibly involved, but what the most limiting factor is for the specific patient on a particular test protocol.

### *Fatigability in SMA*

Muscle weakness, defined as 'the failure to generate the desired force', and muscle atrophy have been appointed as hallmark symptoms of SMA since the first description of the disease in 1891[4]. Despite the fact that

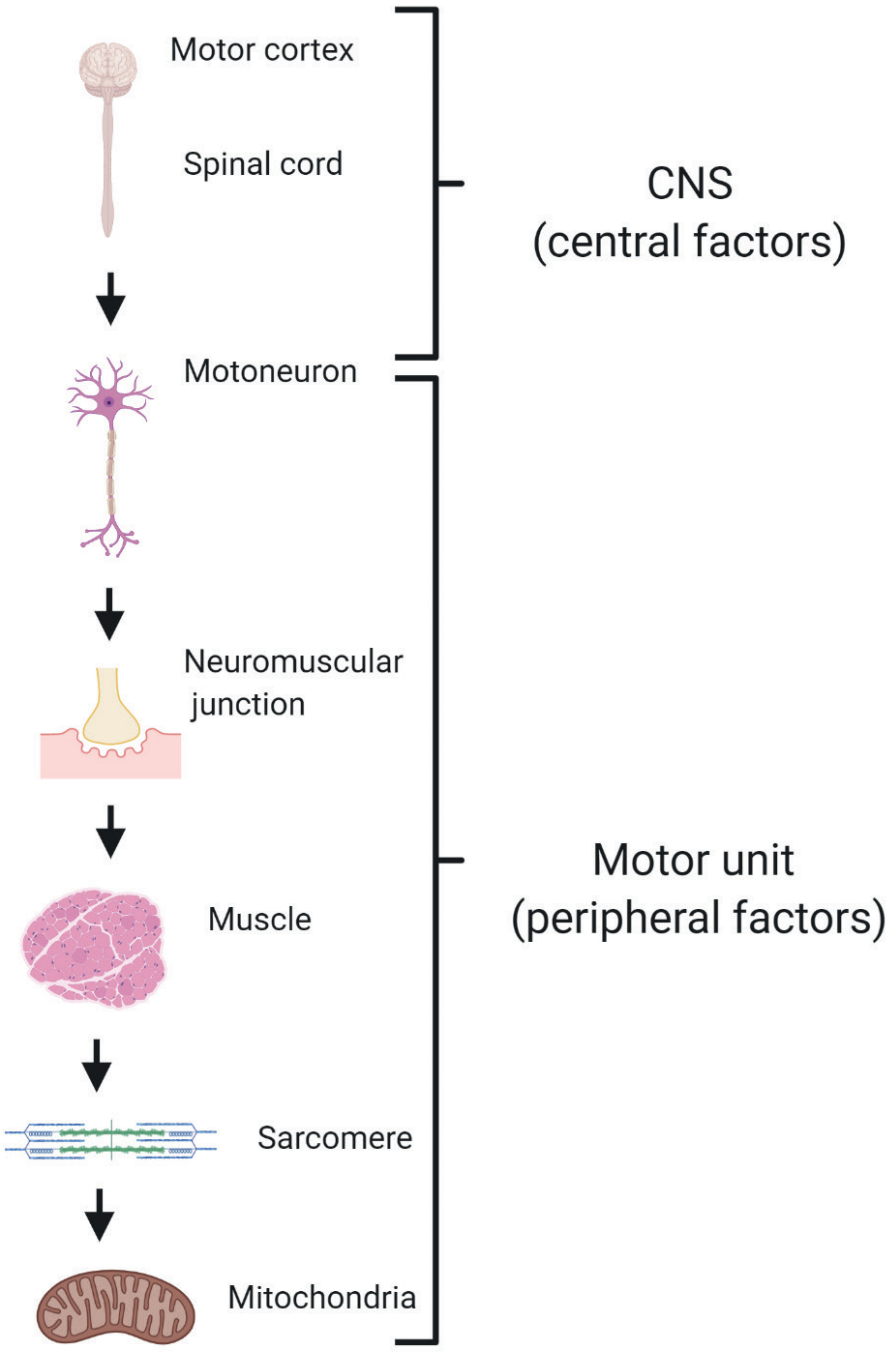


**Figure 1.** central and peripheral factors

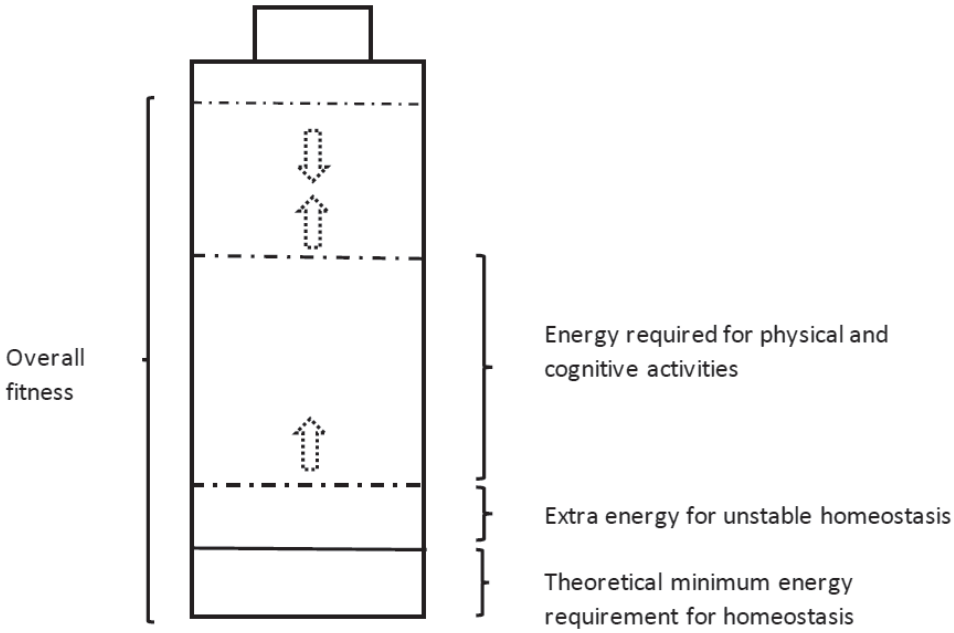


**Figure 2.** Hypothesized mechanism for fatigability

fatigability was already linked to SMA in 1989[93], it took a long time before it was acknowledged as an important *additional* impairment of daily life functioning in patients with SMA. This delay might be explained by the fact that neuromuscular experts are less familiar with exercise tests, in contrast to functional scales and muscle strength. Exercise testing in severely weakened neuromuscular patients is challenging and has been advised against for a long time because of the fear of exercise induced muscle damage and disabling fatigue[94]. Until recently, therapeutic strategies have focused on the most severe SMA type 1 patients with survival and motor development as primary endpoints. Although fatigability was taken into account in the development and validation of the HFSME and CHOP INTEND, by limiting the test duration and avoiding certain strenuous starting positions, it took until 2010, before fatigability itself was quantified by means of a functional task[58,65]. Montes et al. reported a decrease in walking speed during the 6MWT and thus demonstrating for the first time the clinical relevance of fatigability in SMA. This subsequently led to an increasing need for SMA specific fatigability tests for both ambulatory and non-ambulatory patients that could be used in natural history studies and clinical trials. Fatigability was mentioned

spontaneously by approximately 40% of the patients with SMA that were included between 2010-2014 in the Dutch SMA database (Wadman et al. unpublished data). Frequently mentioned problems perceived as fatigability included proceeding with repetitive tasks such as chewing, writing, flexing the arm while drinking and walking. These complaints are reminiscent of problems encountered by patients with Myasthenia Gravis who are characterized by weakness that is triggered by repetitive tasks. In Myasthenia Gravis, fatigability is caused by loss or decreased function of acetylcholine receptors resulting in a decrease in endplate potential amplitudes that fall below the threshold required for muscle fibre action potential generation during repetitive nerve depolarisations and neuromuscular transmission failure[95]. The involvement of neuromuscular junction dysfunction in SMA was shown in animal studies and post-mortem studies that demonstrated an abnormal anatomy of the neuromuscular junction (i.e. at the most distal part of the axon)[96-99]. Subsequently, Wadman et al. studied neuromuscular junction function in SMA by using repetitive nerve stimulation. A train of 10 supramaximal nerve stimulations was given at a frequency of 3 Hz and recordings were made from m. nasalis, m. abductor digiti minimi, m. flexor carpi radialis and m. trapezius. A relative decrease of ≥ 10% between the fifth and first stimulus was defined as a ´decremental response´. They found pathological decrement in approximately half of patients with SMA type 2-3, suggesting that neuromuscular junction dysfunction is indeed common and may contribute to fatigability[100]. Therapeutic interventions for fatigability, especially for older and less severely affected patients, have gained interest. Drugs that ameliorate NMJ function, may represent drug candidates for the treatment of fatigability in patients with SMA. Exercise training, alone or in combination with pharmacological treatment, has emerged as a potential intervention for inherited neuromuscular diseases, including SMA. Training may optimize resources in available muscle tissue or remaining metabolic function, improve neuromuscular junction function and counteract further muscle deterioration secondary to inactivity and neurodegeneration[101-103]. To test the efficacy of possible treatment strategies for fatigability in SMA patients new reproducible and valid outcome measures to quantify fatigability in SMA patients and to investigate the relationship between NMJ dysfunction and fatigability in SMA patients are crucial.

### *Development of outcome measures*

Before an outcome measure can be used in clinical practice or clinical trials, its measurement properties, i.e. reliability (*the degree of similarity between*

*repeated measurements*), validity (*the degree to which an instrument measures the construct it purports to measure*) and responsiveness (*the ability of an instrument to detect change over time in the construct to be measured)* should be established separately for each target population and considered adequate[104]. The development of a new outcome measure is required when existing scales do not cover the construct intended to be measured. Content validity, the degree to which the content of an instrument is an adequate reflection of the construct to be measured, is considered the most important measurement property and the first step in the development of a new outcome measure or in the use of an existing outcome measure for other purposes or in other patient populations[105]. For example, we recently demonstrated a large variability in evidence for adequate measurement properties of the 6 Minute Walk Test among different chronic pediatric conditions[106]. Clinical management and trial design for SMA require reliable and valid outcome measures that are meaningful and sensitive to change due to disease course and treatment.

## Aims and outline of this thesis

Given the high prevalence of fatigability in children and adults with SMA, the development of new drugs aimed to reduce fatigability and the absence of valid and reliable outcome measures to assess meaningful changes in fatigability, the aims of this thesis are to:

1) *Quantify* fatigability in children and adults with SMA across the clinical spectrum

   In **Chapter 2**, we describe the development and content validation of the Endurance Shuttle Tests to asses fatigability in patients with SMA. We use five methodological steps according the COSMIN guidelines including a scoping review of scientific literature, expert panels and pilot studies. We define fatigability in SMA, built a theoretical construct and validate the endurance tests in healthy persons, children with motor disabilities and patients with SMA.

In **Chapter 3**, we assess construct validity and test-retest reliability of the Endurance Shuttle Nine Hole Peg Test, Endurance Shuttle Box and Block Test and the Endurance Shuttle Walk Test. Additionally, we compile and validate the Endurance Shuttle Test Combined Score allowing comparison of patients with varying severity on their individual most relevant endurance test.

2) *Characterize* fatigability in children and adults with SMA and gain more insight into associated factors

In **Chapter 4**, we investigate motor unit reserve capacity during performance of the Endurance Shuttle Tests. We apply surface electromyography on muscles of the arm and leg to determine exercise intensity levels and change in muscle activation over time.

In **Chapter 5**, we explore correlates of fatigability. We investigate whether lower levels of muscle strength and motor function, impaired neuromuscular junction function and higher levels of perceived fatigue are associated with increased fatigability on the Endurance Shuttle Test Combined Score.

3) *Treat* fatigability in children and adults with SMA

In **Chapter 6** we determine the level of evidence in favor and/or against physical exercise training in patients with SMA type 3. In this Cochrane systematic review we provide an overview of aerobic exercise and strength training studies in patients with SMA type 3 and determine the certainty of evidence and its implications for practice and research.

In **Chapter 7,** we study safety and efficacy of the acetylcholinesterase inhibitor pyridostigmine on motor function and fatigability in patients with SMA types 2-4. We conduct a phase 2, monocenter, placebo-controlled, double blinded cross-over trial in which patients receive 8 weeks of pyridostigmine and 8 weeks of placebo in random order. Primary outcome measures are changes in Motor Function Measure and repeated nine-hole peg test (R9HPT). Secondary outcome measures are the Endurance Shuttle Test Combined Score (ESTCS), patient-reported treatment effect and adverse events.

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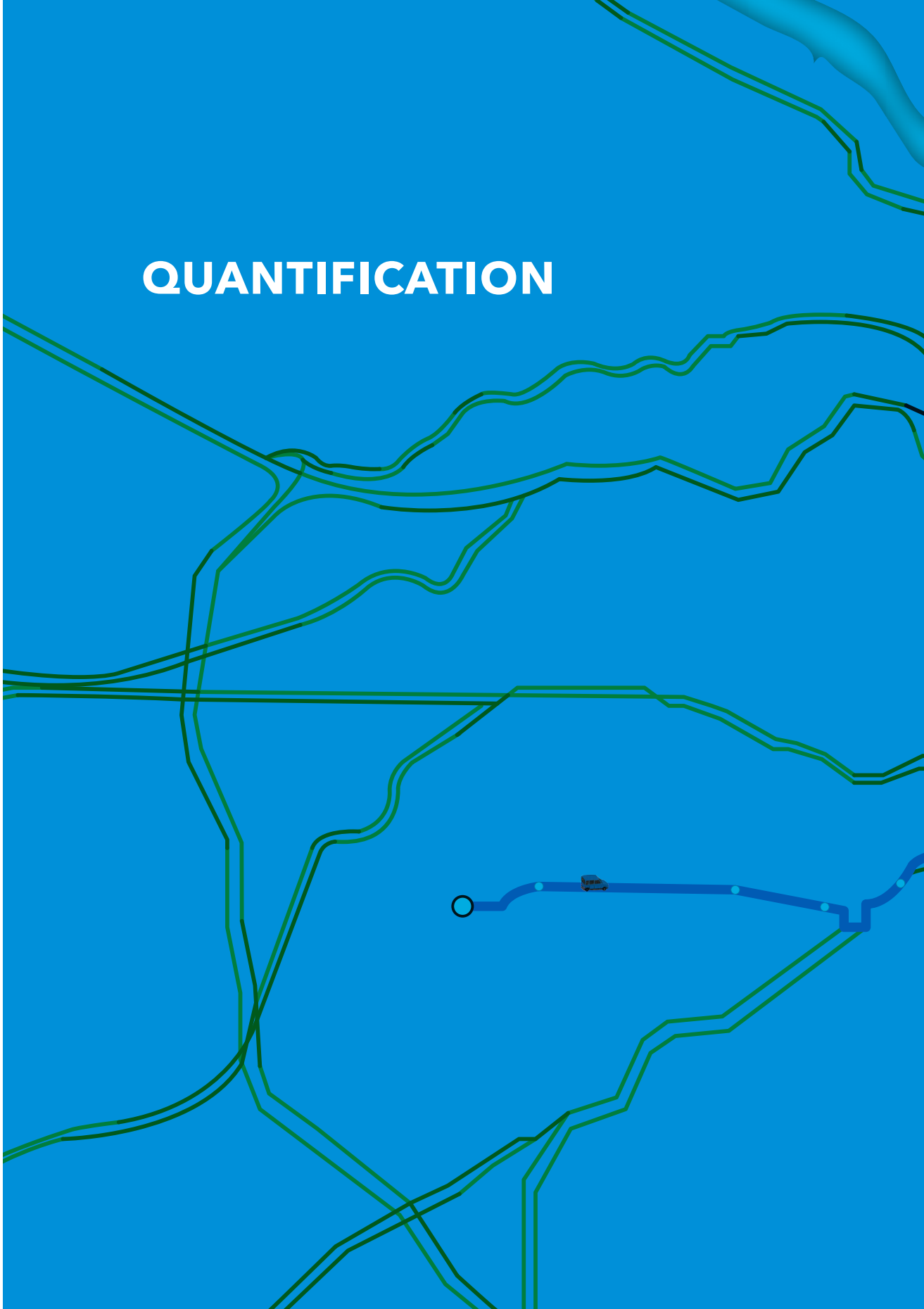
# QUANTIFICATION



Chapter 2



# Assessment of fatigability in patients with Spinal Muscular Atrophy: development and content validity of a set of endurance tests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# Abstract

## Background

Fatigability has emerged as an important dimension of physical impairment in patients with Spinal Muscular Atrophy (SMA). At present reliable and valid outcome measures for both mildly and severely affected patients are lacking. Therefore the primary aim of this study is the development of clinical outcome measures for fatigability in patients with SMA across the range of severity.

## Methods

We developed a set of endurance tests using five methodological steps as recommended by the 'COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN). In this iterative process, data from multiple sources were triangulated including a scoping review of scientific literature, input from a scientific and clinical multidisciplinary expert panel and three pilot studies including healthy persons (N=9), paediatric patients with chronic disorders (N=10) and patients with SMA (N=15).

## Results

Fatigability in SMA was operationalised as the decline in physical performance. The following test criteria were established; one method of testing for patients with SMA type 2-4, a set of outcome measures that mimic daily life activities, a submaximal test protocol of repetitive activities over a longer period; external regulation of pace. The scoping review did not generate suitable outcome measures. We therefore adapted the Endurance Shuttle Walk Test for ambulatory patients and developed the Endurance Shuttle *Box and Block Test* and the - *Nine Hole Peg Test* for fatigability testing of proximal and distal arm function. Content validity was established through input from experts and patients. Pilot testing showed that the set of endurance tests are comprehensible, feasible and meet all predefined test criteria.

## Conclusions

The development of this comprehensive set of endurance tests is a pivotal step to address fatigability in patients with SMA.

## Background

Hereditary proximal Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease caused by homozygous loss of function of the survival motor neuron 1 (*SMN1*) gene.[1] SMA is characterised by a wide range of disease severity ranging from neonatal respiratory insufficiency and death (SMA type 1), ability to sit without support but inability to walk independently (SMA type 2), problems with or the loss of ambulation (SMA type 3a-b) to relatively mild impairments due to proximal muscle weakness in patients with adult onset disease (SMA type 4).[2] All four SMA types are characterised by progressive muscle weakness and secondary loss of motor abilities over time.[3] In addition to muscle weakness, fatigability has emerged as a rather common but often overlooked complaint among patients with SMA.[4,5] The current taxonomy defines 'fatigability' as the magnitude or rate of change in a performance criterion relative to a reference over a given time of task performance or measure of mechanical output and is the opposite of ´endurance´, which involves the prolonged maintenance of constant or self-regulated power or velocity.[6,7] Patients with SMA refer that they easily fatigue during repetitive activities of daily living such as lifting an arm during eating or walking even short distances. A possible explanation comes from SMA animal models and post-mortem studies that showed abnormal development and maturation of the neuromuscular junction. Neuromuscular dysfunction has been found in at least half of the patients with SMA, suggesting that this may contribute to complaints of fatigability.[5,8-12] Since outcome measures sensitive to change in fatigability are lacking, their development is a pivotal step in a better understandig of fatigabillity in SMA.[13,14] This study aimed to provide the framework for the development of novel clinical outcome measures for fatigability in patients with SMA type across the range of severity. We determined content validity following the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) -guidelines and recommendations by European and American regulatory authorities.[15,16]

## Methods

A set of outcome measures for fatigability was developed according to five methodological steps as recommended by COSMIN (table 1).[17,18] In this iterative process data from multiple sources were triangulated. Sources included a scoping review of scientific literature, input from a scientific

and clinical multidisciplinary expert panel and three pilot studies including healthy persons (N=9), paediatric patients with chronic disorders (N=10) and patients with SMA (N=15). The expert panel consisted of ten clinicians and researchers including paediatric physical therapists (BB, MS), clinical exercise physiologists and movement scientists (JG, LH, HH,TT) and neurologists or neurology residents with ample experience in caring for children and adults with SMA (MS, CW, RW, WP). Three round table discussions took place with different group compositions.

**Table 1.** Methodological steps COSMIN*

| Methodological steps | Questions to be answered | Sources |
|---|---|---|
| Step 1:<br>Definition and elaboration of the construct | 1) Definition of fatigability?<br>2) Target population?<br>3) Purpose of the outcome measure? | • Key papers on fatigability assessment |
| Step 2:<br>Choice of measurement method | 1) Existing measurement that responds closely to construct to be measured?<br>2) Level of measurement?<br>3) Single or multiple measures? | • Scoping review of scientific literature<br>• Expert panel (round table discussion 1) |
| Step 3:<br>Selecting and formulating items | 1) Which activities cause most problems?<br>2) Which available measures reflect these activities? | • Patient report outcome (pilot sample 3)<br>• Expert panel (round table discussion 2) |
| Step 4:<br>Scoring issues | 1) Application in research or clinical practice<br>2) Measurement level? | • Expert panel (round table discussion 3) |
| Step 5:<br>Pilot testing | 1) Comprehensibility?<br>2) Feasibility?<br>3) Relevance? | • Pilot sample 1 (healthy subjects)<br>• Pilot sample 2 (pediatric patients with chronic diseases)<br>• Pilot sample 3 (patients with SMA) |

*COSMIN = 'COnsensus-based Standards for the selection of health Measurement Instruments

## Definition and elaboration of the construct intended to be measured

The first step in the development of a new outcome measure consisted of the operationalization of the theoretical construct in SMA. This included a clear definition of fatigability, a description of the target population and the purpose of the outcome measure and the composition of specific test criteria. The taxonomy for fatigue and fatigability as proposed by Kluger et al was used as a starting point from which a construct for fatigability assessment

in SMA was described. Fatigability was defined as the magnitude or rate of change in a performance criterion relative to a reference over a given time of task performance or measure of mechanical output.[7] Several other key papers on fatigability that used a similar definition and described test methodology were selected to complement the framework.[19-25]



## Choice of measurement method

During the second step we combined the results from a scoping review on available measures for fatigability in patients with SMA with the experiences with fatigability testing by our research group.

### *Scoping review of the literature*

Given the fact that SMA has been associated with fatigability only recently, it was anticipated that a systematic review would not generate significantly more information than a scoping literature search. Peer-reviewed experimental articles written in English were retrieved from Pubmed and Trial.gov up to the first of October 2014. The following search strings was used: *((“muscular atrophy, spinal”[MeSH Terms] OR (“muscular”[All Fields] AND “atrophy”[All Fields] AND “spinal”[All Fields]) OR “spinal muscular atrophy”[All Fields] OR (“spinal”[All Fields] AND “muscular”[All Fields] AND “atrophy”[All Fields])) OR (“Stat Methods Appt”[Journal] OR “sma”[All Fields])) AND (((Fatigability[All Fields] OR Endurance[All Fields]) OR Stamina[All Fields]) OR (“fatigue”[MeSH Terms] OR “fatigue”[All Fields])).* At first, papers were selected that described the measurement of fatigability or endurance in patients with SMA. Secondly, outcome measures were assessed to what extent they complied with the definition and test criteria defined within this study. In the case that no suitable outcome measure were retrieved, the expert panel discussed in the first round table discussion whether other appropriate outcome measure were available that met the clinimetric requirements and could be validated for SMA.

## Selecting and formulating items

During the third step, questionnaires were taken from the pilot sample of patients with SMA to determine which activities of daily living (ADLs) provoked fatigability. In adults, the questionnaire by Straver et al. was used which was originally validated for peripheral nervous system disorders.[26] A similar questionnaire was developed for children based on clinical experience from the expert panel and items from the Child Health Assessment Questionnaire, a validated questionnaire for ADLs in other clinical populations.[27] Patient-reported activities that caused fatigability were clustered into three different

functional domains, namely leg function, upper arm function and hand function. The expert panel assessed in the second round table discussion whether all domains were relevant to SMA and should be included in the development of the set of outcome measures for fatigability.

## Scoring issues

During the fourth step, the expert panel discussed about the composition of the tests, taking into account the application setting (research, clinical practise) and the patient group, and selecting primary outcome parameters. For example, tests are usually shorter in clinical practise, due to time constraints.[17]

## Pilot testing

Patients with SMA were recruited from the Dutch SMA registry(www.treatnmd.eu/patientregistries).[28] This registry contains detailed clinical information of over 300 children and adults with SMA. To minimize selection bias, all eligible patients listed in this register were offered the possibility to participate. All patients had a confirmed homozygous deletion of the *SMN1* gene or a heterozygous *SMN1* deletion in combination with a point mutation on the second *SMN1* allele. In order to be eligible to participate in this study, a subject had to meet all of the following additional criteria: age 8-60 years; ability to follow test instructions and no exercise restrictions. Two patients with SMA declined participation due to frequent hospital visits in the recent past and fear of increased fatigue. Patient controls were recruited from a school for special education in Utrecht. Healthy controls were recruited from the University of Applied Sciences and the University Medical Center Utrecht. The outcome measures for fatigability were pilot-tested on *'comprehensibility'* ('Are test instructions to participants unambiguous and well understood?') and *'feasibility'* (measurement completion, acceptability and perceived burden) in three consecutive pilot samples of healthy controls (pilot sample 1), paediatric patients with chronic diseases (pilot sample 2) and patients with SMA (pilot sample 3). 'Measurement completion rate' was defined as the number of participants able to complete the test without premature discontinuation caused by motivational issues or a-specific physical complaints.[17] 'Acceptability' was defined as the willingness to perform the test again in the future and was assessed with a 'Visual Analogue Scale'.[29] Perceived burden was assessed with the OMNI scale for perceived exertion.[30] The third round table discussion was used to discuss pilot data and if necessary to make small adjustments to the protocol.

# Results

## Definition and elaboration of the construct intended to be measured

Fatigability is subdivided in ´physical fatigability' and 'cognitive fatigability' which are measured in different ways.[7,21,24,31] 'Physical fatigability is primarily measured by quantifying the decline in one or more aspects of motor performance such as peak force, power, speed and accuracy while cognitive fatigability is measured by quantifying the decline in processing speed and sustained attention over time during a sustained complex information processing task. Given the fact that patients with SMA complain about sustaining physical activities, we decided to focus on physical fatigability defined as a decline in performance such as peak force, power, speed and accuracy. A number of methods have been described to measure fatigability during different types of performances including:

1) Continuous performance of a prolonged task:[19,21]
   a) Intermitted submaximal exercise protocol which mimics activities such as walking or cycling in which fatigability develops over a longer period
   b) Continuous maximal protocol which mimics activities such as lifting heavy objects or sprinting
2) Comparing performance on a probe task before and immediately after prolonged performance of a separate fatigue inducing task.[32]

Fatigability is experienced by patients with SMA as the inability to perform prolonged repetitive tasks during activities of daily life. These complaints are reminiscent of those of patients with myasthenic syndromes, which are caused by reduced efficiency of neuromuscular junction.[33] Moreover, SMA is characterised by structural and physiological abnormalities of the neuromuscular junction as shown by post-mortem studies and the presence of pathological decrement upon repetitive nerve stimulation supporting the hypothesis that neuromuscular junction dysfunction is associated with fatigability in SMA and should be the focus of fatigability test development. The extent of fatigability may vary according to the method of testing.[22,24] Therefore, test protocols should be used that mimic daily life activities that provoke fatigability in patients. Consequently a set of predefined test-criteria were composed (table 2).

**Table 2.** test criteria for SMA and candidate outcome measures

| | **Methodology** | **Type** | **Protocol** | **Standardization** | **Intensity** | **Test duration** | **External regulation of pace** |
|---|---|---|---|---|---|---|---|
| Pre-defined test criteria | Generic applicable | Mimic daily life activities | Repetitive tasks | Yes | Submaximal | >75 sec* | yes |
| RNHPT | +/- | + | + | + | + | + | - |
| Sustained MVC during 60 seconds | - | - | - | + | - | + | - |
| Sustained MVC during 15 seconds | + | +/- | - | + | - | - | - |
| Masticatory function | - | - | - | + | - | - | - |
| 6MWT | - | + | + | +/- | + | + | - |
| ESWT | - | + | + | + | + | + | + |

RNHPT = Repeated Nine Hole Peg Test, MVC = Maximal Voluntary Contraction, 6MWT = 6 Minute Walk Test, ESWT = Endurance Shuttle Walk Test, Mn = Mean value, *Gastin et al. 2010 (64)

## Choice of measurement method

The scoping review search performed on the 1st of October 2014 retrieved 109 records in Pubmed and no additional records in trial.gov. All records were screened on title and abstract. Seven papers were included describing 4 different methods to assess fatigability in SMA (Appendix 1): Sustained maximal voluntary contraction for 60 seconds[34] , Sustained maximal voluntary contraction for 15 seconds[35], Masticatory endurance[36] and the Six Minute Walk Test (6MWT).[37-40] We recently reported our experience with the *repeated Nine Hole Peg Test* (rNHPT) as a measure for fatigability of arm and hand function in patients with SMA. Given the promising results, the r9HPT was included in the assessment of potential outcome measures derived from the review. Recently, this study was published.[41]

### *Evaluation of selected outcome measures*

All five different outcome measures from the methods above, defined fatigability as the decrease in physical performance, which was in accordance with the definition used in this study. There was however a large difference in methods of testing with regards to target muscles, type of exercise,

intensity and duration (table 2). The rNHPT and the 6MWT, both submaximal repetitive tasks, met most predefined criteria and provided proof of principle that including an endurance element holds promise as a mode to measure fatigability objectively in patients with SMA. The authors of the 6MWT and the rNHPT use similar methodology in which subjects are instructed to deliver maximal performance and change in velocity or distance is assessed as primary outcome measure. The simple instruction and relatively short test period (1.5-6 minutes) make them particularly useful to detect fatigability in the individual patient with SMA. There were, however, several intrinsic clinical properties of both tests which made them less appropriate to assess the construct of fatigability as defined in this study: The intensity was not standardized and might fluctuate between maximal and submaximal intensity within and between subjects depending on disease severity and motivation;[42,43] The change in velocity as primary outcome measure did not directly reflect the inability to sustain prolonged repetitive task during ADLs such as frequently reported by patients; Both tests did not cover the subgroup of non-ambulatory patients with antigravity function of the arms, who primarily experience problems with repetitively lifting the arm while drinking or eating. The expert panel discussed whether potential non-validated outcome measure for fatigability were available that were more standardized on performance and used meaningful outcome parameters for endurance capacity. The methodology of the Endurance Shuttle Walk Test (ESWT) was proposed by one of the experts with experience in chronic pulmonary disease.

### *The Endurance Shuttle Walk Test*

Revill et al. developed a externally controlled constant paced walking test to assess endurance capacity in patients with chronic obstructive pulmonary disease[44] (Appendix 1). The expert panel judged the methodology of ESWT as superior to all other outcome measures with regards to external regulation of pace, test duration and standardization of intensity. Since the ESWT could only be used in ambulatory patients, it was concluded that alternative outcome measures using the same methodology should be ideally used for endurance testing in non-ambulatory patients. Since no such outcome measure were available, it was decided to select existing scales that corresponded well with reported activities by patients and incorporate them in to the methodology of the ESWT.

## Selecting and formulating items

Patients with SMA reported a great number of different activities on the domains of leg function, upper arm function and hand function (table 3). The expert panel therefore decided that all domains should be included in the development of the set of outcome measures for fatigability. The ESWT was selected to cover the activities related to leg muscles. The upper arm domain mainly comprehended activities lifting an object while the hand function domain mainly included activities performed at the table while moving around the lower arm and hand. To cover activities of the upper arm and hand function, the expert panel decided to apply the methodology of the ESWT to the Nine Hole Peg Test and the Box and Block Test resulting in the Endurance Shuttle Nine Hole Peg test (ESNHPT) and the Endurance Shuttle Box and Block Test (ESBBT). The Nine Hole Peg Test, originally developed to assess distal arm function demonstrated good feasibility and sensitivity to detect fatigability in patients with SMA type 2.[41,45,46] The Box and Block Test, a measure for upper limb motor function, represented antigravity activities of the arms such as brushing teeth, eating a sandwich and lifting a cup.[47,48]

**Table 3.** Daily life activities provoking fatigability clustered per functional domain

| Leg function | Proximal arm function | Hand function |
|---|---|---|
| *Walking* | *Lifting a cup* | *Writing* |
| *Climbing stairs* | *Brushing teeth* | *Eating a sandwich* |
| *Cycling* | *Throwing a ball* | *Typing* |
| *Swimming* | *Fishing* | *Riding a power driven wheelchair* |
| *Showering* | *Holding phone to ear* | *Cutting (scissors)* |
| *Playing soccer* | *Washing hair* | *Drawing* |
| *Running* | *Carrying a bag* | *Painting* |
| *Putting clothes in the washing machine* | *Shoe polishing* | *Playstation* |
| | *Dish washing* | *Using cutlery* |
| | *Using cutlery* | *Driving car with mini joystick* |
| | *Showering* | *Moving things on the table* |
| | *Cooking* | *Using Mousepad* |
| | *Vacuum cleaning* | *Taking money out of wallet* |
| | *Washing clothes* | *Clapping hands* |
| | *Riding a hand driven wheelchair* | *Fixing screws* |
| | *Swimming* | *Putting on make-up* |
| | *Hanging clothes to dry* | *Moving objects on wheelchair table* |

## Scoring issues

In accordance with the original ESWT, Time to Limitation (Tlim (sec)) was chosen by the expert panel as the primary outcome measure of all three endurance tests (round table discussion 3). With the aim to eventually use the set of tests both in research and clinical practise time constraints in the latter had to be taken in account.[17] To improve both motivation for and feasibility of tests, maximum test duration was shortened from 20 minutes to 10 minutes. Based on clinical experience, it was expected that 10 minutes would be a sufficient time period to measure fatigability.

## Pilot testing

### *Pilot-test sample 1 and 2*

Eight healthy adults and one adolescent (mean age = 28.7, 50% female) performed all three endurance tests. Respectively 30% and 44% of the subjects could not continue at an intensity level of 85% for at least 10 minutes during the ESWT and ESBBT. Early termination was primarily caused by subtle coordinative errors due to the high velocity at which the motor task was performed. Therefore, intensity level of 85% was not considered valid for the assessment of fatigability in patients with SMA. Assessment at a 65% intensity level was considered too easy. It was therefore decided to set the intensity level at 75% for all tests. Consecutively a second pilot study was performed in 10 children with neuromuscular diseases and other motor disabilities (Developmental Coordination Disorder (N=1), Cerebral Palsy (N=2), SMA (N=2), Duchenne Muscular Dystrophy (N=2), Spina Bifida (N=1), Acquired Brain Injury (N=1) and Spinal Cord Injury (N=1)) to determine the feasibility of the endurance tests. All participants showed good comprehensibility and acceptability of the tests without any adverse events. Three children (SMA (n=2) , Spinal Cord Injury (n=1) demonstrated a decreased time to limitation.

### *Pilot-test sample 3*

Fifteen patients with SMA type 2 (n=8), type 3a (n=5) and type 3b (n=3) aged 10-49 and with a broad range in clinical severity (Hammersmith Functional Motor Scale Expanded score = 0-66) (table 4) performed 1,2 or 3 of the endurance shuttle tests (i.e. ESNHPT, ESBBT, ESWT) tests depending on their level of motor function. The comprehensibility, acceptability and measurement completion of all three tests were excellent despite moderate to severe self-reported muscle fatigue. All subjects were strongly motivated to perform well on the test and willing to do the test again in the context of future studies. Beforehand, it was expected that at least 50% of the subjects would end the

test prematurely because of fatigability. Although most subjects did show signs of fatigability at the end of the test reflected by decrease in coordination, compensatory movements and perceived exertion, the drop –out rate was lower than expected on the ESNHPT (31%), ESBBT (45%) and ESWT (50%). The ESWT showed a trend towards ceiling effect (Tlim (Mn) = 462/600 seconds). It was observed that during the ESBBT subjects were actively compensating for fatigability by leaning on the box.

**Table 4.** pilot sample 3

| | **ESWT** | **ESBBT** | **ESNHPT** |
|---|---|---|---|
| Sample size | 4 | 9 | 13 |
| SMA type | | | |
| 2 | 0 | 3 | 6 |
| 3a | 1 | 3 | 4 |
| 3b | 3 | 3 | 3 |
| Age | | | |
| yrs (min.-max.) | 26.2 (10-37) | 20.8 (10-37) | 23.9 (10-49) |
| Gender | | | |
| Male | 2 | 6 | 8 |
| Female | 1 | 3 | 5 |
| HFMSE | | | |
| 0-66 | 52 (44-66) | 31 (4-66) | 22 (1-66) |
| Time to Limitation | | | |
| 0-600 | 555 (462-600) | 373 (83-600) | 457 (52-600) |
| Reduced time to limitation | | | |
| Yes | 50% | 44,4% | 30,8% |
| No | 50% | 55,6% | 69,2% |
| Measurement completion | | | |
| Yes | 100% | 100% | 100% |
| No | 0% | 0% | 0% |
| Comprehensibility | | | |
| Yes | 100% | 100% | 100% |
| No | 0% | 0% | 0% |
| Acceptability | | | |
| 0-10 (min. – max.) | 9.2 (7.4-10) | 9.6 (7.9-10) | 8.9 (4.9-10) |
| Perceived burden | | | |
| Muscle fatigue | 7 (6-9) | 4.9 (3-9) | 4.5 (1-10) |

ESWT = Endurance Shuttle Walk Test, ESBBT = Endurance Shuttle Box and Block Test, ESNHPT = Endurance Shuttle Nine Hole Peg Test

### The Endurance Shuttle Tests: materials and procedures

To improve the validity of the tests, the protocol was modified on two important aspects. First, the maximal test duration was lengthened to 20 minutes for all tests (in accordance with the original ESWT procedure). Second, we decided that compensatory movements (e.g. leaning on the box during the performance of the box and block test) was no longer allowed. Materials and procedures for the set of endurance tests were described (Appendix 2)



## Discussion

This study aimed to provide the framework for the development of novel clinical outcome measures for fatigability in patients with SMA across the range of severity. The major strength of this study includes the use of the methodological steps as recommended by the COSMIN guidelines to systematically develop a set of endurance tests for patients with SMA with a specific emphasis on content validity.[17] Content validity is the degree to which the content of an instrument is an adequate reflection of the construct to be measured and without it, it is difficult to select appropriate outcome measures for trials or other types of interventions.[49] It is therefore recommended by the US Food and Drug Administration and the European Medicines Agency to establish content validity before evaluating other measurement properties.[15,16] The content validity of the endurance shuttle tests was established by combining evidence from scientific literature with patient reported outcome and the expertise from health care professionals and scientists, which will potentially lead to both valid and clinically meaningful outcome measures.

An important aim of this study was to develop one methodology for a broad clinical spectrum that would enable comparison between severely and mildly affected patients and with that facilitate future study trial inclusion. The methodology of the ESWT, originally validated for pulmonary disease was adjusted and applied to other motor tasks to meet with the specific disease characteristics of SMA. The ESWT speed was originally derived from a time consuming four component process including a second ISWT and a regression equation including maximal predicted oxygen uptake. Although Hill et al. simplified this method by directly using maximal walking speed it still included a second exercise test.[50] We questioned the validity of this method because of the risk of inducing fatigability prior to the test and therefore decided to use muscle power as the parameter to determine exercise intensity in SMA. Time in which 10 meter, 9 pegs or 10 blocks could be transferred were taken

as maximal performance measure. It was decided not to adjust for the weight of the blocks and pegs or body weight, since both materials were very light and body weight is fixed in daily life activities as well. The convergent validity of this modified method with the original procedures and the comparability between patients with mild and severe muscle weakness need to be further analysed in future studies.

We decided to include motor tasks because we wanted to generate clinical relevant outcome measures and patients with SMA generally have normal coordinative function. The use of motor task within endurance tests potentially causes validity issues. For example, a subject might drop out because of motor coordination difficulties rather than fatigability. To confirm construct validity, it will be important to monitor other parameters of fatigability such as perceived exertion, motor behaviour and change in strength and electromyography response.[38,40,51,52]

Besides the clinimetric properties, the practical application of a new measurement test is an important aspect in the development of outcome measures for clinical practice. Ideally, an outcome measure is suitable for both day-to-day clinic purposes and clinical trials. For this purpose, an instrument needs to be easy to use in a limited time period, acceptable and feasible for the individual subject, while at the same time, applicable to a large part of the study population. The endurance tests have demonstrated to be comprehensible and acceptable for both healthy subjects and patients with a wide range of severity in an age range of 10-49 years. Based on our clinical experience and an upcoming large study on validity and reliability (Bartels et al. in progress), we expect the endurance tests to be suitable for subjects aged 6 years and older for those being able to move around their dominant hand on their wheelchair table as minimal motor function. The additional burden and time consumption in the context of endurance tests as part of the already extensive trial assessments asks for a clear rationale about the efficacy in terms of function accompanied by the selection of the most appropriate tests. In order to be able to measure clinically relevant improvement in endurance, endurance tests that mimic long-term activities are required.

In the current literature, the concept of fatigability and fatigue are often used interchangeably with different terms such as fatigue,[25] fatigability[7], neuromuscular fatigue,[53] perceived fatigability,[54] physiological fatigue,[55] physiological fatigability[19], physical fatigue,[20] peripheral fatigue,[22] muscle fatigue[21,56,57] and so on. The lack of standard definitions and the inconsistency of terminology hamper the advancement in our understanding of the pathophysiological background of

fatigability in SMA and the development of appropriate outcome measures. The taxonomy used in this study was particularly suitable to standardize definitions and clarify the different concepts and means of measurements as a prelude to the development of an outcome measure for fatigability in SMA. The taxonomy makes an important distinction between ´perception´ and ´performance´, which are measured at a different level. Perception of fatigue is defined as the subjective sensations of weariness, increasing sense of effort, mismatch between effort expended and actual performance or exhaustion while fatigability is about decline in either physical or mental performance. Although there is a clear distinction in definitions and means of measure, endurance performance is regulated by an interaction between fatigability and perceptions of fatigue and influenced by physchological factors, peripheral limitations and central factors.[6,58-60] Therefore, a psychophsyiological approach is needed when interpreting the outcome of endurance testing in patients with SMA. Based on both pre-clinical and clinical data it was hypothesised that fatigability would be associated with neuromuscular junction dysfunction in at least half of the patients with SMA and therefore, similar to the approach in myasthenic syndromes, best provoked with a repetitive submaximal prolonged motor task. Although Spinal Muscular Atrophy is primary characterised by loss of motor neurons, involvement of other systems such as autonomic dysfunction and altered muscle metabolism is reported and might demand additional methods of testing to capture fatigability in SMA.[61,62] Fatigability in SMA could also be related to an increased energy cost of movement due to progressive muscle weakness and secondary deconditioning.[20,23] Therefore, the individual disease course and physical activity levels should be taken in account when measuring fatigability and its change in time.

We decided to limit our search for existing outcome measures to a scoping review in SMA although a systematic review in the entire range of neuromuscular diseases could have generated other endurance measures. Based on experience by the expert panel and the specific characteristics of SMA regarding clinical variability and complaints of fatigability, it was anticipated that a time consuming systematic review would give very limited additional information as hardly any endurance testing has been developed in neuromuscular diseases. The involvement of patients in rare disease clinical trial design is increasingly becoming a priority.[63] Although established methods such as face-to-face meeting and focus groups were not applied yet, extensive questionnaires provided a valuable insight in the patient perspective on fatigability. In the further development of the endurance tests, patients will continue to play an important role.

## Conclusions

Fatigability has emerged as an important dimension of physical impairment in patients with SMA. The development of a comprehensive set of endurance tests is a pivotal next step to facilitate intervention studies on fatigability and address this important complaint in patients with SMA. We developed a set of endurance tests for both non-ambulatory and ambulatory children and adults with SMA which meet predefined specific criteria to achieve three main objectives: 1) quantify endurance; 2) generate clinical relevant outcome parameters and; 3) cover a large part of the clinical spectrum of SMA. Reliability and construct validity need to be investigated in future studies.

## List of abbreviations

| | |
|---|---|
| 6MWT | Six Minute Walk Test |
| ADLs | Activities of Daily Living |
| COSMIN | COnsensus-based Standards for the selection of health Measurement INstruments |
| ESBBT | Endurance Shuttle Box and Block Test |
| ESWT | Endurance Shuttle Walk Test |
| ISWT | Incremental Shuttle Walk Test |
| r9HPT | repeated Nine Hole Peg Test |
| SMA | Spinal Muscular Atrophy |
| Tlim | Time to Limitation |

## Declarations

### *Ethics approval and consent to participate*

The Medical Ethics Committee of the University Medical Centre Utrecht in the Netherlands approved the research protocol. Written informed consent was obtained from all subjects and their parents.

## Consent for publication

Written consent for publication was obtained from all subjects and their parents with regards to images used.

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# Appendix 1

## Description of outcome measures considered for selection

### Sustained maximal voluntary contraction for 60 seconds

Milner-Brown studied fatigability of knee extensors and ankle dorsiflexors in 15 patients with neurogenic muscle weakness including five patients with SMA aged 16-55 years and 20 healthy controls aged 18-55 years. Subjects were asked to exert maximum force against a electromechanical device incorporating a force transducer and maintain maximum effort for one minute. Fatigue Index (FI) was expressed as the percentage decrease in maximal force at the end of 60 seconds. The mean FI of both ankle dorsiflexion (50 % ± 15 versus 34% ± 13) and knee extensors (62% ± 17 versus 46% ± 15%) of patients was significantly greater than in controls ($p < 0.01$) and characterised by a steep decrease in performance by patients from 30 to 60 seconds.

### Sustained maximal voluntary contraction for 15 seconds

Iannacone et al. studied fatigability of knee flexors, knee extensors, elbow flexors and elbow extensors in 72 ambulatory and non- ambulatory patients with SMA aged 5-57 years and 24 healthy controls aged 5-32 years. Subjects were asked to push or pull against a fixed myometer as hard as possible and hold for 15 seconds, while given audio feedback. The maximal voluntary contraction times 15 seconds represented 100% of endurance or no fatigability. Endurance was expressed as the area under the curve for each maximal voluntary contraction. The authors found a large variability in endurance (AUC of 50-90%) with a similar response in patient and controls.

### Masticatory endurance

Granger et al. studied masticatory muscle endurance in 15 patients with juvenile onset SMA aged 6-20 years and 15 age- and sex-matched healthy controls. Subjects were asked to hold a 60% sub-maximum bite force level for as long as possible while being timed. Patients with SMA (11.1 seconds) fatigued faster than controls (17.9 seconds) ($p = 0.03$).

### The six minute walk test (6MWT)

Montes et al. studied fatigability during ambulation in patients with SMA type 3 aged 4-49 year in 4 separate studies. Subjects were instructed to walk as far as possible along a 25-m course during 6 minutes. Encouragements during

the test were standardized according to the American Thoracic Society (ATS) – guidelines. Distance walked each minute and time to complete each 25-m segment were recorded. Montes et al. found a range of 11-21% decrease in walking distance between the 6th and the 1st minute.



## The Repeated Nine Hole Peg Test (r9HPT)

Stam et al. studied fatigability of the arm and hand in fifty two patients aged 7-72 years with SMA type 2-4, 17 healthy aged 6-73 years and 29 disease controls aged 8-76 years. Subjects were asked to perform five consecutive rounds of the Nine-Hole Peg Test as fast as possible without a break [41]. The time required to complete each round was recorded and compared to the first round. Time needed to complete each round during the five-round task increased in 65% of patients with SMA type 2, 36% of type 3a, 22% of type 3b/4, 31% of disease controls and 6% of healthy controls. Patients with SMA type 2 performed the test significantly more slowly (+27%) than all other groups ($p<0.005$). This study was published recently and given the promising results, the r9HPT was included in the assessment of potential outcome measures

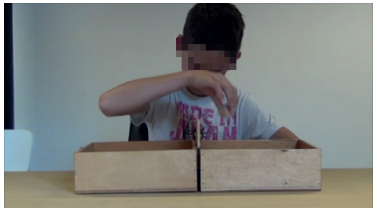
## The Endurance Shuttle Walk Test

Subjects walked at 85% intensity, derived from the walking speed at 85% of peak $VO_2$ uptake during a separate Incremental Shuttle Walk Test (ISWT). Subject were instructed to continue walking on a 10 meters shuttle course until too tired or breathless to continue with a cut off time of 20 minutes. Subjects were given no indication of how long they were walking and were not informed of the 20 minutes limit. Walking speed was externally regulated by a beep signal and the test was terminated prematurely when subjects failed two times in a row to reach the other side within time. The ESWT demonstrated good test-retest reliability and sensitivity to change after a seven week rehabilitation program.

# Appendix 2

## The Endurance Shuttle Tests: materials and procedures

*Description of the materials and procedures needed to perform the endurance tests*

| | ESWT | ESBBT | ESNHPT |
|---|---|---|---|
| |  |  |  |
| **Test Material** | • Straight corridor<br>• 10 meter walking course<br>• 4 cones<br>• Metronome | • Box and Block Test<br>• 200 blocks<br>• Adjustable table<br>• Metronome | • Nine Hole Peg Test<br>• Adjustable table<br>• Metronome |
| **Maximal performance estimation** | • Walk as fast as possible and turn at the line between the cones before the beep<br>• 5-10 trials, 30 seconds breaks<br>• Fastest time out of three attempts < 10% difference | • Transfer 10 blocks over the partition as fast as possible before the beep<br>• 5-10 trials, 30 seconds breaks<br>• Fastest time out of three attempts < 10% difference | • Place and return the nine pegs as fast as possible before the beep<br>• 5-10 trials, 30 seconds breaks<br>• Fastest time out of three attempts < 10% difference |
| **Intensity level** | • 75% individual intensity (s) = maximal time(s)/0.75 and then converted into the matching metronome number | • 75% individual intensity (s) = maximal time(s)/0.75 and then converted into the matching metronome number | • 75% individual intensity (s) = maximal time(s)/0.75 and then converted into the matching metronome number |
| **Maximal duration** | • 20 minutes | • 20 minutes | • 20 minutes |
| **Instruction Assessor** | • Cover each time 10 meters before the beep<br>• Continue as long as possible within safety margins<br>• Try to speed up in case of one failure | • Transport each time 10 blocks before the beep<br>• Continue as long as possible<br>• Try to speed up in case of one failure | • Place and return 9 pegs each time before the beep<br>• Continue as long as possible<br>• Try to speed up in case of one failure |

Chapter 3



# Fatigability in Spinal Muscular Atrophy: Validity and reliability of Endurance ShuttleTests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## Abstract

### Objective

To determine construct validity and test-retest reliability of Endurance Shuttle Tests as outcome measures for fatigability of remaining motor functions in children and adults with Spinal Muscular Atrophy (SMA) across the severity spectrum

### Methods

We assessed the Endurance Shuttle - Nine Hole Peg Test (ESNHPT), - Box and Block Test (ESBBT) and – Walk Test (ESWT) in 61 patients with SMA types 2-4, 25 healthy controls (HC) and 15 disease controls (DC). Convergent validity, discriminative validity and test-retest reliability were investigated. Additionally, we compiled the Endurance Shuttle Combined Score (ESTC) by selecting the most relevant endurance test of each individual.

### Results

54%, 70% and 73% of patients with SMA demonstrated increased fatigability on the ESNHPT, ESBBT and the ESWT. Endurance response in SMA was characterized by a decrease in muscle strength, an increase in muscle fatigue and an increase in motor adaptions, thereby confirming convergent validity. Patients with SMA showed increased drop-out rates and a shorter endurance time compared to HC and DC demonstrating good discriminative validity. Test-retest reliability was moderate to excellent (ICC's ranging from .78 to .91) with a trend towards better performance on retest. The ESTCS increased sample size and drop-out rate up to 100% and 85%.

### Conclusions

Fatigability is an important additional dimension of physical impairments across the severity spectrum in children and adults with SMA. The EST's are reliable and valid to document fatigability of walking, proximal- and distal arm function in SMA and thus are promising outcome measures for use in clinical trials.

## Introduction

Hereditary proximal Spinal Muscular Atrophy (SMA) is a severe neuromuscular disorder with predominantly infantile or childhood onset and is caused by deficiency of the survival motor neuron (*SMN*) protein due to loss of function of the *SMN1* gene[1]. SMA is characterised by progressive loss of muscle strength and motor function with a large clinical variety ranging from severe hypotonia in the first months of life (type 1), stalled gross motor development but the ability to sit without support (type 2), difficulties with or the loss of ambulation later in life (type 3) to relatively mild impairments in adulthood (type 4)[2-5]. Fatigability, defined as the inability to sustain repetitive physical activities, is increasingly being recognized as an important additional dimension of physical impairments and a target for therapeutic interventions[6-9]. Research into the effect of both SMN-augmenting treatment strategies and pharmacological compounds specifically targeting skeletal muscle on fatigability is hampered by the lack of sensitive and clinically relevant outcome measures for the assessment of fatigability[10-13]. Therefore, we recently established content validity and feasibility of the Endurance Shuttle Tests[7, 14, 15]. The *primary* objective of this study was to determine construct validity and reliability of the Endurance Shuttle - Walk Test, - Box and Block Test and – Nine Hole Peg Test as outcome measures for fatigability of walking, proximal- and distal arm function in SMA types 2-4. The *second* objective was to compile and evaluate the Endurance Shuttle Combined score to increase sensitivity and provide one single outcome measure for a broad range of phenotypes.

## Methods

### Subjects

Patients with SMA type 2, 3a, 3b and 4 were recruited from the Dutch national SMA registry (ww.treatnmd.eu/patientregistries)[2, 16]. To minimize selection bias, all eligible patients from a total of more than 300 enrolled in this register were invited to participate. All patients had a confirmed homozygous deletion of the *SMN1* gene or a heterozygous *SMN1* deletion in combination with a disabling point mutation on the second *SMN1* allele. Disease controls with another (genetically) confirmed neuromuscular disease were recruited from the paediatric neuromuscular outpatient clinic at the University Medical Center Utrecht and from Rijndam Rehabilitation Center in Rotterdam, the Netherlands. Healthy controls were recruited from the HU University of

Applied Sciences, the University Medical Center Utrecht and through the subject's social network of family, friends and schoolmates. Inclusion criteria were an age between 8-60 years and the ability to follow test instructions. Subjects were excluded if they had a history of Myasthenia Gravis or another neuromuscular disorder known to cause fatigability or affect neuromuscular junction function, if they used drugs that change neuromuscular transmission, or if they had other medical problems that could interfere with the outcomes of the testing.

## Standard Protocol Approvals, Registrations, and Patient consents

The Medical Ethics Committee of the University Medical Centre Utrecht in the Netherlands approved the research protocol (NL48715.041,14). Written informed consent was obtained from all subjects and their parents if they were under 18 years old.

## Study design

The study consisted of three visits (V1,V2,V3) within approximately six weeks (table 1). At V1 we documented baseline characteristics and subjects practiced the endurance tests during one minute to reduce the learning effect on test-retest reliability. At V2 and V3, subjects performed respectively test 1 (test) and test 2 (retest) at home or at the exercise laboratory in our hospital (both under supervision), depending the subjects preference. There was at least one week resting period between V2 and V3.

**Table 1.** Study design

| | V1 | V2 | V3 |
|---|---|---|---|
| Baseline | | | |
| Demographics | X | | |
| Medical history | X | | |
| Muscle strength | X | | |
| Endurance Shuttle Tests | | | |
| Practice test | X | | |
| Endurance test 1 (test) | | X | |
| Endurance test 2 (re-test) | | | X |

## Muscle strength

We assessed muscle strength of 22 muscle groups on both sides using a slightly modified Medical Research Council (MRC) score (i.e. no distinction between MRC 0 and 1; in both cases we used a score of 1) and calculated the MRC sum score (Range: 44-220)[2]. We calculated a sub score for the upper limb strength using 11 muscle groups of the upper limb on both sides (22-110).



## Endurance Shuttle Tests

The Endurance Shuttle - Nine Hole Peg Test (ESNHPT), – Box and Block Test (ESBBT) and - Walk Test (ESWT) were performed according to standardized procedures as previously described[7]. In short, we instructed subjects to repeatedly place and return 9 pegs in 9 holes, move 10 blocks over a partition or walk 10 meters at 75% of their previously determined, individualized maximum speed. The individual rounds were paced by auditory signals. The test was ended when the subject was not able to keep up the pre-set pace during two consecutive shuttles or when the maximal duration of 20 minutes was reached (test completion). Subjects performed all tests they were physically capable of in a predetermined order starting with the ESNHPT followed by the ESBBT and the ESWT. Subjects recovered between tests for at least 30 minutes. Fourteen out of 25 (56%) HC performed tests for the duration of 10 (rather than 20) minutes. This test duration was chosen for the initial protocol but was later changed into 20 minutes to optimize outcome[7]. We corrected for differences in test duration during statistical analysis. For each performed Endurance Shuttle Test (EST), we documented two outcomes 'drop-out' (Yes/No) and 'time to limitation' (Tlim) (sec). Drop-out was defined as the inability to endure the maximum duration of 20 minutes. We also documented test acceptability, defined as the willingness to perform the endurance test again in the future using a visual analogue scale (VAS) with a range of 0-10[17].

## Fatigability parameters

We compared muscle strength, self-reported fatigue and motor adaptations before and directly after each EST. We determined the dominant side by documenting the hand that the subject used for writing or picking up a pen.

### *Changes in muscle strength*

For change in muscle strength, we performed quantitative hand held myometry (type CT 3001, C.I.T. Technics, Groningen) according standardized procedures to measure maximal voluntary contraction (MVC) of five muscle groups of the dominant arm (shoulder abduction, elbow flexion, wrist extension, hand grip

and pinch grip in subjects that performed the ESNHPT and ESBBT and of the dominant leg (hip flexion, hip abduction, knee extension, knee flexion and ankle dorsal flexion in subjects that performed the ESWT[18].

### *Self-reported fatigue*

Subjects reported on general and local muscle fatigue with the OMNI scale of perceived exertion (0-10)[19].

### *Motor adaptations*

We video-taped all patients during each EST to capture motor adaptations. Two assessors (BB, LH) independently compared four different aspects of performance of the first two and last two rounds of each EST: the disability to use different parts of the body together smooth and efficiently; increase in compensatory movements (i.e. movements used habitually to achieve functional motor skills when a normal movement pattern has not been established or is unavailable); increase in synkinesis (e.g. non-functional involuntary movement of muscles or limbs accompanying a voluntary movement) and decrease of the ability to move against gravity[20, 21]. 'Motor adaption' was assumed when at least one aspect was scored as abnormal and 'no motor adaptation' when all aspects were normal. The assessors resolved any disagreements through discussion.

## Statistical Analysis

### Construct validity

Construct validity refers to the degree to which the scores of an instrument are consistent with predefined hypotheses regarding relationships to scores of other instruments (convergent validity) or differences among relevant groups (discriminative validity)[15].

### *Convergent validity*

To determine convergent validity, we used a linear mixed model (LMM) to assess muscle strength and self-reported fatigue in SMA while accounting for within-subject clustering with a random intercept. Time (0 and 1) was added to the model as fixed effect. Subsequently, we added 'drop-out´ and the interaction between ´time´ and ´drop-out´ as fixed effects to determine the effect of drop-out on muscle strength and self-reported fatigue. The association between drop-out and motor adaptations was studied with

Pearsons Chi Square and Fisher's exact test. We hypothesized that subjects with SMA would demonstrate a lower muscle strength, higher self-reported fatigue and more motor adaptations directly after the EST compared to before.

### *Discriminative validity*



We used the log-rank test to study whether the ESWT and ESBBT could discriminate between SMA and HC and the ESNHPT between SMA, HC and DC. Event probabilities were estimated using Kaplan Meyer estimates. Group differences in age (between SMA, HC and DC) and muscle strength (between SMA and DC) were tested with Mann-Whitney U test. We hypothesized that patients with SMA would demonstrate increased drop-out rates and shorter endurance time compared to HC and DC.

## Reliability

For test-retest reliability, we calculated the two-way mixed intra-class correlation coefficients (ICC), type consistency. We defined ICC's as 'excellent' if the lower bound of the 95% CI > 0.80, 'high' if it ranged between 0.7 – 0.8, and 'moderate' if it ranged between 0.5 – 0.7[22]. For agreement between test completion of test 1 and test 2, we calculated Cohen's kappa considering a kappa of 40-60 % as moderate, 60-80% as substantial and > 80% as excellent agreement[22]. Due to repeated measurements of the time-to-event outcome (i.e. trial 1 and 2), we used a linear mixed Cox model with a Gaussian distribution to account for intra-individual clustering [23]. The linear mixed Cox model estimated the effect of retest (i.e. trial 2) on the probability of dropout and is expressed as hazard ratio. As visual illustration of test – retest effect on the dropout probability, we modeled the first test (i.e. trial 1) using a parametric Weibull model. Subsequently, we reduced the estimated Weibull hazard rate with the hazard ratio from the linear mixed Cox model.

## The Endurance Shuttle Test Combined Score (ESTCS)

We compiled the ESTCS based on the scores of the separate EST's. Patients performed, depending on their physical capability, either one (ESNHPT), two (ESNHPT, ESBBT) or all three (ESNHPT, ESBBT, ESWT) endurance tests. To compare between the most relevant endurance test of each individual, we selected the EST that corresponded with the highest level of motor function for each patient. Therefore, the ESNHPT was selected for patients with only hand- and forearm function, the ESBBT for non-ambulatory subjects who could lift their arm against gravity and the ESWT was selected for patients who could walk. For each selected EST, we documented two outcomes i.e.

'Drop-out (Yes/No) and 'Time to limitation' (Tlim) (sec). The final combined outcome was adjusted for test type. We checked for normality of residuals and model assumptions. All statistical analyses were performed using SPSS for Windows (version 24.0, SPSS Inc, Chicago, Ill) and R for windows (package coxme version 2-2.10, Terry M. Therneau (2018).

### Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

## Results

### Subject characteristics

Sixty-one patients with SMA, 25 healthy controls and 15 disease controls completed the study (table 2). Three participants were excluded due to perceived burden (after V1: SMA; N=1 ), personal circumstances (after V2: HC; N=1) and an injury not related to the study (after V2: DC; N=1). The ESNHPT, ESBBT and ESWTT were all well accepted by patients with SMA (9.0 (1.6), 8.9 (1.5), 9.1 (1.1)) and HC (9.0 (1), 9.2 (1), 9.3 (1)), respectively. The ESNHPT was moderately accepted by DC (5.8 (2.9). Both SMA and DC demonstrated a large variation in levels of muscle strength and ambulation. Patients with SMA who performed the ESNHPT were significantly older than DC ($p = .001$). General muscle strength and upper limb strength were not significantly different between SMA and DC ( $p = 0.6$, $p = 0.7$).

### Construct validity and Reliability

In this section we will describe outcomes of validity and reliability per separate EST and for the ESTCS.

#### *Endurance Shuttle Tests*

#### *ESNHPT*

We observed an increase in general fatigue and local muscle fatigue of the upper arm, lower arm and hand after the test in patients with SMA (table 3). We did not find a decrease in muscle strength. Motor adaptation occurred more frequently in patients with SMA with drop-out (p=.000). Drop-out was significantly higher in SMA compared to HC and DC (p=.000) (Figure 1A). Drop-out was different between SMA type 2, type 3a and type 3b-4 (p=.001) (Figure 1B). The test-retest reliability was moderate (table 4). Agreement on

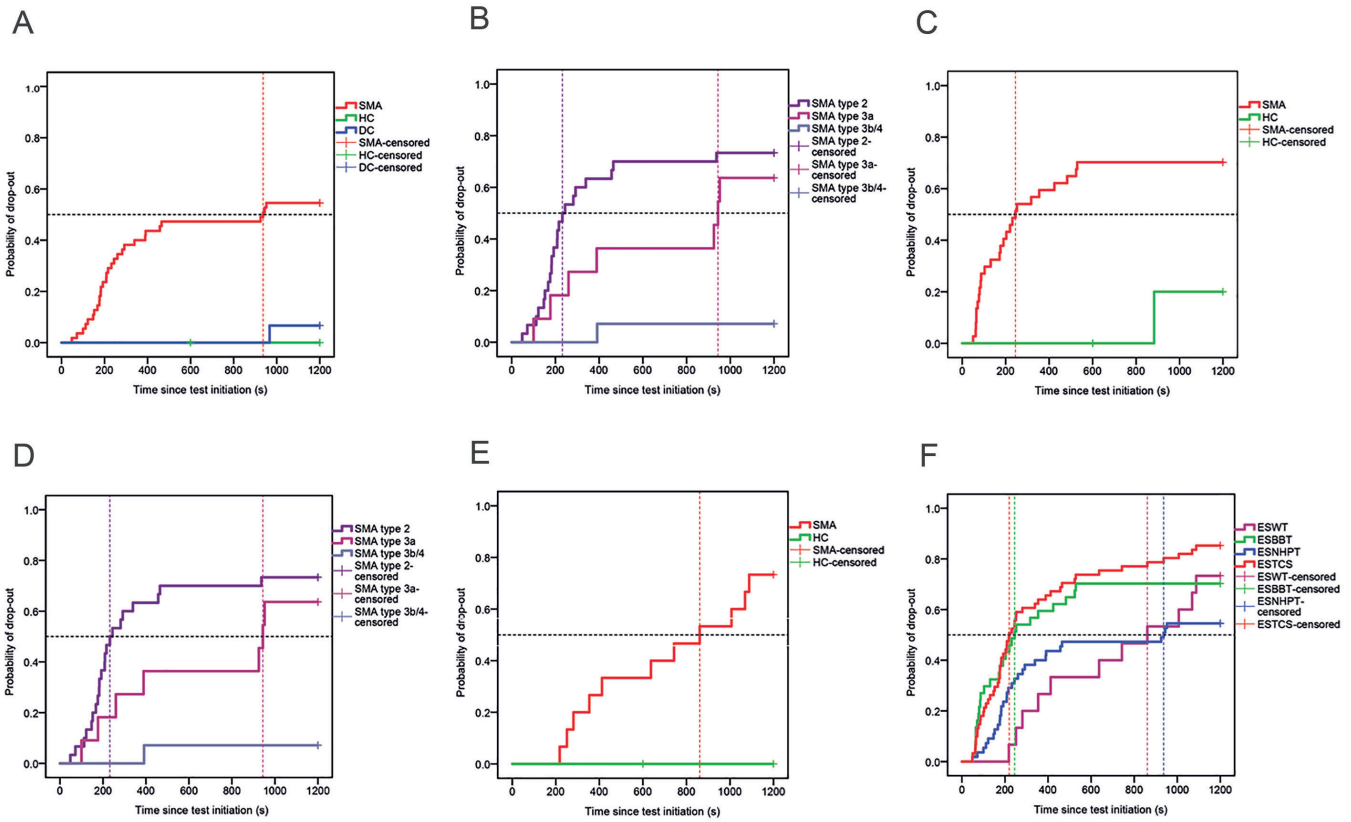


**Figure 1A-E. Kaplan-Meier curves of the Endurance Shuttle Tests**

Probability of drop-out since test initiation on the Endurance Shuttle Nine Hole Peg Test (ESNHPT) (A-B), Endurance Shuttle Box and Block Test (ESBBT) (C-D), Endurance Shuttle Walk Test (ESWT) (E) and a composite figure of all separate Endurance Shuttle Tests and the Endurance Shuttle Test Combined Score (ESTCS) (F). SMA: all patients with SMA; HC: Healthy Controls and DC: Disease Controls. SMA sub groups: SMA type 2, SMA type 3a and SMA type 3b and 4. Subjects that completed the Endurance Shuttle Tests are censored. The intersection of the horizontal and vertical dashed lines depict the median time to drop-out.

**Table 2.** Demographics and Clinical Characteristics of Participants

| | SMA (N=61) | | | | HC (N=25) | | | PC (N=15) |
|---|---|---|---|---|---|---|---|---|
| | **ESWT (N=15)** | **ESBBT (N=37)** | **ESNHPT N=55)** | **ESTCS (N=61)** | **ESWT (N=20)** | **ESBBT (N=20)** | **ESNHPT (N=24)** | **ESNHPT (N=15)** |
| Subtype/ Diagnosis | 3a: N=3<br>3b: N=11<br>4: N=1 | 2: N=11<br>3a: N=10<br>3b: N=15<br>4: N=1 | 2: N=30<br>3a: N=11<br>3b: N=14 | 2: N=31<br>3a: N= 14<br>3b: N=15<br>4: N=1 | NA | NA | NA | LGMD:N=7<br>BMD:N=1<br>DMD:N=7 |
| Gender (F:M) | 4:11 | 17:20 | 34:21 | 34:27 | 12:8 | 14:6 | 15:9 | 3:12 |
| Age (years) | 28.4 (12.4) | 28.4 (14) | 28 (14.4) | 28.7 (14.3) | 21.8 (9.6) | 21.6 (7.9) | 21.1 (9.6) | 14.0 (3.6) |
| MRC-scale (44-220) | 191 (15) | 161 (32) | 134 (39) | 138 (39) | 220 (1) | 220 (1) | 220 (1) | 144 (44) |
| MRC-scale upper limb (22-110) | 98 (8) | 86 (14) | 75 (18) | 76 (17) | 110 | 110 | 110 | 79 (21) |
| Level of ambulation | NA: N=12<br>CA: N=2<br>HA: N=1 | NA: N=12<br>CA: N=2<br>HA: N=3<br>NFA: 1<br>NOA: N=19 | NA: N=9<br>CA: N=1<br>HA: N=2<br>NFA: 1<br>NA: N=42 | NA: N=12<br>CA: N=2<br>HA: N=3<br>NFA: N=1<br>NOA: N=43 | NA: N=20 | NA: N=20 | NA: N=24 | NA: N=4<br>CA: N=3<br>NOA: N=8 |

ESWT = Endurance Shuttle Walk Test; ESBBT = Endurance Shuttle Box and Block Test; ESNHPT = Endurance Shuttle Nine Hole Peg Test; ESCT = Endurance Shuttle Composite Test; SMA = Spinal Muscular Atrophy; Subtype 3a = clinical symptoms < 3yrs; Subtype 3b = clinical symptoms > 3 yrs; HC = Healthy Controls; DC = Disease Controls; MRC = Medical Research Council; LGMD = Limb Girdle Muscular Dystrophy; BMD = Becker Muscular Dystrophy; CMT = Charcot Marie Tooth. DMD = Duchenne Muscular Dystrophy. NA = Normal Ambulation, CA = Community Ambulation, HA = Household Ambulation, NFU = Non Functional Ambulation, NOA = Non Ambulation.

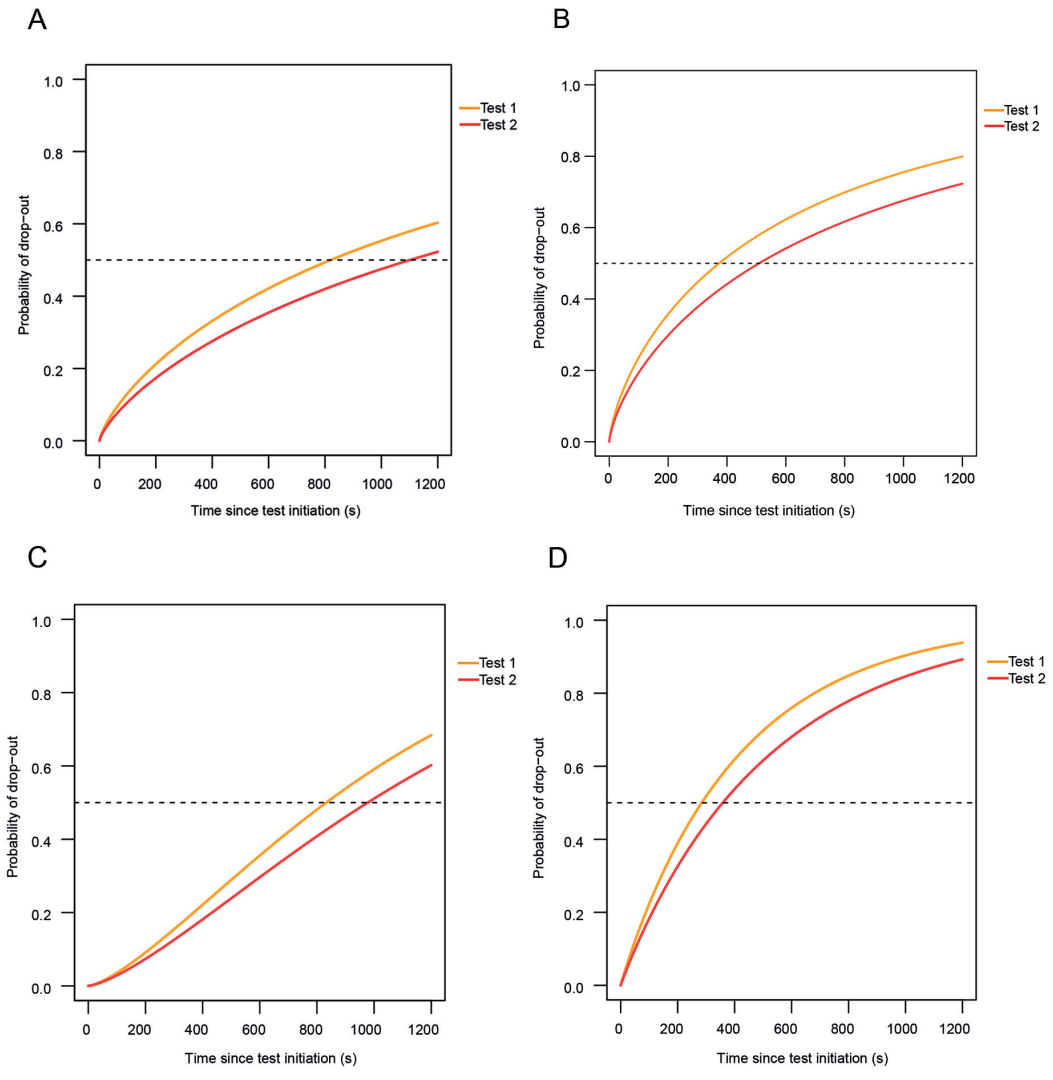


**Figure 2A-D. Illustration of test and re-test effect on the Endurance Shuttle Tests**
Parametric Weibull curves of the Endurance Shuttle Tests. Probability of drop-out since test initiation on the Endurance Shuttle Nine Hole Peg Test (A), Endurance Shuttle Box and Block test (B), Endurance Shuttle Walk Test (C) and Endurance Shuttle Test Combined Score (D) at test 1 (blue) and test 2 (red) (B) in patients with SMA. The horizontal dashed line depicts the median time to drop-out.

**Table 3.** Construct validity

| | **ESNHPT** | **ESBBT** | **ESWT** |
|---|---|---|---|
| Discriminative validity | SMA – HC - DC | SMA - HC | SMA - HC |
| Time to limitation (Mdn (s)) | SMA (N=55): 937 (48-1200)<br>HC P1 (N=15): 600<br>HC P2 (N=9): 1200<br>DC (N=15): 1200 (967 –1200) | SMA (N=37): 245 (50-1200)<br>HC P1 (N=15): 600<br>HC P2 (N=5): 1200 | SMA (N=15): 861 (218-1200)<br>HC P1 (N=12): 600<br>HC P2 (N= 8): 1200 |
| Drop-out (%) | SMA: 54.5%<br>HC: 0%<br>DC: 6.7%<br>SMA type 2: 73.3%<br>SMA type 3a: 63.6%<br>SMA type 3b: 7.1% | SMA: 70.3 %<br>HC: 5%<br>SMA type 2: 100%<br>SMA type 3a: 80%<br>SMA type 3b-4: 44% | SMA: 73.3%<br>HC: 0% |
| Convergent validity | SMA | SMA | SMA |
| Muscle strength (N) | No decrease | SA: -5.5 (2.1), p=.013 | KF: - 8.9 , p=.011 |
| Perceived fatigue (0-10) | G: +1.1 (0.2), p=.000<br>UA: +2.6 (0.3), p=.000<br>LA: +2.8 (0.4), p=.000<br>H: +2.4 (0.4), p=.000 | UA: +2.6 (0.4), p=.000)<br>LA: +2.1 (0.4), p=.000<br>H: +1.0 (0.3), p=.000 | G: + 3.7 ( 0.77), p=.000<br>UL: + 4.0 (.92), p=.001 |
| Motor adaptation (Yes) | No drop-out: 26%<br>Drop-out: 96% | No drop-out: 33%<br>Drop-out: 96% | No drop-out 75%<br>Drop-out: 100% |

ESWT = Endurance Shuttle Walk Test, ESBBT = Endurance Shuttle Box and Block Test, ESNHPT = Endurance Shuttle Nine Hole Peg Test, HC = Healthy Controls, DC = Disease Controls, P1 = protocol 600 seconds, P2 = protocol 1200 seconds, KF = Knee Flexion, SA = Shoulder Abduction, G = General, UL = Upper Limb, UA = Upper Arm, LA = Lower Arm, H = Hand.

test completion between test 1 and test 2 was substantial. We observed a trend towards better performance on retest but this was not significant (figure 2A).

### *ESBBT*

We observed a decrease in muscle strength of shoulder abduction and an increase in muscle fatigue of the upper arm, lower arm and hand after the test in patients with SMA (table 3). We didn't find a significant difference between patients with and without drop-out. Motor adaptation occurred more frequently in patients with SMA with drop-out (p=.000). Drop-out was significantly higher in SMA compared to HC (p=.000) (table 1C). Drop-out was different between SMA type 2, type 3a and type 3b-4 (p=.001) (Figure 1D). The test-retest reliability was high (table 4). Agreement on test completion between test 1 and test 2 was excellent. We observed a trend towards better performance on retest but this was not significant (Figure 2B).

### *ESWT*

We observed a decrease in muscle strength of knee flexion, an increase in general muscle fatigue and upper leg muscle fatigue, and an increase in motor adaptations after the test in patients with SMA (table 3). We didn't find a significant difference between patients with and without drop-out. Drop-out was significantly higher in SMA compared to HC (p = .000) (Figure 1E). The test-retest reliability was high and agreement on test completion between test-retest was excellent (table 4). We observed a trend towards better performance on retest but this was not significant (Figure 2C).

**Table 4.** Reliability

| Test-retest reliability | ESWT (N=15) | ESBBT (N=37) | ESNHPT (N=55) | ESTCS (N=61) |
|---|---|---|---|---|
| Endurance time (ICC) | .91 (.77 – .97) | .86 (.75-.93) | .78 (.66 - .87) | .71 (.57 - .81) |
| Test Completion (kappa) | .84 (.55 – 1.00) | .80 (.59 – 1.00) | .74 (.56 - .92) | .57 (.26 - .88) |
| Survival curves (HR) | .83 (.31 – 2.25), p=.071 | .80 (.43 – 1.5), p=.49 | .79 (.44 – 1.42), p=.44 | .76 (.49 – 1.18), p=.22 |

ESWT = Endurance Shuttle Walk Test, ESBBT = Endurance Shuttle Box and Block Test, ESNHPT = Endurance Shuttle Nine Hole Peg Test, ESTCS = Endurance Shuttle Test Combined Score, ICC = Intra Class Coefficient, HR = Hazard Ratio.

### Endurance Shuttle Test Composite Score

Drop-out (85%) was significantly higher and Time to limitation (220, 95% CI 174 – 266) significantly lower on the ESTCS compared to the separate EST's (p=.002) (Figure 1F). The test-retest reliability and agreement between test 1 and test 2 were moderate (table 4). We observed a trend towards better performance on retest but this was not significant (Figure 2D).

## Discussion

The primary objective of this study was to determine construct validity and reliability of the EST's in patients with SMA. Results of our study indicate good convergent validity of EST's to assess fatigability and good discriminative validity between patients with SMA, HC and DC. Even with similar muscle strength, higher frequency of drop-out and shorter endurance time in patients with SMA were present compared to disease controls. These results indicate that fatigability is an important dimension of physical impairment in SMA separate from muscle strength.

The high prevalence of fatigability we report in both mildly and severe affected patients with SMA is consistent with recent studies that reported increased fatigability in ambulatory patients with SMA type 3 using the 6-minute walk test (6MWT) and in type 2 patients with the repetitive Nine Hole Peg Test (r9HPT)[24, 25]. The 6MWT and the r9HPT however, do not cover the large severity spectrum of SMA and use different methodologies which make them difficult to compare. Therefore, we developed a set of endurance shuttle tests based on the same construct using the same methodology in patients with mild, moderate and severe motor impairments[7]. The ESNHPT showed increased sensitivity of approximately 64% to capture fatigability during fine motor tasks in patients with SMA type 3a compared to 36% using the r9HPT[26]. The ESBBT is the first validated and sensitive fatigability test for proximal arm function in SMA and may be complementary to outcome measures that focus on arm motor function such as the Revised Upper Limb Measure (RULM), by adding the dimension of endurance[27].

Few studies have addressed the prevalence of fatigability and the variability in endurance capacity between ambulatory patients[25, 28]. Our results show that most ambulatory patients do show fatigability during walking, but that the moment at which that occurs is highly variable. The fact that respectively over 80% of the patients with SMA were able to walk for more than 6 minutes at a constant walking speed during the ESWT, does suggest that the currently

used 6MWT might not be sensitive to capture fatigability in patients with moderately limited ambulatory capacity. The ESWT could be a good alternative to capture change in endurance in ambulatory patients.

The reliability of the EST's was good (ICC's .78 - .91) and similar to the r9HPT and 6MWT (ICC's .71 - .99)[26, 29]. Reliability of the ESNHPT was slightly lower than the ESBBT and ESWT which was explained primarily by a learning effect we observed in some videos. We did not detect a learning effect in a previous study on the value of the r9HPT to document fatigability in SMA, so we anticipated that a practice session of 1 minute would be sufficient to correct for motor learning[26]. Based on the findings in this study, a complete practice test of the entire duration of 20 minutes should be applied in the future.

Ideally, outcome measures can be used across the severity spectrum of SMA without large floor- and ceiling effects. These and previously published data of motor function and endurance suggest that current performance measures are not sensitive to capture possible changes at the extreme ends of the spectrum of physical abilities[26, 30]. A commonly used method to counteract this problem in functional scales, adding items to both ends of the hierarchical scale, is not applicable to exercise testing[27, 31, 32].

The second objective of this study was to develop a combined score that would allow comparison of patients with varying severity on their individual most relevant endurance test, thereby increasing sensitivity and circumventing subgroup analysis with less statistical power. The ESTCS increased sensitivity to detect fatigability and increased sample size compared to the ESNHPT (+31% , N= +6 ), the ESBBT (+15%, N= +24) and the ESWT (+12%, N= +46). At the same time, test-retest reliability of the ESTCS was slightly lower compared to the reliability of the individual EST's. This implies that in the choice between a separate EST and the ESTCS, the size and heterogeneity of the study sample and the degree of reliability and sensitivity that are necessary to demonstrate trial efficacy have to be taken in account.

An important strength of this study was the application of survival analysis to quantify fatigability in SMA which gave us the opportunity to include patients with severe fatigability that could only sustain the specific endurance test for a short amount of time. The alternative method that looks at change over time such as the 6MWT or repetitions such as the r9HPT might underestimate fatigability because patients that drop out early are often not included in the analysis. The use of hazard ratios is an innovative approach to test reliability and can be used to determine efficacy of clinical trials by calculating the difference with the hazard ratio of the treatment- versus placebo group. Longitudinal natural history studies and data from clinical trials are now

required to determine whether the EST's are sensitive to detect clinically meaningful changes over time.

We were not able to determine discriminative validity of the ESWT and the ESBBT between SMA and DC since few patients with Muscular Dystrophy we included were able to walk or lift their arms against gravity. Disease controls are generally hard to recruit and difficult to match with SMA on the severity and distribution of muscle weakness. Despite the limited number of DC's, we made a first step to explore differences in fatigability response between subjects with SMA and other neuromuscular diseases. The lower endurance time in patients with SMA compared to DC is in line with previous results using the repetitive nine hole peg test[24]. The available data suggest that the dramatic deterioration in muscle performance that we observed in many subjects with SMA, is not present to the same extent in disease controls even with similar muscle strength, but this needs further confirmation.

In conclusion, the Endurance Shuttle Tests are reliable and valid to assess fatigability in patients with SMA across the spectrum of disease severity. This makes them promising outcome measures for application in standard care and clinical trials in patients with SMA.

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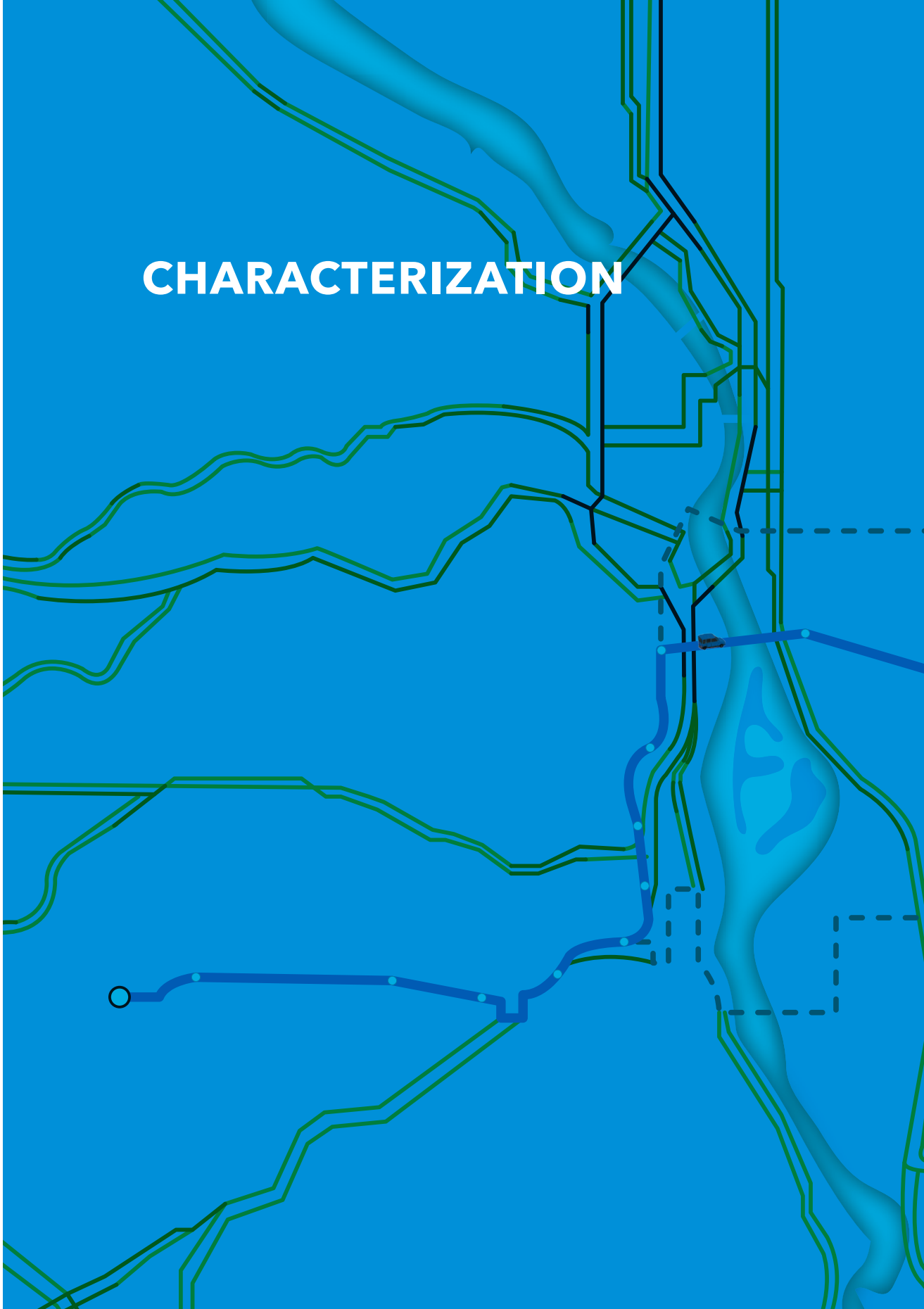
# CHARACTERIZATION



Chapter 4

# Motor unit recruitment reserve during fatiguing endurance performance in SMA

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*Submitted*

# Abstract

## Introduction

Fatigability in patients with hereditary proximal Spinal Muscular Atrophy (SMA) types 2-4 may be associated with low motor unit reserve capacity.

## Methods

Muscle activation during the Endurance Shuttle Tests (ESTs) was assessed with sEMG in 70 patients with SMA and 19 healthy controls. Motor unit reserve capacity was defined by a decrease in median frequency or increase in amplitude. Linear mixed effects models were used to determine exercise intensity and change in muscle activation over time.

## Results

Patients demonstrated increased fatigability compared to controls at submaximal but higher intensity levels compared to controls (upper extremities: 8-61%, lower extremities: 41-82%). Both patients with SMA types 2 and 3 and controls demonstrated motor unit reserve capacity in several muscle groups during all ESTs.

## Discussion

A subgroup of patients with SMA reveals motor unit reserve capacity during ESTs. The heterogeneity in muscle activation implies more than one underlying cause of fatigability.

## Introduction

Hereditary proximal Spinal Muscular Atrophy (SMA) is caused by a deficiency of survival motor neuron (SMN) protein due to the homozygous deletion of the *SMN* 1 gene[1]. SMA has a broad spectrum of severity ranging from neonatal respiratory insufficiency and death (type 1), inability to walk independently (type 2), problems with or loss of ambulation (type 3) to mild impairments in adults (type 4)[2]. Degeneration of α-motor neurons is the pathological hallmark of the disease, but other constituents of the motor unit such as the neuromuscular junction and the muscle are also affected[3–5]. SMA is characterized by progressive muscle weakness, limitations in motor function and reduced endurance during repetitive tasks[6–10].We recently developed and validated a panel of endurance tests (ESTs) for quantitative evaluation of endurance in patients with SMA types 2-4. We demonstrated increased fatigability in up to 85% of patients with SMA and in none of the healthy controls[11,12]. Disease controls showed significantly higher endurance levels, indicating that fatigability is not secondary to weakness but a specific feature in SMA. Dysfunction of the neuromuscular junction is a likely cause of fatigability in SMA but other mechanisms may also contribute[11,13].

Surface electromyography (sEMG) recordings from muscles during endurance testing may provide insight into the mechanisms underlying fatigability in SMA. Muscle activation, indicated by frequency changes and amplitudes of the EMG-signal can reliably be measured during both standardized voluntary contractions as well as during cyclic dynamic tasks[14–21]. Fatigability in healthy persons during dynamic tasks is characterized by a shift towards lower median frequencies due to slower muscle fiber conduction velocities and towards higher amplitudes due to increased firing rates, recruitment and synchronization of motor units, followed by a decrease[22–24]. This pattern is the reflection of the cumulative recruitment of larger motor neurons until maximal capacity, after which a decrease in amplitude occurs resulting in task failure[21]. It is unknown if the same pattern during execution of fatiguing motor tasks would be observed in patients with SMA.

Here, this matter was investigated. We conducted continuous sEMG recording from upper and lower extremity muscles during ESTs execution in patients with SMA types 2-4 and healthy controls. We tested two specific hypotheses: 1) patients with SMA types 2-4 would perform the ESTs at submaximal but higher levels of muscle activation compared to healthy controls and 2) patients with SMA are able to recruit motor unit reserve capacity during fatiguing motor tasks. Support for the latter hypothesis would

identify preserving, if not expanding this reserve capacity as a potential therapeutic target in clinical care for SMA types 2-4.

# Methods

## Subjects

Data was collected as part of a cross-sectional study on fatigability in Spinal Muscular Atrophy[11]. We invited patients with SMA type 2, 3a, 3b and 4 registered in the Dutch SMA registry (www.treatnmd.eu/patientregistries) to participate in this study. The SMA classification system (i.e. type 1-4) is based on the age of onset and the best of two achieved motor milestones. All had a confirmed homozygous deletion of the SMN1 gene. We recruited healthy controls through the HU University of Applied Sciences, the University Medical Center Utrecht and the subject's social network of family, friends and schoolmates. The inclusion criteria were: 1) age between 8 and 60 years and 2) the ability to follow test instructions. The exclusion criteria were: 1) history of a disorder which affects the neuromuscular junction (NMJ) function, 2) use of medication that affects NMJ function and 3) other medical problems that could influence endurance shuttle test results.

## Standard Protocol Approvals, Registrations and Patient Consents

All participants and their parents signed informed consent. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht in the Netherlands (NL48715.041.14).

## Study design

The study consisted of three visits within approximately 6 weeks. At the first visit we documented baseline characteristics and subjects performed a practice test[11]. At the second and third visit participants performed ESTs and retests at the participant's home or at the exercise laboratory in our hospital, depending the subject's preference. Visit two and three were separated by at least one week of rest[11].

## Endurance shuttle tests

We used three different validated endurance shuttle tests to assess fatigability: the Endurance Shuttle Nine Hole Peg Test (ESNHPT) for distal arm function; the endurance shuttle box and block test (ESBBT) for proximal arm function and the endurance shuttle walk test (ESWT) for leg function[6,11]. The execution of these

ESTs has been described in detail elsewhere[11]. In short, we first determined the individual's maximum test intensity level by asking him/her to perform one cycle of an endurance test at maximum speed (i.e. one cycle of putting nine pins in holes for the ESNHPT; of transporting 10 blocks from a bin over a partition into an adjacent bin for the ESBBT; of walking 10 meters repeatedly for the ESWT). Participants then repeated the cycle of an EST at 75% of their maximum speed until they consecutively twice failed to complete a cycle within the defined time period, paced by auditory signals. Participants were not informed about the maximal duration of the test. During the pilot phase of the development of the ESTs[6] the maximal duration was 10 minutes. Thereafter this was adjusted to 20 minutes. Participants performed all tests or the most difficult two tests they physically could do, always keeping the same sequence of tests; ESNHPT, ESBBT and ESWT. A resting period of at least 30 minutes was taken between two tests to fully recover. Maximal voluntary contraction (MVC) strengths of four relevant maneuvers were measured before the EST with a handheld dynamometer using the break test (CT 3001; C.I.T. Technics, Groningen, The Netherlands) or with manual muscle testing in some muscles of patients. Contractions lasted for about three seconds. Standardized starting positions in a fixed sequence, proximal to distal, were maintained.



## sEMG registration

We used surface electromyography (sEMG) at visit V2 of the ESNHPT and ESBBT, and at visit V3 during the ESWT. Muscle activation was continuously measured with wireless Bio Radio (Great Lakes Neurotechnologies, Cleveland, Ohio, USA) bipolar four channel sEMG during both MVCs and the ESTs. Each performed cycle during an EST was manually online marked in the sEMG signal.

### *Electrode placement*

We used self-adhesive Ag/AgCl Discs (*3M™* Red *Dot™,* 0.9mm electrode, 1.8 mm gel, 50 mm disk) with 20 mm inter-electrode distance. Skin preparation procedures included removal of hair if necessary and rubbing and cleaning of the skin with alcohol (70% denatured ethanol incl. 5% isopropanol). We placed standardized electrodes on the following muscles for the ESNHPT/ ESBBT: m. Deltoideus pars anterior (DE), m. Biceps Brachii (BB), m. Flexor Digitorum Superficialis (FD) and m. Extensor Digitorum Superficialis (ED). For the ESWT we recorded the m. Rectus Femoris (RF), m. Biceps Femoris (BF), m. Tibialis Anterior (TA) and m. Gastrocnemius (GA).[25,26] Electrodes were placed on the dominant side of the body parallel to the fiber direction. Reference electrodes were placed on the spina scapulae and spina iliaca anterior

superior for the ESNHPT/ESBBT and ESWT respectively. Wires were secured with tape on the skin to prevent cable movement artifacts.

### *Signal acquisition and processing*

We used Biocapture software at a sampling rate of 1000 samples/s and amplified with a gain of 1000, to measure real time muscle activation during the ESTs. The sampling resolution was 6 μV per least significant bit. An anti-aliasing filter, set to 250 Hz was implemented in the recording system. Raw sEMG data was offline detrended, bidirectionally high pass filtered with a fourth order filter at 20Hz and filtered with a 50 Hz notch filter. Lastly, it was rectified using custom programs written in MATLAB R2016b. Markers were manually checked on presence and position. The mean root mean square (RMS) amplitude was calculated over an overlapping moving window (100 samples). We calculated the median frequency (Fast Fourier Transformation) for every cycle. Maximum RMS amplitudes of the Maximal Voluntary Contractions (MVC) were calculated over a 500 samples moving window. RMS amplitudes per cycle were normalized to the MVC of the corresponding muscle to determine exercise intensity over the EST. Raw RMS amplitudes were used to determine time related changes.

## Statistical analysis

The first ten minutes of all sEMG signals were analyzed. First, we aimed to assess the overall group differences between patients with SMA type 2, type 3a, type 3b/4 and controls performing ESNHPT and ESBBT. Due to a smaller sample size the overall group difference between all patients with SMA and controls performing ESWT were assessed. Mean differences in muscle activation, as quantified by the median frequencies or the natural logarithm of RMS (lnRMS) amplitudes, were estimated using linear mixed effects (LME) models for the four muscles described above for the ESNHPT, ESBBT and the four muscles for the ESWT. We back-transformed the lnRMS amplitudes by multiplying estimates by a smearing factor to reduce back-transformation bias[27]. Secondly, we assessed whether muscle activation changed over time (i.e. over the course of the 10-minute endurance test), and whether the effect over time was different between patients with SMA and controls. We constructed an LME model with group, time and their interaction as fixed effects and a random intercept and slope for time per individual. An unstructured covariance type was chosen. All statistical analyses were performed using SPSS and the level of significance was set to 0.05.

## Results

In total, 70 participants with SMA and 19 controls completed the study. Participant characteristics per EST are summarized in table 1. Age and gender were similar in patients with SMA and healthy controls (ESNHPT age: p=0.173, gender: p=0.712; ESBBT age: p=0.198, gender p=0.287; ESWT age: p=0.206, gender p=0.241). Results of muscle activation measured with sEMG on different muscles are described per EST below.



### sEMG ESNHPT

#### *Median frequency*

sEMG recordings from muscles of patients with SMA type 2 and SMA type 3a at onset of the ESNHPT showed significantly higher median frequencies of ED compared to controls (figure 1A). Median frequencies of DE significantly decreased over time in patients with SMA type 2(-0.225 95%CI -0.738 - 0.287 p= 0.003), 3a (-0.189 95%CI -0.755 - 0.377, p=0.019) and 3b/4 (-0.222 95%CI -0.713 - 0.268, p= 0.002) compared to controls (0.264 95%CI 0.076 – 0.452, p=0.006). Median frequencies of BB significantly decreased over time in patients with SMA type 2 (-0.136 95% CI -0.397 – 0.125, p=0.000) compared to controls (0.167 95%CI 0.072 – 0.262, p= 0.001). Median frequencies of ED significantly increased over time in patients with SMA type 3a (0.140 95%CI -0.150 – 0.429, p=0.035) compared to controls (-0.065 95%CI -0.164 – 0.034, p=0.196) (figure 2A). Results of the linear mixed model analyses can be found in supplementary table 2.

#### *RMS amplitude*

All subjects performed the ESNHPT at a submaximal intensity level (8-60%MVC). Exercise intensity of all muscles was inversely correlated to SMA severity, $r_S$ = -0.764 (DELT), -0.775 (BB), -0.803 (FD), -0.811 (ED) all $p$<0.000 (figure 1B). Highest exercise intensity was measured in the most severe sub group of patients with SMA type 2 followed by SMA type 3a, 3b/4 and controls. Amplitudes of the sEMG signal of DE significantly decreased more over time in patients with SMA type 2 (-0.033 95%CI -0.047 - -0.019, p=0.000) and 3b/4 (-0.022 95%CI -0.176 - -0.009, p=0.05) compared to controls (-0.014 95%CI -0.009 – 0.019, p= 0.000). Amplitudes measured in DE significantly increased over time in patients with SMA type 3a (0.016 95% CI 0.001 – 0.031, p=0.000) (figure 2B). Amplitudes measured in BB significantly decreased less over time in patients with SMA type 3a (-0.010 95%CI -0.022 – 0.002, p=0.023) compared to controls (-0.019 95%CI -0.023 - -0.015, p=0.000). Amplitudes measured in

**Table 1.** Participant characteristics

| | SMA (N=70) | | Controls (N=19) | | | |
|---|---|---|---|---|---|---|
| | ESNHPT | | ESBBT | | ESWT | |
| **Characteristics** | **SMA (n=66)** | **Controls (n=19)** | **SMA (n=45)** | **Controls (n=16)** | **SMA (n=17)** | **Controls (n=17)** |
| Subtype/diagnosis(n) | | - | | - | | - |
| Type 2: | 4 | | 14 | | 0 | |
| Type 3a: | 14 | | 13 | | 2 | |
| Type 3b: | 18 | | 17 | | 14 | |
| Type 4: | 0 | | 1 | | 1 | |
| Gender (m:f) | 28:38 | 9:10 | 24:21 | 6:10 | 12:5 | 8:8 |
| Age, y, mean (SD) | 26.9 (14.0) | 23.0 (9.6) | 26.8 (13.7) | 23.2 (7.8) | 28.8 (11.9) | 24.0 (9.8) |
| HFMSE, mean (SD) | 18.5 (22.0) | - | 29.6 (22.8) | - | 54.8 (7.2) | - |
| Strength (N), mdn (min-max) | D(n=30):40.5(2.0-38.5)<br>BB(n=65):22.5(3.5-47.0)<br>FD(n=63):15.0(1.0-167.0)<br>ED(n=65):7.5(0.5-149.0) | D: 132.0(40-243.5)<br>BB: 213.5(83.0-361.5)<br>FD: 167.0(57.5-274.5)<br>ED: 127.0(46.5-196.0) | D(n=33):41.5(6.5-122.0)<br>BB(n=45):39.0(12.0-356.0)<br>FD(n=45):40.0(1.5-171.5)<br>ED(n=45):17.0(3.5-149.5) | D: 136.5 (40.0-244.0)<br>BB: 219.8 (76.5-386.0)<br>FD: 147.5(53.5-297.5)<br>ED: 117.0(46.0-186.0) | RF(n=17):25.5(16.5-201.0)<br>BF(n=17):117.0(26.5-237.0)<br>TA(n=17):215.5(52.5-319.0)<br>GA(n=17) MRC 5 | RF(n=13):359(262-437)<br>BF(n=13):275(123-343)<br>TA(n=13):316(213-364)<br>GA(n=17): MRC 5 |
| Drop out (%) | | 0 | | 13 | | 0 |
| Type 2: | 71 | | 93 | | - | |
| Type 3a: | 50 | | 85 | | 50 | |
| Type 3b/4: | 17 | | 44 | | 36 | |
| Time to limitation, s, mdn (IQR) | P1 (n=10): 600 (548)<br>P2 (n=56): 639 (1001) | P1 (n=13): 600 (0)<br>P2 (n=6): 1200 (0) | P1 (n=6): 272 (412)<br>P2 (n=39): 194 (1096) | P1 (n=13): 600 (0)<br>P2 (n=3): 579 (-) | P1 (n=2): 600 (0)<br>P2 (n=15): 861 (846) | P1 (n=11): 600 (0)<br>P2 (n=5): 1200 (-) |
| Excluded (n) * | MDF: 0<br>RMS-MVC: 24 | MDF: 0<br>RMS-MVC: 4 | MDF: 0<br>RMS-MVC: 5 | MDF: 0<br>RMS-MVC: 1 | MDF: 0<br>RMS-MVC: 5 | MDF: 0<br>RMS-MVC: 5 |

ESNHPT= Endurance Shuttle Nine Hole Peg Test, ESBBT= Endurance Shuttle Box and Block Test, ESWT= Endurance Shuttle Walk Test, SMA = Spinal Muscular Atrophy; subtype 3a: clinical symptoms <3yrs; subtype 3b: clinical symptoms >3yrs, HFMSE = Hammersmith Functional Motor Scale Expanded, P1=protocol 600 seconds, P2=protocol 1200 seconds, MDF= median frequency, RMS-MVC= normalized RMS amplitudes, *Number of muscles excluded due to inadequate MVC measurement.

BB significantly increased in patients with SMA type 2 (0.001 95%CI -0.010 – 0.012, p=0.000). Amplitudes measured in FD decreased significantly less over time in patients with SMA type 2 (-0.005 95%CI -0.012 – 0.011, p= 0.000) compared to controls (-0.016 95%CI -0.020 - -0.012, p= 0.000). Amplitudes measured in FD significantly increased over time in patients with SMA type 3a (0.006 95%CI 0.005 – 0.0182, p= 0.000). Amplitudes measured in ED significantly decreased over time in patients with SMA type 2 (-0.005 95%CI -0.014 – 0.004, p= 0.003) compared to controls (-0.010 95%CI -0.017 - -0.010, p= 0.000)(figure 2B). Results of the linear mixed model analyses can be found in supplementary table 2.



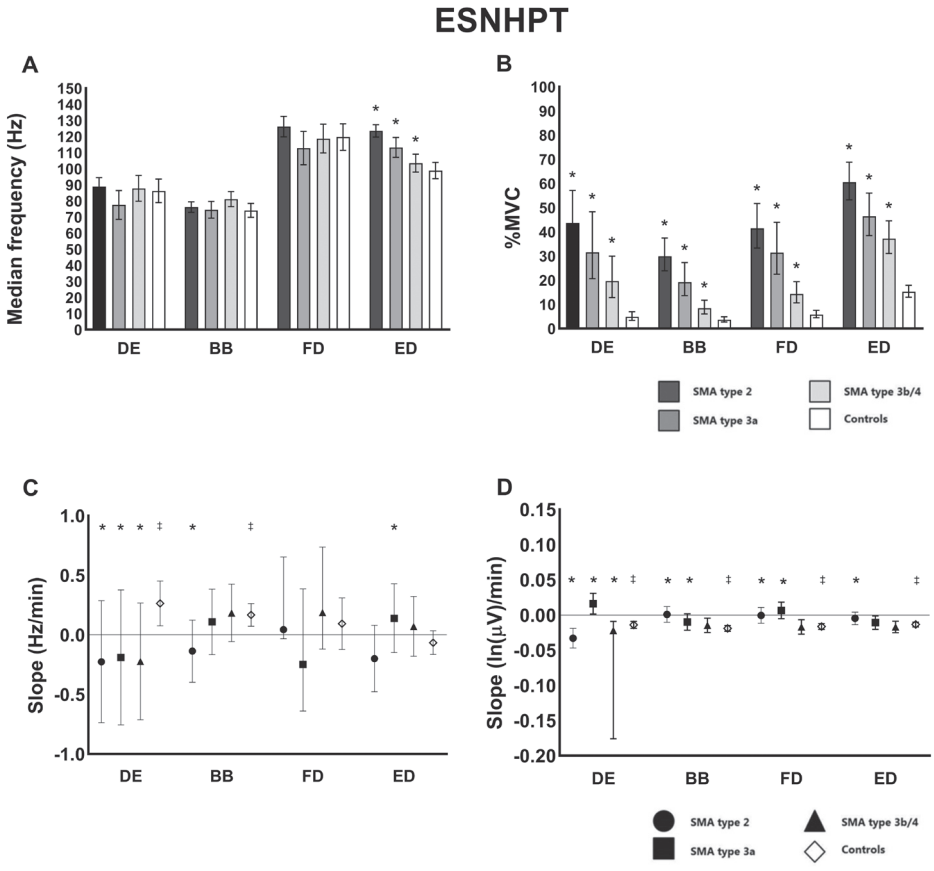


**Figure 1.** Endurance Shuttle Nine Hole Peg Test. A: Median frequency at onset, B: Exercise intensity at onset, C: Slope median frequency over time, D: Slope amplitudes over time
*** =** significantly different from controls (p<.05). ‡ = significantly different from zero (p<.05), DE=m. Deltoideus, BB= m. Biceps Brachii, FD= m. Flexor Digitorum, ED= m. Extensor Digitorum

## sEMG ESBBT

### *Median frequency*

Median frequencies of sEMG recordings from BB, FD and ED muscles of patients with SMA type 2 and SMA type 3b/4 at onset of the ESBBT were significantly higher than in controls. Participants with SMA type 3a showed significant higher median frequencies of BB and ED compared to controls (figure 1C). Median frequencies of DE significantly decreased more over time in patients with SMA type 2 (-5.926 95%CI -7.054 - -4.798, p=0.000), 3a (-0.934 95%CI -1.321 - -0.547, p= 0.000) and 3b/4 (-0.463 95%CI -0.761 - -0.166, p= 0.000) compared to controls (-0.139 95%CI -0.256 - -0.021, p= 0.021) (figure 2C). Median frequencies of BB significantly decreased over time in patients with SMA type 3b/4 (-0.066 95%CI -0.240 – 0.107, p= 0.006) compared to an increase in controls (0.080 95%CI 0.012 – 0.149, p=0.021). Median frequencies of FD significantly decreased more over time in patients with SMA type 3a (-1.150 95%CI -1.790 - -0.510, p=0.000) compared to controls (-0.283 95%CI -0.478 - -0.088, p= 0.004). Median frequencies of ED significantly decreased more over time in patients with SMA type 2 (-1.073 95%CI -1.828 - -0.318, p=0.004) and 3a (-0.612 95%CI -0.871 - -0.354, p=0.000) compared to controls (-0.081 95%CI -0.159 - -0.002, p=0.044). Median frequencies of ED significantly decreased less over time in patients with SMA type 3b/4 (0.046 95%CI -0.152 – 0.245, p=0.038) compared to controls (figure 2C). Results of the linear mixed model analyses can be found in supplementary table 3.

### *RMS amplitude*

All subjects performed the ESBBT at a submaximal intensity level (13-53%MVC). Exercise intensity inversely correlated with SMA severity with the highest percentages measured in the most severely affected patients with SMA type 2 followed by SMA type 3a, 3b/4 and controls, $r_S$ = -0.549 (DELT), -0.645 (BB), -0.693 (FD), -0.773 (ED) all $p$<0.000 (figure 1D). Amplitudes of BB significantly increased over time in patients with SMA type 2 (0.078 95%CI 0.043 – 0.113, p=0.000) and 3b/4 (0.002 95%CI -0.007 – 0.011, p=0.000) compared to a decrease in controls (-0.012 95%CI -0.015 - -0.008, p=0.000). Amplitudes of FD significantly decreased over time in patients with SMA type 3b/4 (0.010 95%CI -0.0198 - -0.003, p=0.043) compared to controls (0.003 95%CI -0.007 – 0.001, p=0.108). Amplitudes of FD significantly increased over time in patients with SMA type 2 (0.060 95%CI 0.022 – 0.098, p=0.000) and 3a (0.010 95%CI -0.003 – 0.023, p=0.004) compared to controls (figure 2D). Amplitudes of ED significantly increased over time in patients with SMA type 2 (0.051 95%CI 0.021 – 0.082, p=0.000) and 3a (0.018 95%CI 0.007 – 0.029,

p=0.000) compared to controls (-0.006 95%CI -0.009 - -0.003, p=0.000). Amplitudes of ED significantly decreased more over time in patients with SMA type 3b/4 (-0.012 95%CI -0.020- -0.003, p=0.030) compared to controls (figure 2D). Results of the linear mixed model analyses can be found in supplementary table 3.



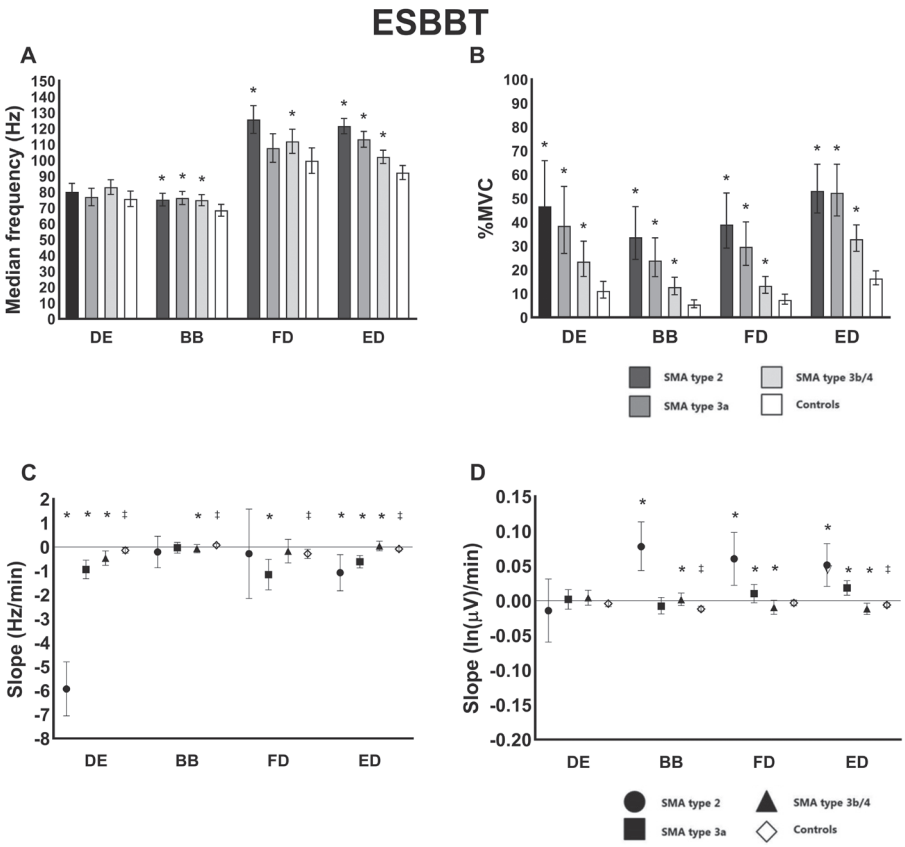


**Figure 2.** Endurance Shuttle Box and Block Test A: Median frequency at onset, B: Exercise intensity at onset, C: Slope median frequency over time, D: Slope amplitudes over time
* = significantly different from controls (p<.05). ‡ = significantly different from zero (p<.05), DE=m. Deltoideus, BB= m. Biceps Brachii, FD= m. Flexor Digitorum, ED= m. Extensor Digitorum

## sEMG ESWT

### *Median frequency*

Median frequencies of sEMG recordings from RF, BF, TA and GA muscles at onset of the ESWT were similar in patients with SMA and controls (figure 1E). However, median frequencies of BF (0.002 95%CI -0.227 – 0.232, p=0.000)

and TA (-0.271 95%CI -0.568 – 0.026, p=0.012) decreased significantly less over time in patients with SMA compared to controls (BF: -0.377 95%CI-0.459– -0.296, p=0.000; TA: -0.515 95%CI -0.622 - -0.408, p=0.000). Median frequencies of GA significantly increased over time in patients with SMA (0.265 95%CI 0.016 – 0.514, p=0.000) compared to controls (-0.074 95%CI 0.162 -0.015, p=0.102) (figure 2E). Results of the linear mixed model analyses can be found in supplementary table 4.

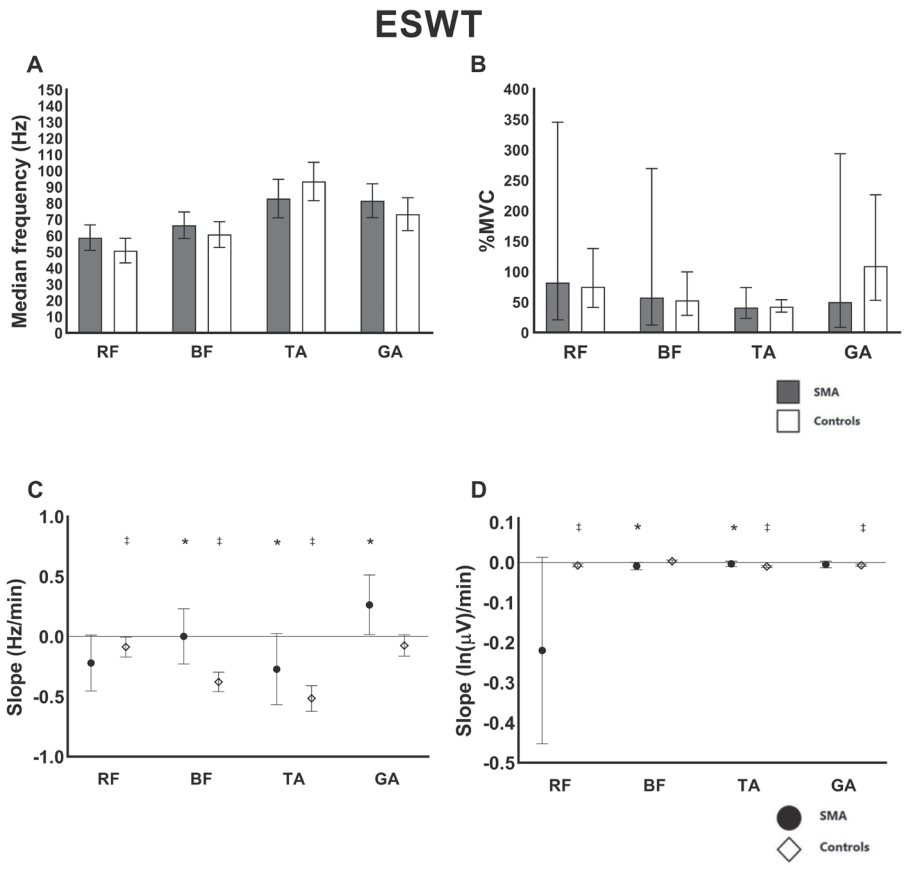


**Figure 3.** Endurance Shuttle Walk Test A: Median frequency at onset, B: Exercise intensity at onset, C: Slope median frequency over time, D: Slope amplitudes over time
* = significantly different from controls (p<.05). ‡ = significantly different from zero (p<.05), RF= m. Rectus Femoris, BF= m. Biceps Femoris, TA= m. Tibialis Anterior, GA= m. Gastrocnemius

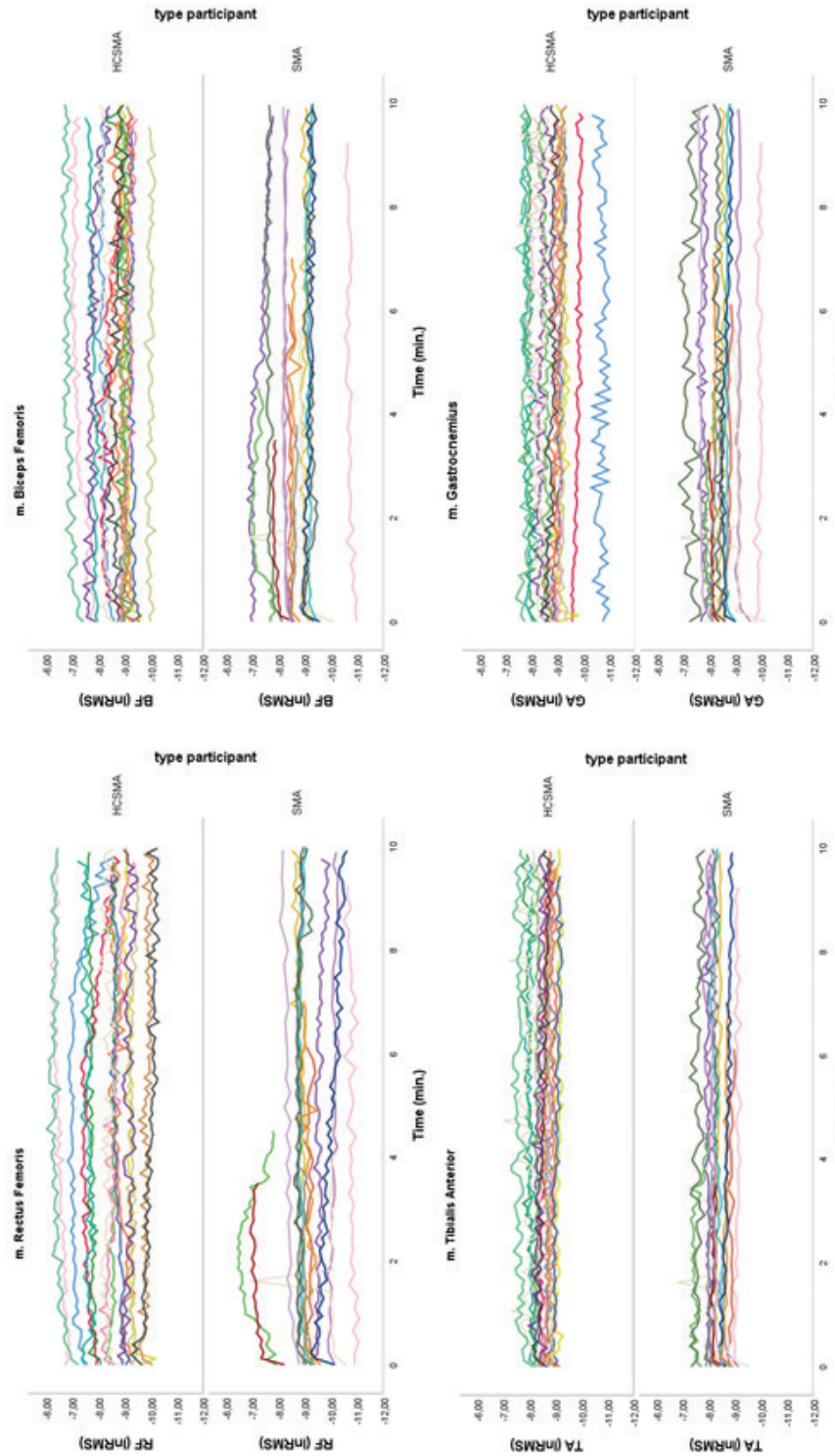


**Figure 4.** Individual muscle activation (lnRMS amplitudes) per cycle of ESWT in four muscles of patients with SMA and controls (HC)

### *RMS amplitude*

All patients performed the ESWT at a submaximal intensity level (41-82%MVC). Exercise intensity was not significantly different between patients with SMA and controls (figure 1F). Amplitudes of RF (-0.008 95%CI -0.005 – 0.012, p=0.000), BF (-0.009 95%CI -0.018 – 0.000, p=0.000), TA (-0.004 95%CI -0.011 – 0.003, p=0.004) and GA (-0.006 95%CI -0.009 - -0.004, p=0.000) significantly decreased over time in patients with SMA. At the individual level, we observed both patients and healthy controls with an increase and decrease of amplitude over time (figure 4). Results of the linear mixed model analyses can be found in supplementary table 4.

## Discussion

This study employed sEMG to investigate motor unit reserve capacity during execution of quantitative endurance tests in patients with SMA types 2-4. Our results suggest the availability of motor unit reserve capacity in upper and lower extremities during fatiguing submaximal endurance performance in some, but not all patients with SMA. As such, this study has identified a potential therapeutic target in clinical care for patients with SMA types 2-4 in the form of preserving, if not expanding this reserve capacity.

The ESTs were performed at submaximal intensity, relative to the movement MVCs (figure 1B, 2B, 3B). This confirms the validity of the tests as endurance tests for all SMA disease subtypes. In the ESNHPT and ESBBT, the relative exercise intensities turned out to be inversely related to SMA severity with the highest percentages of MVC measured in patients with SMA type 2. This finding is in accordance with a recent study that measured trunk muscle activation during unsupported sitting in patients with SMA[28]. Specifically, Peeters and co-workers reported three times higher activation levels in trunk muscles in patients with SMA type 2 and 3 during execution of a reaching or a daily task than in controls[28]. Our sEMG recordings from muscles of the upper-extremities and legs of the patients during EST execution showed a range of (patho)physiological trends in the time courses of median frequencies and amplitudes, respectively. The most remarkable changes over time were found in median frequencies and RMS amplitudes of patients with SMA type 2 during the ESBBT (figure 2C-D). For example, a decrease in median frequency of 5.7 Hz per minute in the DE muscle stands out against a reported change of 5.5 Hz per two hours in m. Deltoideus measured in healthy subjects during a repetitive shoulder flexion task[29]. However, as no published reference

values for fatigability during cyclic dynamic tasks are available, the absolute magnitude of change in median frequency and amplitude over time should be interpreted with some caution.

A progressive decrease in median frequency of the sEMG signal during execution of a physical task is commonly thought to be indicative of muscle acidification associated with recruitment of motor units of fast-twitch anaerobic muscle fibers [21]. We observed such a decrease in median frequency over time in sEMG recordings from the *m. Deltoideus* during ESNHPT, in *m. Deltoideus*, *m. Flexor Digitorum* and *m. Extensor Digitorum* during ESBBT, and in *m. Rectus Femoris* and *m. Tibialis Anterior* during ESWT execution, respectively. Conversely, any progressive increase in sEMG signal amplitude would be indicative of motor unit reserve capacity progressively recruited to delay task failure. This was observed in sEMG recordings from the *m. Deltoideus* and *m. Flexor Digitorum* during ESNHPT and in the *m. Biceps Brachii*, *m. Flexor Digitorum* and *m. Extensor Digitorum* during ESBBT execution, respectively. As such, the latter findings do not reject the hypothesis under investigation that patients with SMA type 2-4 may be able to recruit motor unit reserve capacity during fatiguing motor tasks.

Montes and co-workers previously examined the effect of time on the amplitude of sEMG recordings from leg muscles during a six minute walk test (6MWT) in patients with SMA type 3[30]. They found a decrease in amplitude at minute six compared to the first minute of the 6MWT[30]. It was suggested that this could possibly be explained by a limited motor unit reserve capacity. We observed a similar pathophysiological response of fatigability of the leg muscles with an overall mean decrease in amplitude and median frequencies in RF and TA muscles of SMA patients. However, at the individual level, increases in amplitudes over time were found in some lower extremity muscles of patients (figure 4). Together, these findings demonstrate variability in phenotypic symptomology and presentation between patients.

This study has confirmed submaximal exercise intolerance in patients with SMA type 2-4 while sEMG recordings showed pattern heterogeneities over time between individuals. This variability suggests a multifactorial causal base underlies fatigability in SMA type 2-4, including central and peripheral factors. One evident cause of fatigability may be dysfunction of the neuromuscular junction, with a prevalence of 40-50% of patients with SMA[3]. As such, we may have expected to find constant sEMG median frequencies concomitant with decreasing amplitudes in every other patient. However, our results did not show this. This may be explained by individual compensational strategies during ESNHPT and ESBBT execution. Movements as lateral bending of the trunk

and elevation of the shoulder were allowed. The reported patterns of muscle activation should therefore be interpreted as component of a complex motor task execution rather than informing on isolated muscle performance. More in depth laboratory research on isolated function of individual muscles in patients may reveal neuromuscular junction dysfunction by decreasing amplitudes.

Another potential causal factor underlying fatigability in SMA type 2-4 may perhaps be decreased oxidative capacity of skeletal muscle. A number of mouse and human studies have reported evidence for mitochondrial dysfunction in SMA[4,5,31]. Specifically, Miller and co-workers[5] analysed the transcriptome of spinal motor neurons in a transgenic pre-symptomatic SMA mouse model and found altered mitochondrial function. Ripolone and co-workers[4] reported downregulated mitochondrial biogenesis in quadriceps and paraspinal muscle biopsy samples from patient with SMA type 1-3. Clearly, more research of this particular subject matter is warranted to establish whether or not oxidative abnormalities found in SMA mouse models and muscle biopsies may contribute to exercise intolerance in human SMA.

For the ESWT test, a handheld dynamometer was generally used to determine MVC. This method is considered reliable and valid; i.e. in RF in young healthy subjects[32]. We did, however, experience difficulties to measure maximal strength of the GA muscle in patients and leg muscles in general in healthy controls. This may have led to under-estimation of MVC and thereby overestimation of exercise intensity of ESWT for these muscles in these subjects. Similarly, the large range in estimated exercise intensities in RF and BF muscles in patients with SMA may well reflect variation in muscle strength between individuals (table 1). Lastly, studies in healthy subjects report diverse EMG-length relations for the RF muscle at varying knee joint angles[33]. As MVCs were assessed in either supine or in a sitting position while patients were in upright position during ESWT, this may have contributed to variance of strength and sEMG activation confounding normalization.

In conclusion, we have found evidence that some patients with SMA type 2, 3 and 4 have reserve capacity in motor unit recruitment, yet still experience exercise intolerance during submaximal activities of daily life. Preserving, if not expanding this reserve capacity may therefore present a potential therapeutic target in clinical care for some patients with SMA types 2-4.

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# Additional tables

**Table 2.** ESNHPT

| Variable | Median Frequency | | | | RMS amplitude | | | |
|---|---|---|---|---|---|---|---|---|
| | β | 95% CI | | *p* | β | 95% CI | | *p* |
| | | lower bound | upper bound | | | lower bound | upper bound | |
| **m. Deltoideus** | | | | | | | | |
| Time*controls (ref.) | 0,263984 | 0,075689 | 0,45228 | 0,006 | -0,01418 | -0,019 | -0,009054 | 0,000 |
| Time*SMA type 2 | -0,48937 | -0,813804 | -0,164943 | 0,003 | -0,01877 | -0,028 | -0,009828 | 0,000 |
| Time*SMA type 3a | -0,45299 | -0,830694 | -0,075288 | 0,019 | 0,030081 | 0,0204 | 0,039812 | 0,000 |
| Time*SMA type 3b/4 | -0,48652 | -0,788872 | -0,184169 | 0,002 | -0,00788 | -0,157 | -0,000006 | 0,05 |
| **m. Biceps Brachii** | | | | | | | | |
| Time*controls (ref.) | 0,167395 | 0,072386 | 0,262405 | 0,001 | -0,019 | -0,023 | -0,015 | 0,000 |
| Time*SMA type 2 | -0,30352 | -0,469219 | -0,137824 | 0,000 | 0,0198 | 0,0127 | 0,0268 | 0,000 |
| Time*SMA type 3a | -0,05827 | -0,238123 | 0,121584 | 0,525 | 0,009 | 0,0013 | 0,0166 | 0,023 |
| Time*SMA type 3b/4 | 0,017593 | -0,128401 | 0,163588 | 0,813 | 0,0044 | -0,002 | 0,0106 | 0,169 |
| **m. Flexor Digitorum** | | | | | | | | |
| Time*controls (ref.) | 0,062972 | -0,073115 | 0,199059 | 0,364 | -0,01635 | -0,020419 | -0,01229 | 0,000 |
| Time*SMA type 2 | n.a. | n.a. | n.a. | n.a. | 0,015841 | 0,008747 | 0,022935 | 0,000 |
| Time*SMA type 3a | n.a. | n.a. | n.a. | n.a. | 0,022848 | 0,015127 | 0,03057 | 0,000 |
| Time*SMA type 3b/4 | n.a. | n.a. | n.a. | n.a. | -0,00062 | -0,006869 | 0,005621 | 0,845 |
| **m. Extensor Digitorum** | | | | | | | | |
| Time*controls (ref.) | -0,06519 | -0,164081 | 0,033693 | 0,196 | -0,01361 | -0,016872 | -0,01035 | 0,000 |
| Time*SMA type 2 | -0,13336 | -0,31305 | 0,046333 | 0,146 | 0,008755 | 0,003064 | 0,014446 | 0,003 |
| Time*SMA type 3a | 0,204905 | 0,014554 | 0,395256 | 0,035 | 0,002611 | -0,003584 | 0,00885 | 0,409 |
| Time*SMA type 3b/4 | 0,136575 | -0,01538 | 0,288529 | 0,078 | -0,00339 | -0,008398 | 0,001623 | 0,185 |

ESNHPT=Endurance Shuttle Nine Hole Peg Test, SMA=Spinal Muscular Atrophy, n.a.=not applicable

**Table 3.** ESBBT

| Variable | Median Frequency | | | | RMS amplitude | | | |
|---|---|---|---|---|---|---|---|---|
| | β | 95% CI | | *p* | β | 95% CI | | *p* |
| | | lower bound | upper bound | | | lower bound | upper bound | |
| **m. Deltoideus** | | | | | | | | |
| Time*controls (ref.) | -0,138514 | -0,256197 | -0,020830 | 0,021 | -0,00039 | -0,003430 | 0,002700 | 0,802 |
| Time*SMA type 2 | -5,787041 | -6,797315 | -4,776767 | 0,000 | n.a. | n.a. | n.a. | n.a. |
| Time*SMA type 3a | -0,795716 | -1,065029 | -0,526403 | 0,000 | n.a. | n.a. | n.a. | n.a. |
| Time*SMA type 3b/4 | -0,324759 | -0,504743 | -0,144774 | 0,000 | n.a. | n.a. | n.a. | n.a. |
| **m. Biceps Brachii** | | | | | | | | |
| Time*controls (ref.) | 0,080307 | 0,011914 | 0,148700 | 0,021 | -0,012 | -0,015000 | -0,008000 | 0,000 |
| Time*SMA type 2 | 0,282358 | -0,871123 | 0,306406 | 0,347 | 0,0898 | 0,0583 | 0,1213 | 0,000 |
| Time*SMA type 3a | -0,106274 | -0,262943 | 0,050395 | 0,184 | 0,0041 | -0,004 | 0,0125 | 0,342 |
| Time*SMA type 3b/4 | -0,146505 | -0,251142 | -0,041869 | 0,006 | 0,0135 | 0,0078 | 0,0191 | 0,000 |
| **m. Flexor Digitorum** | | | | | | | | |
| Time*controls (ref.) | -0,282722 | -0,477585 | -0,087859 | 0,004 | -0,00325 | -0,007225 | 0,000718 | 0,108 |
| Time*SMA type 2 | 0,005321 | -1,665913 | 1,676554 | 0,995 | 0,063431 | 0,029316 | 0,097547 | 0,000 |
| Time*SMA type 3a | -0,867433 | -1,313012 | -0,421854 | 0,000 | 0,013308 | 0,004173 | 0,022444 | 0,004 |
| Time*SMA type 3b/4 | 0,112689 | -0,185164 | 0,410543 | 0,458 | -0,0063 | -0,012397 | -0,000194 | 0,043 |
| **m. Extensor Digitorum** | | | | | | | | |
| Time*controls (ref.) | -0,080811 | -0,159356 | -0,002266 | 0,044 | -0,00617 | -0,009379 | -0,002964 | 0,000 |
| Time*SMA type 2 | -0,991974 | -1,668339 | -0,315609 | 0,004 | 0,057583 | 0,030019 | 0,085146 | 0,000 |
| Time*SMA type 3a | -0,531236 | -0,71118 | -0,351293 | 0,000 | 0,024428 | 0,017049 | 0,031807 | 0,000 |
| Time*SMA type 3b/4 | 0,127151 | 0,006978 | 0,247325 | 0,038 | -0,00545 | -0,010381 | -0,000526 | 0,030 |

ESBBT= Endurance Shuttle Box and Block Test, SMA=Spinal Muscular Atrophy, n.a. = not applicable

**Table 4.** ESWT

| Variable | Median Frequency | | | | RMS amplitude | | | |
|---|---|---|---|---|---|---|---|---|
| | β | 95% CI | | *p* | β | 95% CI | | *p* |
| | | lower bound | upper bound | | | lower bound | upper bound | |
| **m. Rectus Femoris** | | | | | | | | |
| Time*controls (ref.) | -0,12671 | -0,195651 | -0,057765 | 0,000 | -0,0083 | -0,011559 | -0,005042 | 0,000 |
| Time*SMA | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| | | | | | | | | |
| **m. Biceps Femoris** | | | | | | | | |
| Time*controls (ref.) | -0,37743 | -0,458728 | -0,296121 | 0,000 | 0,002906 | -0,000424 | 0,006237 | 0,087 |
| Time*SMA | 0,379775 | 0,231705 | 0,527845 | 0,000 | -0,01183 | -0,017895 | -0,005768 | 0,000 |
| | | | | | | | | |
| **m. Tibialis Anterior** | | | | | | | | |
| Time*controls (ref.) | -0,51474 | -0,621758 | -0,407728 | 0,000 | -0,01017 | -0,012595 | -0,00775 | 0,000 |
| Time*SMA | 0,243734 | 0,054209 | 0,43326 | 0,012 | 0,006417 | 0,002007 | 0,010826 | 0,004 |
| | | | | | | | | |
| **m. Gastrocnemius** | | | | | | | | |
| Time*controls (ref.) | -0,07364 | -0,161936 | 0,014657 | 0,102 | -0,00638 | -0,008785 | -0,003974 | 0,000 |
| Time*SMA | 0,338543 | 0,177778 | 0,499308 | 0,00 | n.a. | n.a. | n.a. | n.a. |

ESWT= Endurance Shuttle Walk Test, SMA=Spinal Muscular Atrophy, n.a.=not applicable

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Chapter 5

# Correlates of fatigability in patients with spinal muscular atrophy




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*Submitted*

# Abstract

## Objective

To determine the associations between fatigability and muscle strength, motor function, neuromuscular junction (NMJ) function and perceived fatigue in patients with spinal muscular atrophy (SMA).

## Methods

Fatigability was defined as the inability to continue a 20-minute submaximal repetitive task of either walking or proximal- or distal arm function and expressed as 'drop-out' on the Endurance Shuttle Test Combined Score (ESTCS). We assessed muscle strength with the MRC sum score, motor function with the Hammersmith Functional Motor Scale Expanded (HFMSE) and Motor Function Measure (MFM), NMJ-function with repetitive nerve stimulation of the accessory- and ulnar nerve and perceived fatigue with the PROMIS fatigue short form questionnaire in 61 children and adults with SMA types 2-4. We applied Cox regression analysis to explore the associations between fatigability and these factors.

## Results

The hazard of drop-out on the ESTCS decreased with respectively 0.8%, 2% and 1.3% for each point increase in the MRC sum score, the HFMSE score and the MFM percentual score. However, we observed prominent fatigability with preserved muscle function and vice versa in 13%-16% of patients. We did not find an association between neuromuscular junction dysfunction of the accessory- ($p$ = .37) and ulnar nerve ($p$ = .063) and fatigability, which could be due to a large number of missing values. Perceived fatigue in SMA was comparable to reference values and was not associated with fatigability ($p$ = .52).

## Conclusions

Fatigability in SMA is associated with, yet not equivalent to muscle strength and motor function.

## Introduction

Hereditary proximal Spinal Muscular Atrophy (SMA) is a progressive neuromuscular disorder caused by loss of function of the *SMN1* gene and the resulting degeneration of motor neurons in the spinal cord[1,2]. SMA is characterized by stunted gross motor development followed by slow decline of motor function[3,4]. It shows a wide range of severity, with neonatal (SMA type 1) infantile (SMA types 2-3) to adult onset (SMA type 4) types[1,5]. The most prominent clinical hallmarks of types 2-4 are axial and proximal muscle weakness but patients also lack the endurance to repeatedly perform functional tasks[6]. Fatigability, is an important physical impairment of daily life activities but has received relatively limited attention[6-9]. To study fatigability, we recently validated three endurance tests that cover walking-, arm- and hand function, employ the same methodology and allow combined analysis as the Endurance Shuttle Test Combined Score (ESTCS)[6,10]. With this approach, we demonstrated increased fatigability in 85% of patients with SMA. The causes of fatigability in patients with SMA have not been elucidated. Fatigability may be a secondary manifestation of impaired motor neurons and muscle loss but it may also be caused by impaired neuromuscular transmission[11-15]. Patients with SMA report increased perceived fatigue but the association with fatigability has not been studied in non-ambulatory patients[16-18]. The objective of this study was to gain more insight into factors associated with fatigability to facilitate clinical management and clinical trial design aiming at reducing these complaints.

## Methods

We collected data as part of a large cross-sectional study on fatigability in spinal muscular atrophy[6,10]. We recruited subjects with SMA type 2-4 between 8-60 years old through the Dutch SMA registry (www.treatnmd.eu/patientregistries)[5]. Exclusion criteria were a history of neuromuscular diseases or the use of medication that affect neuromuscular junction function (e.g. myasthenia gravis) or medical conditions incompatible with exercise or the inability to perform any of the endurance tests. The research protocol was approved by the Medical Ethics Committee of the University Medical Center Utrecht in the Netherlands (NL48715.041.14) and written informed consent was obtained from all adults and children and/or their parents.

### *Standard Protocol Approvals, Registrations, and Patient Consents*

The research protocol was approved by the Medical Ethics Committee of the University Medical Center Utrecht in the Netherlands (NL48715.041.14) and written informed consent was obtained from all adults and children and/or their parents.

### *Demographics and medical history*

Clinical characteristics were obtained using questionnaires as described previously (table 1)[4,5]. We used multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA kit P021-B1-01, MRC-Holland) to confirm homozygous loss of function of the *SMN1* gene and to assess *SMN2* gene copy number.

### *Fatigability*

We assessed fatigability with the Endurance Shuttle Test Combined Score (ESTCS)[10]. The ESTCS is compiled based on the scores of three different endurance shuttle tests that match the different levels of motor function in patients. Ambulatory patients performed the Endurance Shuttle Walk Test (ESWT). Non-ambulatory patients who were able to lift their arm performed the Endurance Shuttle Box and Block Test (ESBBT) and non-ambulatory patients with only distal arm function performed the Endurance Shuttle Nine Hole Peg Test (ESNHPT). In summary, we instructed subjects to either walk 10 meters (ESWT), move 10 blocks over a partition (ESBBT) or place and return 9 pegs in 9 holes (ESNHPT) at 75% of their individualized maximum speed. Each individual round was paced by auditory signals. The test was terminated when the subject was not able to keep pace for two consecutive rounds or when the maximal duration of 20 minutes was reached. We documented 'drop out' (Yes/No) and 'Time to drop- out (sec) for each performed EST. Drop-out was defined as the inability to sustain the maximal duration of 20 minutes.

### *Muscle strength*

We used the Medical Research Council (MRC) Scale with a slight modification (i.e. MRC 0 and 1 were both scored as 1) to assess muscle strength of 22 muscle groups of both sides[4,5]. MRC scores were used to calculate an MRC sum score (min-max: 44-220).

### *Motor function*

We used both the Hammersmith Functional Motor Scale Expanded (HFMSE) and the Motor Function Measure (MFM) to assess motor function in the

broadest possible range of SMA severity. The HFMSE is a gross motor scale developed for SMA and especially suitable for patients with type 2 and type 3 with higher levels of functioning[19]. It consists of 33 items that can be scored on a 3 point scale (0-2), i.e. with scores ranging from 0 to 66[20]. The MFM was developed for neuromuscular diseases and is more sensitive in capturing remaining motor function in weaker subjects with SMA[21]. It consists of 32 items that can ben scored on a 4-point scale (0-3). MFM total score was calculated as percentage of the maximum possible score (0-100%)[22].



### *Neuromuscular junction function*

One of the authors (HSG) performed repetitive nerve stimulation (RNS) to evaluate the function of neuromuscular junction. We used a standardized protocol that assessed the accessory and ulnar nerves (recordings from m. trapezius respectively m. abductor digiti minimi (ADM) unilaterally, in SMA patients from 12 years of age of the dominant arm as described previously[11]. We used Keypoint equipment (Dantec Keypoint; Medtronic Inc., Skovlunde, Denmark) and Viking equipment (Nicolet VikingSelect; VIASYS Healthcare Inc., Middleton, WI). We assessed the maximal distal compound maximal action potential (CMAP) obtained during supramaximal nerve stimulation and subsequently applied RNS using a train of 10 supramaximal nerve stimulations at 3 Hz when distal CMAP amplitudes were > 1.0 mV. Quality of individual RNS data were checked by one of the authors (RIW) and abnormal NMJ function was defined as an amplitude decrease between the first and the fifth response of ≥ 10%.

### *Perceived fatigue*

We asked subjects aged 8-17 years to indicate perceived fatigue by filling out the 'Patient Reported Outcomes Measurement Information System (PROMIS) pediatric short form V1.0 – Fatigue 10a (PROMIS Ped SF Fatigue 10a). The PROMIS Ped SF Fatigue 10a consists of 10 items representing the most informative items from the PROMIS item bank[23,24]. The PROMIS Fatigue item banks assess a range of self-reported symptoms, from mild subjective feelings of tiredness to an overwhelming, debilitating, and sustained sense of exhaustion that likely decreases one's ability to execute daily activities and function normally in family or social roles. Subjects were asked to score on a 5- point scale how often they experienced certain complaints during the past 7 days. For subjects aged ≥ 18 years we used the PROMIS V 1.0 short form 8a (PROMIS adult SF fatigue 8a). The PROMIS adult SF fatigue 8a consists of 8 items representing the most informative items from the PROMIS item bank.

T-scores (normal reference mean = 50, sd = 10) were calculated using response pattern scoring through Scoring Service (https://www.assessmentcenter.net/ac_scoringservice). Scores above +2 standard deviations were classified as abnormal.

### *Statistical analysis*

Quantitative descriptive statistics (median, minimum and maximum) were used to present demographics and clinical characteristics. The association between the independent variables muscle strength (MRC- sum score), motor function (HFMSE score, MFM percentual score), neuromuscular junction function (decrement accessory nerve, decrement ulnar nerve), perceived fatigue (PROMIS fatigue T-score) and the depended variable drop-out on the ESTCS were analyzed with univariate Cox Regression analysis and expressed as hazard ratio's (HR) . To illustrate, a HR of 0.95 for MRC indicates that the hazard of drop-out during the ESTCS is reduced by 5% for each unit increase in MRC. Missing data were imputed using multiple imputations and Predictive Mean Matching (PMM). Random numbers were produced by the Mersenne-Twister algorithm using R version 3.3.2 (2016-10-31). In the imputation model, we included gender, age, SMA type, CMAP amplitude of the accessory- and ulnar nerves, presence of decrement during repetitive stimulation of the ulnar and accessory nerves, HFMSE, MFM, MRC sum score, PROMIS FAT T-score, natural logarithm of Endurance Time, and Drop-out Yes/No. Results were pooled across imputations (N = 25) using Rubin's rules[25]. We used scatterplots to illustrate the strength of the association between muscle strength, motor function and time to drop out on the ESTCS. $p$ values < 0.05 were significant. We used SPSS (IBM SPSS Statistics version 24; IBM Inc., Chicago, IL) for statistical analysis.

### *Data availability statement*

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Results

### *Patient characteristics*

We included 61 patients with SMA types 2-4. Patient characteristics are summarized in table 1.

### *Fatigability*

Fifteen patients (25%) performed the ESWT, 22 patients (36%) performed the ESBBT and 24 patients (39%) performed the ESNHPT. Individual scores were combined into the ESTCS. Eighty-five percent of the patients demonstrated fatigability on the ESTCS (table 1).

**Table 1.** clinical characteristics

| | **Subjects (N=61)** |
|---|---|
| **Age (years)** | 27.0 (8-59) |
| **Gender (F/M)** | 34:27 |
| **SMA subtype** | |
| 2 | N=31 |
| 3a | N=14 |
| 3b | N=15 |
| 4 | N=1 |
| **Fatigability** | |
| Drop-out (%) | 85 |
| Time to limitation (sec) | 220 (48-1200) |
| **Muscle strength** | |
| MRC sum score (44-220) | 123 (76 – 206) |
| **Motor function** | |
| HFMSE (0-64) | 7 (0-64) $_{(N=55)}$ |
| MFM (0-100%) | 44 (17-99) $_{(N=60)}$ |
| **Perceptions of fatigue** | |
| Total group | |
| PROMIS SF Fatigue (T-score) | 52.3 (30-72) $_{(N=55)}$ |
| Children | |
| PROMIS Ped SF Fatigue 10a | 48.5 (30 – 69) $_{(N=16)}$ |
| Adults | |
| PROMIS SF Fatigue 8a | 52.2 (33 – 72) $_{(N=39)}$ |

SMA subtype based on age of onset and maximum function achieved [1]: 2 = 7-18 months and sits but never stands; 3a = 1.5 -3 years and stands and walks; 3b = >3 years and stands and walks; 4 = 10-30 years and stands and walks. MRC = Medical Research Council; HFMSE = Hammersmith Functional Motor Scale Expanded; MFM = Motor Function Measure; MRC = Medical Research Council; PROMIS = Patient-Reported Outcomes Measurement Information System; SF = Short Form; Ped = Pediatric

## *Associated factors*

### Muscle strength

Levels of muscle strength are summarized in table 1. The hazard of drop-out on the ESTCS decreased with approximately 0.8% for each point increase on the MRC-sum score (HR = .992 (.967 - .993), $p$ = .02). We also detected patients with relatively spared muscle strength but poor endurance and vice versa: respectively 16% of the subjects demonstrated above-average (> median score of the group) MRC sum score but below-average (< median score of the group) endurance time (Figure 1 quadrant D). On the other hand, 18% of the subjects demonstrated below-average (< median score of the group) MRC sum score but above-average (> median score of the group) endurance time (Figure 1 quadrant A).

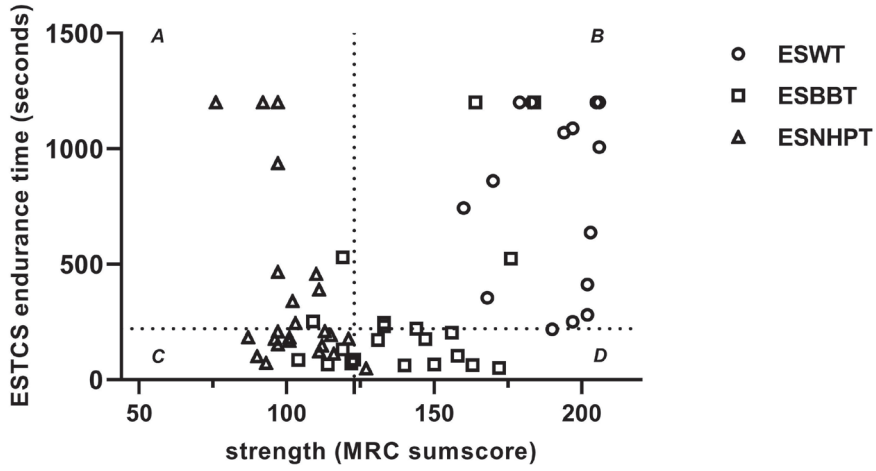


**Figure 1.** Distribution of muscle strength scores
Distribution of individual values of independent variables (X-axis) and dependent variable (Y-axis). MRC = Medical Research Council. ESTCS = Endurance Shuttle Test Combined Score. Dashed lines are median group scores. Quadrant A: low strength and high endurance; B: high strength and high endurance; C: low strength and low endurance; D: high strength and low endurance.

### Motor function

Levels of motor function are summarized in table 1. The hazard of drop-out on the ESTCS decreased with approximately 2% and 1.3% for each point increase on the HFMSE score (HR = .980 (.964 - .991), $p$ = .002) and MFM percentual score ( HR = .987 (.977 - .997), $p$ = .015). We detected patients with relatively spared motor function but poor endurance and vice versa: respectively 13% and 15% of the subjects demonstrated above-average (> median score of the

group) HFMSE score and MFM percentual score but below-average (< median score of the group) endurance time (Figure 2 quadrant D). On the other hand, 15% and 16% of the subjects demonstrated below-average (< median score of the group) HFMSE score and MFM percentual score but above-average (> median score of the group) endurance time (Figure 2 quadrant A).

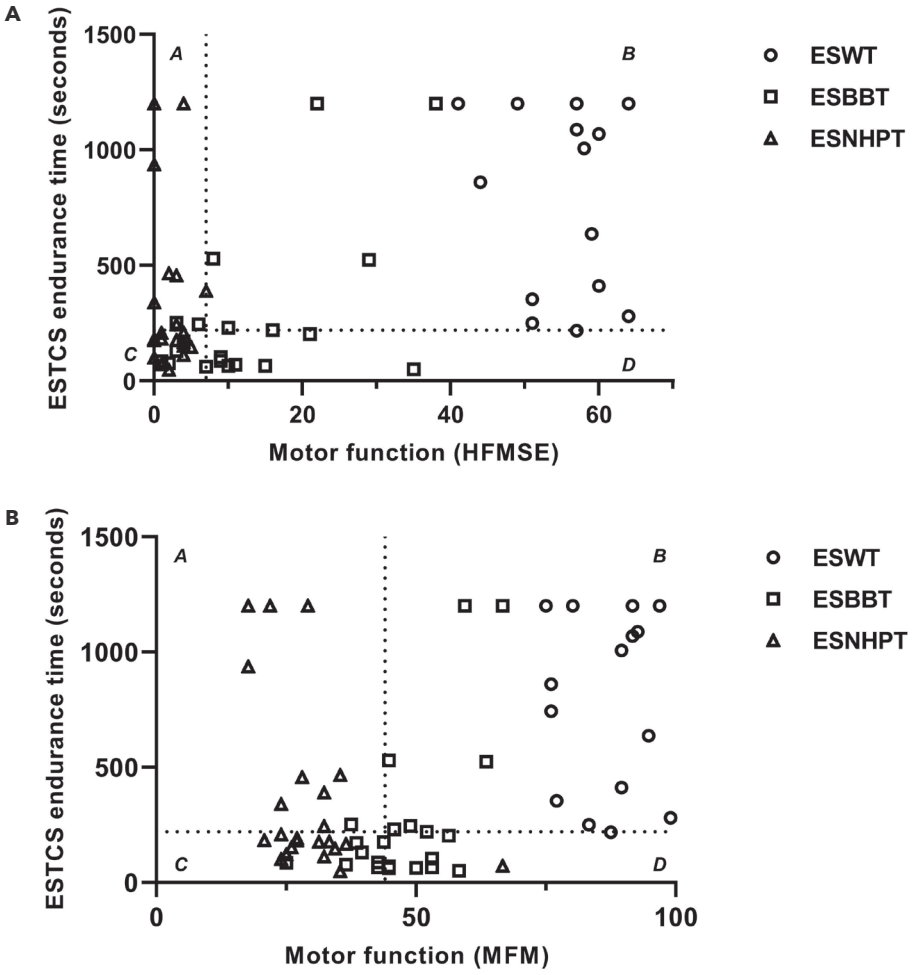


**Figure 2AB.** Distribution of motor function scores
Distribution of individual values of independent variables (X-axis) and dependent variable (Y-axis). HFMSE = Hammersmith Functional Motor Scale Expanded. MFM = Motor Function Measure. ESTCS = Endurance Shuttle Test Combined Score. Dashed lines are median group scores. Quadrant A: low motor function and high endurance; B: high motor function and high endurance; C: low motor function and low endurance; D: high motor function and low endurance.

### Neuromuscular junction function

The results of the nerve conduction study are summarized in table 2. Fifty-three patients were ≥ 12 years and eligible for assessment. One child did not give consent.

*Accessory nerve (m. Trapezius)*

CMAP could not be quantified in 15 patients due to technical difficulties. Twenty-six patients (50%) were excluded from RNS analysis because the CMAP amplitude was <1.0 mV or because RNS was technically impossible. There was no significant association between neuromuscular junction function of the accessory nerve and fatigability (HR = .686 (.299 – 1.574), $p$ = .37).

*Ulnar nerve (m. ADM)*

CMAP could not be quantified in two patients due to technical difficulties. Thirteen patients (26%) were excluded from RNS analysis because of a CMAP amplitude <1.0 mV or because the results of RNS were of poor quality. There was no significant association between neuromuscular junction function of the ulnar nerve and fatigability (HR = .511 (.252 – 1.038), $p$ = .063).

**Table 2.** Repetitive nerve stimulation findings in patients with SMA

| Nerve (muscle group) | Type 2 (N=24) | Type 3a (N=13) | Type 3b (N=14) | Type 4 (N=1) | All types (N=52) |
|---|---|---|---|---|---|
| **Accessory nerve (ADM)** | | | | | |
| Mean CMAP amplitude (mV) (Med, min-max) | 1.8 (0.4 - 4.9) (N=22) | 4.5 (1.7 – 9.3) (N=13) | 8.8 (2.1 – 15) (N=14) | 5.3 - (N=1) | 4.5 (0.4 – 15) (N=50) |
| Patients with decrement ≥ 10% N (%) | 39% (N=13) | 0 % (N=12) | 8% (N=13) | 0% (N=1) | 15% (N=39) |
| **Ulnar nerve (Trapezius)** | | | | | |
| Mean CMAP amplitude (mV) (Mdn, min-max) | 1.4 (0.4 - 5.5) (N=14) | 3.0 (0.5 - 7.8) (N=10) | 6.6 (3.7 - 11.3) (N=12) | 11.3 - (N=1) | 3.7 ( .4 – 11.3) (N=37) |
| Patients with decrement ≥ 10% (%) | 80% (N=5) | 43% (N=7) | 31% (N=13) | 0% (N=1) | 42% (N=26) |

ADM = Abductor Digiti Minimi. CMAP = Compound Muscle Action Potential ; Decrement = difference between amplitude of first and fifth CMAP in a train of 10 at 3Hz stimulation expressed as percentage (%)

### Perceived fatigue

Sixteen children (94%) filled out the PROMIS Ped SF Fatigue 10a and thirty-nine adults (89%) filled out the PROMIS SF Fatigue 8a. Mean T-scores are reported in table 1. One adult (T-score = 72.2) demonstrated an abnormal high level of perceived fatigue ( >2sd) but none of the children (Figure 3). There was no significant association between perceived fatigue and fatigability (HR = .99 (.961 – 1.02), $p$ = .52).



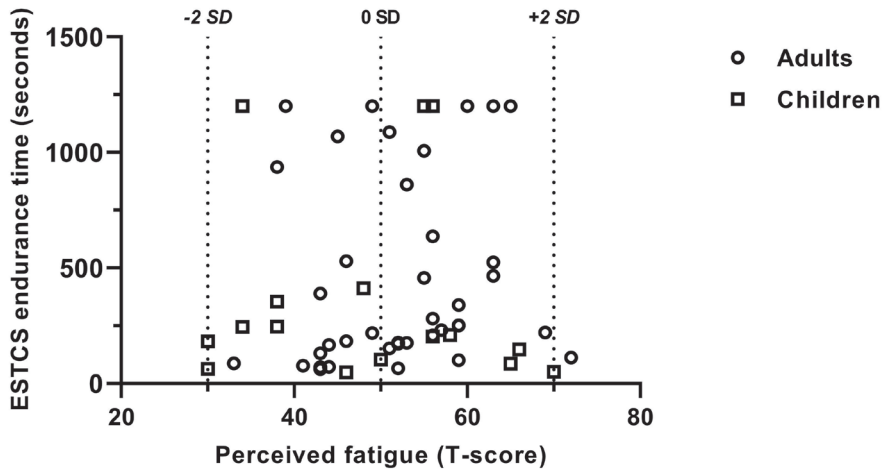


**Figure 3.** Distribution of perceived fatigue scores
ESTCS = Endurance Shuttle Test Combined Score

## Discussion

The objective of this study was to determine the associations between fatigability and muscle strength, motor function, NMJ- function and perceived fatigue in children and adults with SMA type 2-4 across the severity spectrum. We show that fatigability in SMA is significantly associated with muscle strength and motor function. However we also detected prominent fatigability despite preserved muscle function (and vice versa) in a substantial subgroup of patients.

To date, functional scales on muscle strength and motor function such as the HFMSE and MFM have been used to capture meaningful improvements in physical functioning in children and adolescents with SMA who participated in clinical trials[26,27]. With the emergence of fatigability as an important additional dimension of physical impairment in SMA, existing outcome

measures have been adapted and new fatigability tests have been developed and validated[6,10,28,29]. Research into factors associated with fatigability was limited to small cohorts of patients or has focussed on general indicators of disease severity such as disease onset and disease duration. Recent studies have shown that patients with SMA type 3 with early disease onset (type 3a) and/or patients aged 11 years and older were more prone to fatigability during the 6 Minute Walk Test[30,31]. Among patients with SMA type 2 there was no effect of age at disease onset or disease duration upon fatigability of distal arm function during five consecutive rounds of the nine hole peg test[29]. The current study is the first to systematically evaluate fatigability and its associations with muscle weakness and motor function impairments as direct measures of disease severity in a large cohort of patients with SMA.

Fatigability was not significantly associated with NMJ-function in our cohort, but we did find a trend towards an effect of neuromuscular transmission failure of the ulnar nerve upon ESTCS dropout. A recent study reported a strong correlation between the decrease in walking velocity during the 6MWT and absolute decrement values of the axillar nerve in a small cohort of ambulatory patients[32]. This difference in outcome between these two studies may be explained by the high percentage of missing values in RNS due to the technical difficulties in patients with SMA type 2, and may have led to underestimation of the prevalence of NMJ- dysfunction and its association with fatigability in our study sample[11,33]. A contribution of NMJ-dysfunction to fatigability is supported by the results from a randomized double-blind cross-over study on the effects of the acetylcholinesterase inhibitor pyridostigmine on motor function and fatigability in SMA[34,35]. Patients with SMA types 2-4 demonstrated a 70% reduced drop-out risk on the ESTCS under pyridostigmine while motor function did not improve significantly.

Patients with SMA reported similar levels of perceived fatigue compared to the general population despite high prevalence of fatigability. Our results are consistent with a recent study that found no relationship between perceived fatigue and fatigability during the 6MWT in patients with SMA type 2-3[18]. Fatigability and perceived fatigue in SMA should therefore be appreciated as distinct concepts requiring specific diagnostic and therapeutic approaches.

Other factors than muscle weakness and involvement of the NMJ may contribute to fatigability. Several mouse and human studies reported mitochondrial dysfunction in SMA[36-38]. The clinical relevance of mitochondrial abnormalities found in ex-vivo studies have yet to be studied in patients but this could provide an additional pathway to reduce fatigability in SMA.

An important strength of this study is that we used the ESTCS, a selection of endurance tests tailored to the highest level of motor function of the individual patient, to study fatigability and associated factors. The ESTCS allowed us to explore clinical relevant associations with fatigability in a large and heterogenous study sample. Other outcome measures such as the 6MWT and r9HPT only apply to ambulatory type 3 or type 2 patients, which makes it difficult to produce adequate sample sizes. Fatigability in SMA is associated with, yet not equivalent to muscle strength and motor function. This implies that endurance tests should be included in future clinical trials and can be considered for supportive care teams in order to capture meaningful changes in physical functioning that are not covered by motor function scales or strength assessment.



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# TREATMENT

Chapter 6

# Physical exercise training for type 3 spinal muscular atrophy




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# Abstract

## Background

Physical exercise training might improve muscle and cardiorespiratory function in spinal muscular atrophy (SMA). Optimization of aerobic capacity or other resources in residual muscle tissue through exercise may counteract the muscle deterioration that occurs secondary to motor neuron loss and inactivity in SMA. There is currently no evidence synthesis available on physical exercise training in people with SMA type 3.

## Objectives

To assess the effects of physical exercise training on functional performance in people with SMA type 3, and to identify any adverse effects.

## Search methods

On 8 May 2018, we searched the Cochrane Neuromuscular Specialised Register, Cochrane Central Register of Controlled Trials, MEDLINE, Embase, CINAHL, AMED, and LILACS. On 25 April 2018 we searched NHSEED, DARE, and ClinicalTrials.gov and WHO ICTRP for ongoing trials.

## Selection criteria

We included randomized controlled trials (RCTs) or quasi-RCTs lasting at least 12 weeks that compared physical exercise training (strength training, aerobic exercise training, or both) to placebo, standard or usual care, or another type of non-physical intervention for SMA type 3. Participants were adults and children from the age of five years with a diagnosis of SMA type 3 (Kugelberg-Welander syndrome), confirmed by genetic analysis.

## Data collection and analysis

We used standard Cochrane methodological procedures.

## Main results

We included one RCT that studied the effects of a six-month, home-based, combined muscle strength and recumbent cycle ergometry training program versus usual care in 14 ambulatory people with SMA. The age range of the participants was between 10 years and 48 years. The study was evaluator-blinded, but personnel and participants could not be blinded to the intervention, which placed the results at a high risk of bias. Participants performed strength training as prescribed, but 50% of the participants did not achieve the intended

aerobic exercise training regimen. The trial used change in walking distance on the six-minute walk test as a measure of function; a minimal detectable change is 24.0 m. The change from baseline to six months' follow-up in the training group (9.4 m) was not detectably different from the change in the usual care group (-0.14 m) (mean difference (MD) 9.54 m, 95% confidence interval (CI) -83.04 to 102.12; N = 12). Cardiopulmonary exercise capacity, assessed by the change from baseline to six months' follow-up in peak oxygen uptake ($VO_{2max}$) was similar in the training group (-0.12 mL/kg/min) and the usual care group (-1.34 mL/kg/min) (MD 1.22 mL/kg/min, 95% CI -2.16 to 4.6; N = 12). A clinically meaningful increase in $VO_{2max}$ is 3.5 mL/kg/min.

The trial assessed function on the Hammersmith Functional Motor Scale - Expanded (HFMSE), which has a range of possible scores from 0 to 66, with an increase of 3 or more points indicating clinically meaningful improvement. The HFMSE score in the training group increased by 2 points from baseline to six months' follow-up, with no change in the usual care group (MD 2.00, 95% CI -2.06 to 6.06; N = 12). The training group showed a slight improvement in muscle strength, expressed as the manual muscle testing (MMT) total score, which ranges from 28 (weakest) to 280 (strongest). The change from baseline in MMT total score was 6.8 in the training group compared to -5.14 in the usual care group (MD 11.94, 95% CI -3.44 to 27.32; N = 12).

The trial stated that training had no statistically significant effects on fatigue and quality of life. The certainty of evidence for all outcomes was very low because of study limitations and imprecision. The study did not assess the effects of physical exercise training on physical activity levels. No study-related serious adverse events or adverse events leading to withdrawal occurred, but we cannot draw wider conclusions from this very low-certainty evidence.

## Authors' conclusions

It is uncertain whether combined strength and aerobic exercise training is beneficial or harmful in people with SMA type 3, as the quality of evidence is very low. We need well-designed and adequately powered studies using protocols that meet international standards for the development of training interventions, in order to improve our understanding of the exercise response in people with SMA type 3 and eventually develop exercise guidelines for this condition.

# Plain language summary

## Review question

In people with spinal muscular atrophy (SMA) type 3, does physical exercise training improve motor function, cardiovascular fitness, muscle strength, fatigue, physical activity levels, or quality of life, and does it have unwanted effects?

## Background

Physical exercise training could improve the physical fitness of people with SMA type 3 and protect them from muscle wasting due to inactivity and disease progression. However, we do not know whether physical exercise training is safe or what specific parts of an exercise program might be helpful. We reviewed the evidence about the effect of physical exercise training in people with SMA type 3.

## Search date

The evidence is up to date to May 2018.

## Study characteristics

We included one trial that studied the effects of a six-month, home-based training program that combined exercises to increase muscle strength with aerobic exercise training (exercise that increases breathing and heart rate). The aerobic exercise training used in the trial was recumbent cycling training (seated cycling, with back support). The study included 14 people with SMA type 3, all of whom were able to walk. The participants were between 10 years and 48 years old and had SMA type 3 of mild-to-moderate severity. The nature of the intervention made it impossible to hide the treatment group from participants or personnel, which is an important limitation when measurements rely on participant assessments or effort.

## Key results and certainty of the evidence

Participants performed strength training as prescribed, but only half of them completed the full aerobic exercise program.

The effects of physical exercise training in people with SMA type 3 remain unclear, as the evidence is very uncertain.

## Summary of findings table

1 Combined strength and aerobic exercise training compared to usual care in SMA type 3

Comparison 1. Combined strength and aerobic exercise training compared to usual care in SMA type 3

Patient or population: children and adults with SMA type 3
Setting: home-based exercise, clinic follow-up
Intervention: combined strength and aerobic exercise training
Comparison: usual care

| **Outcomes** | **Anticipated absolute effects* (95% CI)** | | **Relative effect (95% CI)** | **Nº of participants (studies)** | **Certainty of the evidence (GRADE)** | **Comments** |
|---|---|---|---|---|---|---|
| | **Risk with usual care** | **Risk with combined strength and aerobic exercise training** | | | | |
| **Outcomes from aerobic exercise training** | | | | | | |
| Walking distance on the 6MWT (m) (a higher score indicates better function)<br>Change from baseline<br>Follow up: 6 months | The mean change in distance walked on the 6MWT in the usual care group was -0.14 m. | The mean change in distance walked on the 6MWT in the training group was 9.54 m more (83.04 less to 102.12 more). | - | 12<br>(1 RCT) | ⊕⊖⊖⊖<br>VERY LOW[a,b,c] | - |
| Cardiopulmonary exercise capacity assessed with $VO_{2max}$ mL/kg/min (a higher score indicates better function)<br>Change from baseline<br>Follow up: 6 months | The mean change in $VO_{2max}$ in the usual care group was -1.34 mL/kg/min. | The mean change in $VO_{2max}$ in the training group was 1.22 mL/kg/min more (2.16 less to 4.6 more). | - | 12<br>(1 RCT) | ⊕⊖⊖⊖<br>VERY LOW[a,b,c] | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Outcomes from strength training** | | | | | | |
| Functional performance assessed with HFMSE<br>Scale from: 0 to 66 (a higher score indicates better function)<br>Change from baseline<br>Follow up: 6 months | The mean change in HFMSE score in the usual care group was 0. | The mean change in HFMSE score in the training group was 2 points more (2.06 points less to 6.06 points more). | - | 12<br>(1 RCT) | ⊕⊕⊖⊖<br>VERY LOW[b,c] | - |
| Muscle strength (MMT total score) assessed with Medical Research Council total MMT score<br>Scale from: 28 to 280 (a higher score indicates greater muscle strength)<br>Change from baseline<br>Follow up: 6 months | The mean change in MMT total score in the usual care group was -5.14. | The mean change in MMT total score was 11.94 more (3.44 less to 27.32 more). | - | 12<br>(1 RCT) | ⊕⊕⊖⊖<br>VERY LOW[b,c] | - |
| **Outcomes from either type of training** | | | | | | |
| Fatigue<br>In children, assessed with PedsQLMFS<br>Scale from:<br>0 to 100 (a higher score indicates less fatigue)<br>In adults, assessed with FSS<br>Scale from: 0 to 7 (a higher score indicates more fatigue)<br>Change from baseline<br>Follow up: 6 months | The trial reported no significant differences between the training group and the usual care group in parent-reported PedsQLMFS score, child-reported PedsQLMFS score, or FFS score in adults, but did not report P values. | | - | 12<br>(1 RCT) | ⊕⊖⊖⊖<br>VERY LOW[a,b,c] | - |
| Physical activity - change from baseline<br>Follow up: 6 months - not reported | No evidence available on physical activity levels | | - | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Quality of life<br>In children, assessed with PedsQLNM<br>Scale from: 0 to 100 (a higher score indicates better quality of life)<br>In adults, assessed with SF-36 subdomains Physical Health (SF-36PH) and Mental Health (SF-36MH)<br>Scales from: 0 to 100 (a higher score indicates better quality of life)<br>Change from baseline<br>Follow up: 6 months | The trial reported no significant differences between the training group and the usual care group in child-reported or parent-reported PedsQLNM score, or in SF-36PH and SF-36MH scores, but did not provide P values. | - | 12<br>(1 RCT) | ⊕⊖⊖⊖<br>VERY LOW[a,b,c] | - |
| Serious adverse events leading to withdrawal<br>Follow up: 6 months | No study-related serious adverse events or adverse events leading to withdrawal occurred. | - | 12<br>(1 RCT) | ⊕⊖⊖⊖<br>VERY LOW[a,b,c] | - |

*The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group in the included study and the relative effect of the intervention (and its 95% CI).

6MWT: 6-minute walk test; CI: confidence interval; FSS: Fatigue Severity Scale; PedsQLMFS: Pediatric Quality of Life Inventory Multi Dimensional Fatigue Scale; PedsQLNM: Pediatric Quality of Life Inventory Neuromuscular Module; RR: risk ratio; SF-36: 36-Item Short-Form Health Survey

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect

## Footnotes

[a]We downgraded the certainty of the evidence once for indirectness. Fifty per cent of the training group did not receive the intended volume of aerobic exercise training owing to decreased exercise tolerability.
[b]We downgraded the certainty of the evidence twice for imprecision. There were few participants (training group N = 5, usual care group N = 7) and the wide CI encompassed large effects in either direction.
[c]We downgraded the evidence once for study limitations. No participant blinding was possible, which placed all outcomes at high risk of bias since all were either effort dependent or participant-reported.

# Background

## Description of the condition

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease caused by a genetic mutation in the survival motor neuron 1 (*SMN1*) gene (5q11.2-q13.3) (Lefebvre 1995). SMA is characterized by degeneration of spinal cord α-motor neurons, which results in progressive proximal muscle weakness, fatigue, scoliosis, nutritional problems, respiratory complications, and severe functional limitations. SMA has a broad clinical spectrum but, in general, can be classified into four clinical types based on age of onset and maximum motor function achieved (Mercuri 2012). With an incidence of one in 10,000 live births, SMA type 1 is the leading genetic cause of infant death, accounting for 60% of all cases of SMA (Verhaart 2017). SMA type 1 is characterized by an onset before six months of age and an inability to sit without support. The onset of SMA type 2 is between seven months and 18 months of age and those affected have the ability to sit independently, but not to walk. SMA type 4 is the mildest form, with the onset of weakness in the second or third decade (Lunn 2008; Mercuri 2012). SMA type 3 (Kugelberg-Welander syndrome) is a relatively mild subtype, with symptom onset typically after 18 months of age, but shows large clinical heterogeneity. SMA type 3 can be further classified into type 3a (clinical symptoms before three years of age) and type 3b (clinical symptoms after three years of age) (Zerres 1997). Children generally reach major milestones, including independent walking, but their level of motor performance varies greatly. Some children are hardly able to stand up from sitting and take a few steps unaided, while others walk well, are able to climb stairs, and mainly experience problems in running and sports (Rudnik-Schöneborn 2001). Long-term follow-up studies (follow-up time of two to 20 years) in people with SMA type 2 and type 3 suggest a very slow deterioration of muscle strength and motor function that takes years to detect (Deymeer 2008; Kaufmann 2012; Piepers 2008; Wadman 2017; Wadman 2018; Werlauff 2012). Nevertheless, about 50% of people with SMA type 3 will lose independent ambulation during the second decade of life and only a small subgroup will remain ambulatory throughout life (Wadman 2017; Mercuri 2012; Russman 1996). In general, people with SMA type 3b perform better on functional outcome measures, such as the six-minute walk test (6MWT) and the Hammersmith Functional Motor Scale - Expanded (HFMSE), in comparison to people with SMA type 3a (Mazzone 2013; Montes 2010). Nusinersen is the only disease-modifying therapy for people with SMA, but its benefits in the mildest phenotype are not yet fully

known, since efficacy was determined in a cohort of patients with more severe muscle weakness (Mercuri 2018). Current standards of care concentrate on SMA-associated complications, such as impaired mobility, scoliosis, fatigue, respiratory infections, and poor nutritional status (Mercuri 2018b).

## Description of the intervention

The intervention under consideration is physical exercise training for children and adults with SMA type 3. Training methods include strength and aerobic exercise training of skeletal muscles. We have not considered respiratory muscle training in people with neuromuscular diseases, as this is the topic of a Cochrane Systematic Review in development (Pedrosa 2015). Types of exercise include, for example, cycling on an ergometer, running on a treadmill, and lifting weights. Physical exercise training aims to increase a person's functional performance, muscle strength, cardiopulmonary exercise capacity and quality of life, and reduce levels of fatigue. These benefits should be achieved without serious adverse events, such as worsening fatigue, pain, or significant increases in levels of biological markers for muscle damage. Suitable comparison interventions are placebo and standard or usual care. The training can be given as monotherapy or in addition to usual practice.



### *Definitions*

- Physical exercise training or physical fitness training: "a planned, structured regimen of regular physical exercise deliberately performed to improve physical fitness. The ability to carry out daily tasks with vigor and alertness, without undue fatigue and with ample energy to enjoy [leisure] pursuits and to meet unforeseen emergencies".
- Physical fitness: "a set of measurable health and skill-related attributes" that includes cardiorespiratory fitness, muscular strength and endurance, body composition, flexibility, balance, agility, reaction time, and power (Caspersen 1985; Garber 2011).
- Strength training: training performed primarily to improve muscle strength and endurance, typically through repeated muscle contractions against resistance (Saunders 2004).
- Aerobic exercise training or cardiorespiratory fitness training: training that consists of an activity or combination of activities using large muscle groups that can be maintained continuously, for example, walking-hiking, running-jogging, cycling-bicycling, or swimming (Pollock 1998).
- Functional performance: performance on functional scores, such as functional strength scores, timed tests, and walking tests.

For physically stronger people with SMA type 3b, physical training is a potentially easily accessible and affordable intervention, which could be provided through exercise groups or personal trainers working together with health practitioners. Those who have significant difficulty with transfers, uneven surfaces, and stairs are more vulnerable to injury and require specialized supervision.

## How the intervention might work

The loss of α-motor neurons in the spinal cord leads to denervation of skeletal muscles, atrophy, and muscle weakness (Mercuri 2012). Functional performance, especially ambulation, deteriorates in most people with SMA type 3, which may lead to inactivity and deconditioning (Wadman 2017). The slow progression of the disease, the relatively preserved residual strength, and a sedentary lifestyle make people with SMA type 3 a promising target population for physical training programs. Training may improve functional performance, muscle strength, and exercise capacity by optimizing resources and metabolic function in available muscle tissue and counteracting further muscle deterioration that occurs with inactivity (Abresch 2012). The effect is likely to depend on the type of training. Strengthening training may increase muscle strength and, as a secondary effect, improve functional performance of anti-gravity activities, such as rising from lying or sitting positions, jumping, and stair climbing. Aerobic exercise training will enhance exercise capacity and improve walking distance and endurance.

Exercise might also have a neuroprotective effect, which could be explained by a relationship between the maturation state of the motor unit and resistance to neuronal cell death. Preclinical studies in SMA mouse models report positive effects of exercise on postnatal maturation of motor units; delayed motor neuron death; and improved motor function and survival (Biondi 2008; Grondard 2005). Biondi 2008 performed a progressive running-wheel training program in SMA type 2-like mice and showed an exercise-induced acceleration of motor-unit maturation at the level of the motor neuron, neuromuscular junction, and muscle fiber, and a delay in motor neuron death. In addition, Grondard 2005 reported a positive effect of exercise on muscle performance measured with a forelimb grip strength-endurance test and physical activity measured with an open-field ambulatory behavior test.

### Why it is important to do this review

Physical exercise training has emerged as a potential intervention for people with inherited neuromuscular disorders for which no curative treatment is as yet available, including people with SMA. Skeletal muscle training may partly counteract disease progression and secondary deconditioning by improvement of functional performance (Voet 2013). At a time when some people with SMA are benefiting from the first approved disease-modifying compound aimed at splicing the *SMN2* gene, and other compounds that directly target skeletal muscle are in development, understanding the effects of conservative treatments remains important. There is currently limited evidence available on physical exercise training in people with SMA type 3. The potential for combination therapies may be better exploited if we first understand the role of exercise therapy when used alone.



### Objectives

To assess the effects of physical exercise training on functional performance in people with spinal muscular atrophy type 3, and to identify any adverse effects.

## Methods

### Criteria for considering studies for this review

#### *Types of studies*

We included randomized controlled trials (RCTs) and quasi-RCTs. Quasi-RCTs are studies that use a quasi-random method to allocate participants to groups, such as alternation, date of birth, or case record number (Higgins 2011). In the 'Discussion' section, we described relevant cross-over studies, case control studies and multi- and single-case reports that fulfilled the same standards as eligible RCTs regarding diagnostic criteria, description of intervention, and outcome measures. We considered trials available in any language, whether available as full-text articles, abstracts, or unpublished data only.

#### *Types of participants*

We included studies in children from the age of five years and adults with a diagnosis of spinal muscular atrophy (SMA) type 3 (Kugelberg-Welander syndrome) who fulfilled the clinical criteria and had a deletion or mutation of the survival motor neuron 1 (*SMN1*) gene (5q11.2-13.2) confirmed by genetic analysis (Lefebvre 1995). Studies of mixed populations, e.g. studies that include

mixed neuromuscular diseases or mixed SMA types, were only eligible for inclusion in the review if they reported results for SMA type 3 separately.

### *Types of interventions*

We included trials that used any form of physical exercise training of skeletal muscles, including aerobic exercise and strength training, carried out for a period of at least 12 weeks, compared with placebo, standard or usual care, or another type of non-physical intervention. We included trials that provided co-interventions to each group equally. We excluded studies of respiratory muscle training or that used a non-exercised limb as a control. We included trials that used training programs standardized on frequency, intensity, time, and type of training, with an incremental exercise protocol.

### *Types of outcome measures*

We included studies that reported outcomes at baseline and at the end of training. We would have reported longer-term outcomes if they had been available.

### *Primary outcomes*

- Walking distance on the six-minute walk test (6MWT; Dunaway 2016; Montes 2010).
- Functional performance, measured with the Hammersmith Functional Motor Scale - Expanded (HFMSE; O'Hagen 2007), Motor Function Measure (MFM; Vuillerot 2013), and timed tests (10-meter walk/run test (10MWT), Gower's time, or Timed Up and Go Test (TUG; Dunaway 2014)).

### *Secondary outcomes*

- Cardiopulmonary exercise capacity, assessed with validated cycle ergometry (W, mL/kg/min) or treadmill testing (mL/min, time to limitation) (Bartels 2015).
- Muscle strength, including maximal isometric and isokinetic voluntary contraction, measured with validated dynamometry (Newton/N*M) and validated Manual Muscle Testing (MMT; an ordinal scale).
- Fatigue, assessed in adults with the Fatigue Severity Scale (FSS; Werlauff 2014) and in children with the Pediatric Quality of Life Inventory Multi Dimensional Fatigue Score (PedsQLMFS; Varni 2004).
- Physical activity, assessed with questionnaires or accelerometry.
- Quality of life, assessed in adults with the 36-Item Short-Form Health Survey (SF-36; Kruitwagen-Van Reenen 2016) questionnaire and in children with

the Pediatric Quality of Life Inventory Neuromuscular Module (PedsQLNM; Iannaccone 2009).
- Serious adverse events leading to withdrawal, such as debilitating fatigue, medical treatment, and hospitalization.

We reported continuous outcomes as changes from baseline.

## Search methods for identification of studies

### *Electronic searches*

The Cochrane Neuromuscular Information Specialist searched the following databases.



- Cochrane Neuromuscular Specialised Register via the Cochrane Register of Studies (CRS-Web; 8 May 2018; Appendix 1).
- Cochrane Central Register of Controlled Trials (CENTRAL) via the CRS-Web (8 May 2018; Appendix 2).
- MEDLINE (1946 to 8 May 2018; Appendix 3).
- Embase (1980 to 8 May 2018; Appendix 4).
- CINAHL Plus (1937 to 8 May 2018; Appendix 5).
- AMED (1985 to 8 May 2018; Appendix 6).
- LILACS (1982 to 8 May 2018; Appendix 7).

The review authors searched the following databases.

- US National Institutes for Health Clinical Trials Registry (www.ClinicalTrials.gov; 25 April 2018; Appendix 8).
- World Health Organization International Clinical Trials Registry Platform (ICTRP) (apps.who.int/trialsearch/; 25 April 2018; Appendix 9).
- NHS Economic Evaluation Database (NHSEED; https://www.crd.york.ac.uk/CRDWeb/; 25 April 2018 (updates until 31 March 2015); Appendix 10).
- Database of Abstracts of Reviews of Effects (DARE; https://www.crd.york.ac.uk/CRDWeb/; 25 April 2018 (updates until March 2015); Appendix 11).

We searched all databases from inception to the present, and did not impose any restriction on language of publication.

### *Searching other resources*

We searched reference lists of review articles and of the included trial for additional references. We also searched for errata or retractions of the included trial.

## Data collection and analysis

### *Selection of studies*

Two review authors (BB and JM) independently screened titles and abstracts of all references identified as a result of the literature searches. We coded the articles as either 'retrieve' (eligible, potentially eligible, or unclear), or 'do not retrieve'. We retrieved the full-text study reports and publications coded as 'retrieve'. Two review authors (BB and JM) independently screened the full-text articles, identified trials for inclusion, and identified and recorded reasons for exclusion of ineligible studies. The review authors resolved any disagreements through discussion or, if required, they consulted a third review author (JdG). We identified and excluded duplicates, and collated multiple reports of the same trial so that each trial rather than each report was the unit of interest in the Cochrane Systematic Review. We completed a PRISMA flow diagram and a 'Characteristics of excluded studies' table.

### *Data extraction and management*

We used a data extraction form to initially pilot one trial included in the review to collect study characteristics and outcome data. One review author (BB) extracted the following study characteristics.

- Methods: study design, total duration of study, details of any 'run in' period, number of study centers and location, study setting, withdrawals, and date of study.
- Participants: number, mean age, age range, gender, severity of condition, diagnostic criteria, baseline characteristics, inclusion criteria, and exclusion criteria.
- Interventions: intervention, comparison, concomitant treatments, and excluded treatments.
- Outcomes: primary and secondary outcomes specified and collected, and time points reported.
- Notes: funding for trial, and notable conflicts of interest of trial authors.

A review author (JM) authored the included trial. Therefore, BB and JdG independently extracted outcome data from this trial. We noted in the 'Characteristics of included studies' table if the trial did not report outcome data in a usable way. We resolved any disagreements by consensus. One review author (BB) transferred data into Review Manager (RevMan) 5 (RevMan 2014). A second review author (JM) checked the outcome data entries. The same review author (JM) spot-checked study characteristics for accuracy against the trial report.

## Assessment of risk of bias in included studies

Two review authors (BB and JdG) independently performed 'Risk of bias' assessments for the included trial using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We resolved any disagreements by discussion. We assessed the risk of bias according to the following domains.

- Random sequence generation.
- Allocation concealment.
- Blinding of participants and personnel.
- Blinding of outcome assessment.
- Incomplete outcome data.
- Selective outcome reporting.
- Other bias.

The review authors graded each study as at high, low, or unclear risk of bias and provided a quote from the study report together with a justification for the judgment in the 'Risk of bias' table. We did not consider it necessary to consider blinding separately for subjective and objective outcomes, as the outcomes in the review were either effort dependent or subjective, and were therefore all at a high risk of bias from a lack of participant blinding. We noted in the 'Risk of bias' table when we based a 'Risk of bias' assessment on unpublished data or correspondence with a trial author. When considering treatment effects, we only took into account the risk of bias that contributed to that outcome.

## Assesment of bias in conducting the systematic review

We conducted the Cochrane Systematic Review according to the published protocol (Bartels 2016). We described any protocol deviations in the Differences between protocol and review section.

## Measures of treatment effect

We analyzed continuous data as mean differences (MDs) with corresponding 95% confidence intervals (CIs).

## Dealing with missing data

We planned to contact trial authors or trial sponsors to verify key study characteristics and, where possible, obtain missing numerical outcome data (e.g. when a trial was available as an abstract only or when SMA subgroup data were not reported separately). We obtained additional information on random sequence generation and allocation concealment from the authors of the included trial (Montes 2015).

We used RevMan to obtain missing standard deviations from P values for the differences between means in the two groups (RevMan 2014).

## Data synthesis

See Appendix 12 for methods of data synthesis described in the protocol (Bartels 2016).

## 'Summary of findings' tables

We planned to create separate 'Summary of findings' tables for aerobic exercise training and strength training. However, we presented findings from the included study, which combined both types of training, in a single table.

- Outcomes from aerobic exercise training
  - Walking distance on the 6MWT
  - Cardiopulmonary exercise capacity
- Outcomes from strength training
  - Functional performance
  - Muscle strength
- Outcomes from either type of training
  - Fatigue
  - Physical activity
  - Quality of life
  - Serious adverse events leading to withdrawal

We used the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness, and publication bias) to assess the certainty of a body of evidence (studies that contribute data for the prespecified

outcomes). We used methods and recommendations described in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), and constructed 'Summary of findings' tables using GRADEpro Guideline Development Tool software (GRADEpro GDT 2015). We provided footnotes to justify our decisions to downgrade or upgrade the quality of the evidence, and we commented where necessary to aid the reader's understanding of the Cochrane Systematic Review.

### Subgroup analysis and investigation of heterogeneity

The included trial, which had 12 participants, was too small to perform subgroup analyses.



### Reaching conclusions

We based our conclusions on findings from the quantitative and narrative review of the included trial. We avoided making recommendations for practice. Our Implications for research section suggests priorities for future research and outlines the remaining uncertainties in the area.

## Results

### Description of studies

#### *Results of the search*

The search retrieved 513 records. After removal of duplicates, we screened the titles and abstracts of 444 records. We identified 10 studies for full-text review, of which we excluded 9 because they were not randomized controlled trials (RCTs). We included one study (Montes 2015). See Figure 1 for a PRISMA flow-chart illustrating the study selection process.

#### *Included studies*

Montes 2015 was an evaluator-blinded RCT that studied the effects of a home-based, combined muscle strength and recumbent cycle ergometry training program in 14 participants with spinal muscular atrophy (SMA) type 3, who were ambulatory and ranged in age from 10 years to 48 years. Three participants had SMA subtype 3a and 11 had subtype 3b. Participants had SMA of mild-to-moderate severity; mean baseline scores on the Hammersmith Functional Motor Scale - Expanded (HFMSE) were 53.4 (standard deviation (SD) 8.9) in the exercise group and 54.0 (SD 8.2) in the usual care group. The investigators randomly allocated participants to exercise or usual care groups

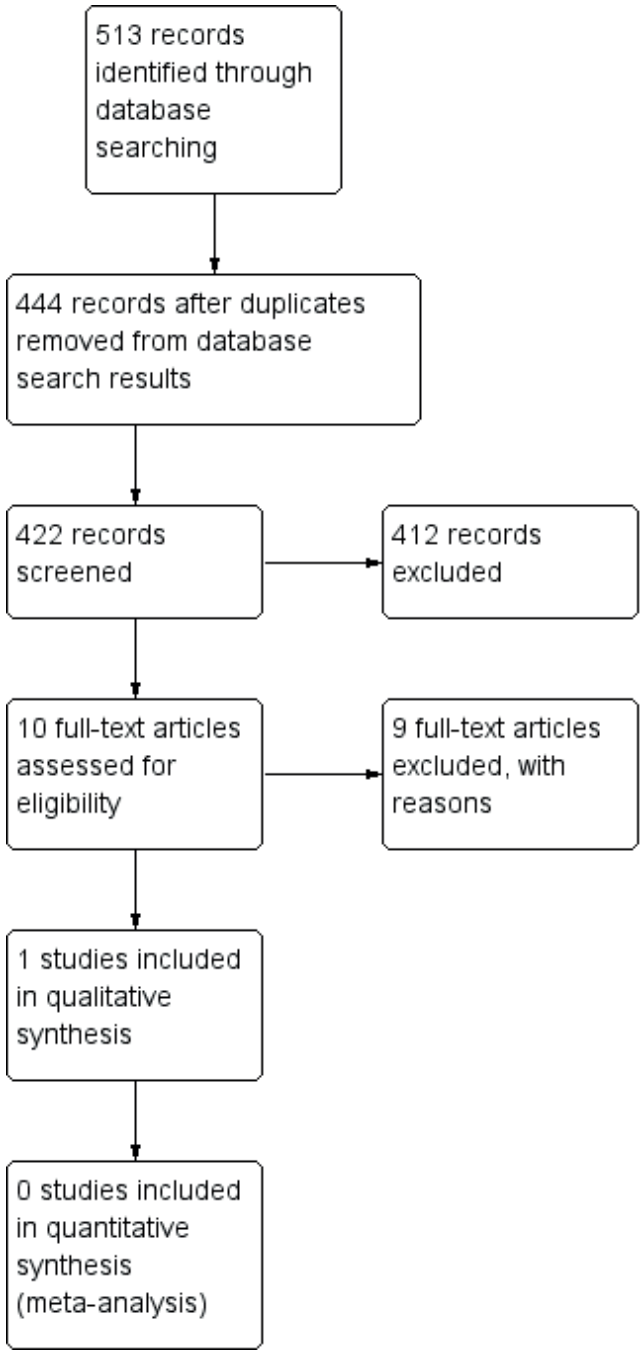


**Figure 1.** Study flow diagram.

after a one-month lead-in period. During months two to seven, the intervention group received training, while the control group continued their usual care. The muscle strength training consisted of a three-times-weekly program of three sets of five-to-six concentric, isometric, or gravity-eliminated exercises, performed at an intensity of 60% to 80% of one repetition maximum. Most participants reported that they performed the strength training program as prescribed, but the trial authors did not mention specific percentages of compliance. The aerobic training consisted of a five-times-weekly program of 30-minute recumbent cycling sessions, performed at an exercise intensity of 5 to 7 on the OMNI Scale of Perceived Exertion. Only one participant after three months and 50% of participants after six months achieved the target exercise volume of 150 minutes per week. The exercise volume of the other participants ranged from 24 minutes to 91 minutes per week at the same time points. After month seven, both groups received the exercise intervention for a further 12 months. Twelve participants completed the first seven months and nine participants completed all 19 months of the trial. The two participants who dropped out during the controlled period were in the training group. The trial authors analyzed the results from the controlled period (months two to seven). They monitored compliance with a heart monitor, participant diary, teleconference and videoconference calls, and via text, phone, and email communications. Program compliance was enhanced by the use of customized illustrated instructional exercise sheets. The investigators asked participants about adverse events, including falls, excessive fatigue, muscle soreness, illness, and other health-related events at every contact, whether in person or by videoconference. The Characteristics of included studies table includes additional information on participants and study design.



### *Excluded studies*

We excluded nine studies that were not RCTs (see Characteristics of excluded studies). The interventions were strength training alone in four studies (Basoglu 2006; Lewelt 2015; McCartney 1988; Milner-Brown 1988), aerobic exercise training alone in one study (Madsen 2015), aquatic therapy incorporated in a comprehensive rehabilitation program in three studies (Cunha 1996; Dahl 2004; Salem 2010), and a combination of functional strengthening exercises and whole body vibration in one study (Vry 2014). Strength training consisted of concentric resistance training either alone (in McCartney 1988 and Milner-Brown 1988) or in combination with gravity-eliminated movements (in Basoglu 2006 and Lewelt 2015). The duration of the interventions varied between eight weeks and 24 months. The target frequencies ranged from two to seven times

per week, with durations of 18 minutes to 60 minutes. The target frequency was fully achieved in one study (Vry 2014), partly achieved in two studies (Lewelt 2015; Madsen 2015), and not reported in six studies (Basoglu 2006; Cunha 1996; Dahl 2004; McCartney 1988; Milner-Brown 1988; Salem 2010). Descriptions of training parameters were incomplete: in four studies with regards to intensity (Basoglu 2006; Cunha 1996; Dahl 2004; Salem 2010), and in three studies with regards to time (Basoglu 2006; McCartney 1988; Milner-Brown 1988). Four studies reported on the occurrence of adverse events (Lewelt 2015; Madsen 2015; McCartney 1988; Vry 2014). Four studies included participants with other neuromuscular diseases, such as muscular dystrophies, polyneuropathies, and myopathies (Dahl 2004; McCartney 1988; Milner-Brown 1988; Vry 2014). Four studies used home-based exercise programs (Basoglu 2006; Lewelt 2015; Madsen 2015; Vry 2014), in four studies participants exercised at an outpatient clinic or university (Cunha 1996; Dahl 2004; McCartney 1988; Salem 2010), and one study did not specify the setting (Milner-Brown 1988). Most studies were limited by inadequate research design: one was a non-randomized controlled clinical trial (Madsen 2015), seven were multiple-case studies (Basoglu 2006; Cunha 1996; Dahl 2004; Lewelt 2015; McCartney 1988; Milner-Brown 1988; Vry 2014), and one

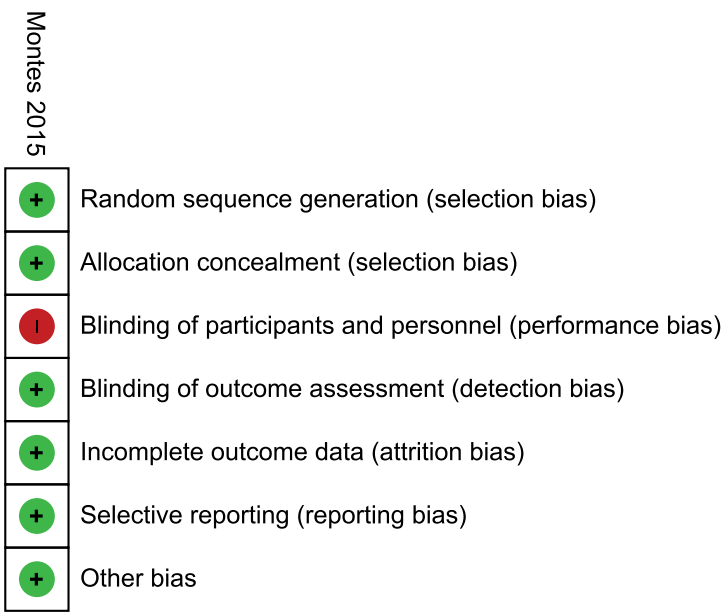


**Figure 2.** Risk of bias summary: review authors' judgements about each risk of bias item for the one included study. Green (+) = low risk of bias; red (-) = high risk of bias.

was a single-case study (Salem 2010). Two non-RCTs fulfilled the standards regarding diagnostic criteria, description of intervention, and outcome measures (Lewelt 2015; Madsen 2015).

Lewelt 2015 investigated the effect of a home-based supervised strength training program in a pilot study of nine children with SMA type 2 (N = 6) and SMA type 3 (N = 3), aged 10.4 (SD 3.8) years, during a 12-week period. Training sessions lasted 45 minutes to 60 minutes (including a 5-minute warm-up and cool down). Participants exercised three times weekly on non-consecutive days and performed two sets of 15 repetitions, with a recovery period of at least 5 minutes between sets. All participants performed concentric and gravity-eliminated exercises of shoulder and elbow flexion and extension. Ambulatory participants also exercised hip flexion, hip extension, and knee extension. Resistance was achieved using ankle and wrist weights, body weight, and variation in level of assistance. Each exercise was increased by 0.08 kg increments until the participant scored between 6 and 8 on the Children's OMNI Resistance Exercise Scale of Perceived Exertion (where 0 = extremely easy and 10 = extremely hard; Robertson 2005). Treatment fidelity, percentage of people with SMA willing to participate, progression of exercise workload, reported pain, and perceived exertion were used to determine feasibility and safety. The study included extremity composite scores of Manual Muscle Testing (MMT), quantitative myometry, and the HFMSE as outcome measures for strength and motor function.

Madsen 2015 studied the effect of a 12-week, home-based aerobic exercise training program in a clinical controlled trial of six participants with SMA and nine healthy controls, aged 19 to 58 years. Participants trained on a cycle ergometer, performing 30-minute training sessions (including 3 to 5 minutes of warm-up) at target heart rate, corresponding to an oxygen uptake ($VO_2$) of 60% to 75% of maximal ($VO_{2max}$). The number of sessions per week was gradually increased from two to four, aiming to reach a total of 42 sessions in 12 weeks. The trialists monitored compliance using weekly calls or emails, a training diary, and by downloading exercise data from pulse watches. Outcome measures were $VO_{2max}$, measured with an incremental exercise test; activities of daily living (ADL) functioning, assessed with a questionnaire; hand-held myometry of four muscle groups; body composition; and functional tests, including the six-minute walk test (6MWT), six-step stair test, the Timed Up and Go (TUG) test, and the five-times-sit-to-stand test. Creatine kinase was measured three times during the training period as a marker for muscle damage.

## Risk of bias in included studies

See the 'Risk of bias' summary figure for a representation of the review authors' 'Risk of bias' assessments (Figure 2).

### *Allocation (selection bias)*

Montes 2015 randomly assigned participants to the training or usual care group. There was no published information on the method of randomization or allocation concealment, but the first author reported that the trialists created 14 envelopes containing either exercise or usual care allocations. After consent was obtained, a designated research coordinator blindly selected a group assignment slip for each participant from a bin and placed it in an envelope labeled with the subject identification code inside a locked cabinet. We judged the randomization and allocation concealment procedures at low risk of bias.

### *Blinding (performance bias and detection bias)*

Blinding of personnel and participants was not possible because of the nature of the intervention. As all outcomes were to some degree effort-dependent or subjective, we considered them all at high risk of performance bias. To maintain outcome assessor blinding, study personnel, participants, and families were instructed not to discuss study design, group assignment, or the exercise program with the blinded primary evaluator. We judged the blinding procedure for the outcome assessor at low risk of bias.

### *Incomplete outcome data (attrition bias)*

Two participants in the training group did not complete the training period of six months because they found the travel distance of more than 1000 miles too burdensome. The trial authors did not perform intention-to-treat analysis. The trial author informed us, however, that differences in baseline values between the participants that completed the study and those that dropped out were not significant. We therefore concluded that the dropout of these two participants was probably not related to the true outcome at six months.

### *Selective reporting (reporting bias)*

We found no evidence of selective reporting. The trial authors reported results showing significant and non-significant differences, in accordance with the protocol.

### *Other potential sources of bias*

We identified no other potential sources of bias.

## Effects of interventions

All primary and secondary outcomes were from one study (Montes 2015).

### *Combined strength and aerobic exercise training versus usual care in SMA type 3*

Montes 2015 studied a combined strength and aerobic exercise training program (see Summary of findings table 1). The trial reported outcomes after six months of training.

### *Primary outcome measure: walking distance on the six-minute walk test*

The minimal detectable change on the 6MWT is 24.0 m (Dunaway 2016).

The change from baseline in mean distance walked on the 6MWT (m) was not detectably different in the exercise group than in the usual care group (mean difference (MD) 9.54, 95% confidence interval (CI) -83.04 to 102.12, N = 12; very low-certainty evidence; Analysis 1.1). We downgraded the evidence to very low: twice for imprecision, as the sample size was small (there were five participants in the training group) and CIs encompassed large effects in either direction, and once for indirectness, as 50% of the training group did not achieve the intended volume of aerobic exercise training, owing to decreased exercise tolerability. Additionally, the lack of participant blinding represented a study limitation.

#### Functional performance

The trial assessed the change in functional performance from baseline to six months' follow-up, using the HFMSE, 10MWT, and TUG test.

The HFMSE scores revealed no clinically meaningful difference in functional performance between the training group and the usual care group (MD 2.00, 95% CI -2.06 to 6.06; N = 12; Analysis 1.2). The range of possible scores on the HFMSE is 0 to 66 and a clinically meaningful improvement is an increase of 3 points or more (Mercuri 2018).

The change in 10MWT time (s) revealed no clear difference between the training group and usual care group (MD -0.65, 95% CI -1.84 to 0.54; N = 12; Analysis 1.3).

Performance on the TUG test (s) was worse in the training group than in the usual care group (MD 4.28, 95% CI -3.43 to 11.99; N = 12; Analysis 1.4).

We considered the evidence for all functional performance scores of very low certainty, downgrading twice for imprecision, as the sample size was small (there were five participants in the training group), and CIs encompassed

moderate effects in either direction, and once for study limitations, as participants could not be blinded to the intervention.

### *Secondary outcome measures*

#### Cardiopulmonary exercise capacity

A clinically meaningful increase in $VO_{2max}$ is 3.5 mL/kg/min (Myers 2002).
There was no clear difference in the change from baseline in $VO_{2max}$ (mL/kg/min) between the training group and the usual care group (MD 1.22, 95% CI -2.16 to 4.6; Analysis 1.5). We considered the evidence of very low certainty, downgrading three times: twice for imprecision, as the sample size was small (there were five participants in the training group) and CIs encompassed moderate effects in either direction, and once for indirectness, because 50% of the training group did not achieve the intended volume of aerobic exercise training owing to decreased exercise tolerability. Additionally, the lack of participant blinding represented a study limitation.

#### Muscle strength

##### *Manual muscle testing*

Muscle strength was assessed by the change from baseline in MMT score (the Medical Research Council (MRC) 10-point grading scale, expressed as total MMT score (which is the total scores of 28 arm and leg muscle groups, maximal score 280), arm MMT score (score of 12 arm muscle groups, maximal score 120), and leg MMT score (score of 16 leg muscle groups, maximal score 160).
The total MMT score (MD 11.94, 95% CI -3.44 to 27.32; Analysis 1.6), arm MMT score (MD 7.51, 95% CI -0.05 to 15.07; Analysis 1.7), and leg MMT score (MD 4.43, 95% CI -5.64 to 14.50; Analysis 1.8) improved more in the training group than in the usual care group. We graded the certainty of the evidence for muscle strength, expressed as total MMT score, as low, downgrading twice for imprecision, as the sample size was small (there were five participants in the training group) and CIs encompassed moderate effects in either direction, and once for study limitations, as participants could not be blinded to the intervention.

##### *Hand-held dynamometry*

The trial also assessed the change from baseline in muscle strength of individual muscles using hand-held dynamometry. There were no clear differences in muscle strength (kg) between the training group and the usual care group for knee extension (MD -0.73, 95% CI -3.10 to 1.64; Analysis 1.9), knee flexion (MD -0.79, 95% CI -16.24 to 14.66; Analysis 1.10), shoulder abduction (MD -0.40, 95% CI -0.92 to 0.12; Analysis 1.11), elbow flexion (MD

0.29, 95% CI -0.49 to 1.07; Analysis 1.12), or elbow extension (MD -0.34, 95% CI -2.12 to 1.44; Analysis 1.13).

### Fatigue

We could not report CIs for fatigue, as the trial authors did not report P values and the subgroup sample sizes were very small (between four and eight participants). Instead, we provided the raw mean scores for the changes from baseline.

*Children*

The Pediatric Quality of Life Inventory Multi-Dimensional Fatigue Scale (PedsQLMFS) is a scale from: 0 to 100 (a higher score indicates less fatigue).

The mean PedsQLMFS score in the training group (N = 1) increased by 2 points from a baseline of 88 (child report) and by 6 points from a baseline of 51 (parent report). In the usual care group (N = 3), PedsQLMFS scores increased by 3.5 from a baseline of 81.7 (child report) and by 7.3 from a baseline of 75.9 (parent report).

*Adults*

The Fatigue Severity Scale (FSS) has a range from 0 to 7 (a higher score indicates more fatigue).

The mean FFS score at baseline was 4.6 in the training group (N = 4) at baseline, and did not change following training. In the usual care group (N = 4), the FSS score increased by 0.4 from a baseline of 5.0. The trial authors stated that the differences were not significant but did not report P values.

We considered the evidence of very low certainty, downgrading twice for imprecision, as the sample size was small (training group N = 5) and CIs encompassed large effects in either direction, and once for indirectness, as 50% of the training group did not achieve the intended volume of aerobic exercise training owing to decreased exercise tolerability. Additionally, the lack of participant blinding represented a study limitation.

## Physical activity

The included study did not measure physical activity levels.

## Quality of life

We could not report CIs for quality of life, as the trial authors did not report P values and the subgroup sample sizes were very small (between four and eight participants). Instead, we provided the raw mean scores for the changes from baseline.

*Children*

The Pediatric Quality of Life Inventory Neuromuscular Module (PedsQLNM) is a scale from: 0 to 100 (a higher score indicates better quality of life).

The trial reported no significant changes in quality of life either from baseline or between the exercise and control groups over the six-month trial, according to child and parent scores on the PedsQLNM. Mean changes in PedsQLNM score in the training group (N = 1) were a 1-point decrease from a baseline of 90 (child report) and a 5-point increase from a baseline of 68 points (parent report). In the usual care group (N = 3), there was a 0.1-point increase from a baseline of 85.3 points (child report) and a 2.7-point increase from a baseline of 83.0 points (parent report).

*Adults*

The 36-Item Short-Form Health Survey (SF-36) questionnaire subdomains Physical Health (SF-36PH) and Mental Health (SF-36MH) range from: 0 to 100 (a higher score indicates better quality of life).

The mean change in SF-36PH score in the training group (N = 4) was an increase of 0.5 points from a baseline of 35.6 points. In the usual care group (N = 2), there was a decrease of 1.6 points from a baseline of 39.4 points.

The mean change in the SF-36MH score in the training group (N = 4) was an increase of 0.2 points from a baseline of 60.3 points. In the usual care group (N = 2), there was an increase of 0.8 points from a baseline of 54.2 points.

The trial authors stated that differences were not significant, but did not report P values. We considered the evidence of very low certainty, downgrading twice for imprecision, as the sample size was small and CIs encompassed large effects in either direction, and once for indirectness, because 50% of the training group did not achieve the intended volume of aerobic exercise training owing to decreased exercise tolerability. Additionally, the lack of participant blinding represented a study limitation.

## Serious adverse events leading to withdrawal

There were no study-related serious adverse events or adverse events leading to withdrawal. We downgraded the certainty of this evidence to very low for serious imprecision and indirectness of the intervention. Additionally, the lack of participant blinding represented a study limitation.

Non-serious musculoskeletal adverse events, such as falls and muscle soreness, did not significantly differ between the training group and the usual care group (23 versus 35 falls (P = 0.49) and 6 versus 4 muscle soreness events (P = 0.69)).

# Discussion

## Summary of main results

We identified one trial for inclusion in this review. Montes 2015 was a single-blind, randomized, controlled clinical trial that studied the effects of a 6-month, home-based combined cycle ergometry and strength training program in 12 participants with spinal muscular atrophy (SMA) type 3. The comparison group received usual care. The evidence was too uncertain to draw conclusions about the effects of exercise training on walking distance on the six-minute walk test (6MWT), cardiopulmonary exercise capacity, fatigue, quality of life, functional performance (on the Hammersmith Functional Motor Scale - Expanded (HFMSE)), or muscle strength. No study-related serious adverse events occurred in either the training or usual care group; however, the certainty of this evidence was also too low for any conclusions to be drawn.



## Overall completeness and applicability of evidence

This review does not provide sufficient evidence for or against aerobic training or strength training in people with SMA type 3. There is a lack of well-designed studies, as most studies are characterized by small sample sizes, a high risk of bias, and inadequate training prescriptions. The striking difference in efficacy and feasibility findings between the randomized trial included in this review (Montes 2015), and a non-randomized controlled trial reported in the discussion section (Madsen 2015), could arise from multiple factors, including differences in study design, training program, and participant population. The paucity of evidence and variability in study designs make it impossible to conclude whether or not physical exercise training is beneficial for people with SMA type 3.

## Quality of the evidence

### *Training program*

Guidelines recommend that training programs are standardized on the 'Frequency', 'Intensity', 'Time' and 'Type' (FITT) principle and use an incremental exercise protocol (ACSM 2010; Ganley 2011). In Montes 2015, aerobic training consisted of recumbent cycle ergometry (T), five times weekly (F) for 30 minutes (T) at an intensity level of a 5 to 7 on a perceived exertion scale with a range of 10 (I). Workload was increased every two weeks, when perceived exertion would drop below a score of 5. The use of a subjective score to determine the exercise intensity level in the trial was not in accordance with the initial protocol (Montes 2014), in which the trial authors

stated that moderate intensity would be objectively based on peak oxygen uptake ($VO_{2max}$) during a maximal exercise test. The trial author explained that the workload of the recumbent bicycles used by participants at home could only be adjusted on an ascending scale, but not in Watts. Instead, the trial used a perceived exertion scale to guide workload increase. Strength training consisted of five or six concentric, isometric, or gravity-eliminated exercises of hip, ankle, and all shoulder muscles, as well as core muscles (T). Target muscles were individually selected based on the most weakened muscles. Strength training was performed three times weekly (F): three sets each of 8 to 12 repetitions during 30 minutes (T), at an intensity level of 60% to 80% of 1 repetition maximum (I). Workload was increased every two weeks when perceived exertion would drop below a score of 5. The trial authors did not report specific data on individual participants and muscles, which makes it difficult to determine the variability in individual strengthening programs.

### *Diagnostic criteria*

In Montes 2015, all participants had genetically confirmed SMA with a clinical type 3. We therefore considered the quality of the diagnostic criteria to be adequate.

### *Outcomes*

Our overall certainty in the results according to GRADE criteria was very low for all outcomes, which means that further research is very likely to have an important impact on the estimate of effect. Imprecision (a small study sample and wide confidence intervals), indirectness (suboptimal aerobic exercise dosing), and study limitations (lack of participant blinding) were the reasons for the judgement of very low certainty. We need larger, well-controlled studies with optimal exercise dosing to improve the body of evidence.

## Potential biases in the review process

Although it is possible that we missed studies from databases not covered by our searches, the extent of our search and the paucity of exercise studies in SMA make it very unlikely that we overlooked eligible studies.

The involvement of JM who was an author of the one included trial was a potential bias in the review process. We used a third reviewer (JdG) to substitute for JM in extraction of outcome data and 'Risk of bias' assessment for this trial (Montes 2015).

## Agreements and disagreements with other studies or reviews

### *Aerobic exercise training in SMA*

There were both agreements and disagreements between the one included trial by Montes and colleagues and the controlled study by Madsen and colleagues (Madsen 2015; Montes 2015). Neither trial reached optimal training frequency within the predetermined dosing period, for different reasons. In Montes 2015, the investigators aimed to gradually increase the number of sessions to five per week. However, only one participant managed to accomplish this training frequency at three months. At six months, 50% reached a frequency of five sessions per week, while the other 50% reached a frequency of one to three sessions per week. There were no dropouts for adverse events and the protocol was well tolerated. In Madsen 2015, the aim was to gradually increase the number of sessions from two to four per week, but the program had to be modified in two out of eight participants with SMA, owing to fatigue. Two participants experienced adverse events (fall incidents and joint pain) and two participants dropped out of the study due to excessive fatigue. The remaining participants eventually reached a frequency range of 1.7 to 2.6 sessions per week, in comparison to three sessions per week in healthy controls. The training effect between the studies was significantly different. Whereas Montes 2015 found no significant improvement in $VO_{2max}$ in the training group versus the usual care group, Madsen 2015 reported a significant and clinically relevant improvement in $VO_{2max}$ in participants with SMA (from 17 mL/kg/min to 21 mL/kg/min, which was an increase of 27% (standard error (SE) 3%); $P < 0.001$). The two studies were both small, subject to imprecision, and had several differences in study design that could explain the differences in outcomes. Madsen 2015 was at risk of selection from bias from use of a non-randomized sample of eight adults with SMA and matched healthy sedentary control participants. Training type and intensity also differed, as Montes 2015 used a recumbent bicycle and set the intensity at a score of 5 to 7 on a subjective rating scale, while Madsen 2015 used an upright bicycle and an exercise intensity set at 60% to 75% of $VO_{2max}$. The two strategies ('subjective fatigue' and 'oxygen uptake') used to determine exercise intensity in these studies might represent different physiological limitations (peripheral versus cardiopulmonary limitations), and it is difficult to determine whether the training intensities were similar. The studies agree on the fact that people with SMA are vulnerable to exercise-induced fatigue and overexertion, and that optimal titration of exercise dosing has still to be determined. Although not formally tested, there did not seem to be a large difference in baseline disease severity in the intervention groups between

Montes 2015 and Madsen 2015 to explain differences in exercise response: the mean (SD) baseline distances walked in the 6MWT (m) were 389.9 (SD 111.3) in Montes 2015 and 330 (SE 67) in Madsen 2015. The mean ages of participants at baseline were 27 years (SD 14.6) in Montes 2015 and 32.5 years (SE 16.5) in Madsen 2015).

### *Strength training in SMA*

The findings of Montes 2015 were to a large extent in agreement with the multi-case study by Lewelt and colleagues (Lewelt 2015). Participants in both studies tolerated strength training well, as reflected by high completion rates (of 90% to 100%), without an increase in adverse events. Both Montes 2015 and Lewelt 2015 found that there may be improvement in both strength (expressed as total and arm manual muscle testing scores) and motor function (the HFMSE score). Lewelt 2015, however, had several methodological flaws that necessitate caution when interpreting the results. Physical therapists delivering the intervention and often the evaluator were not blinded, which introduced potential performance and detection bias. It is unclear whether the results were entirely representative of people with SMA type 3, because the paper did not report findings in participants with SMA type 2 and SMA type 3 separately. There was also a difference in the mean age at baseline between Montes 2015 and Lewelt 2015 (27 years versus 10.4 years), which makes comparisons between the studies difficult.

### *Physical training in other neuromuscular diseases*

No other systematic reviews on the effects of physical exercise training in people with SMA have been published to date, although there are reviews of exercise for other neuromuscular conditions. Voet and colleagues published a Cochrane Systematic Review on the effects of strength and aerobic exercise training for muscle disease, which at the time of writing is being updated (Voet 2013). Voet 2013 included five studies: two in myotonic dystrophy, one in myositis, one in facioscapulohumeral dystrophy, and one in mitochondrial myopathy. Voet 2013 concluded that moderate-intensity strength training and aerobic exercise training seem feasible but that there was not enough evidence to determine efficacy. Also in 2013, Dal Bello-Haas and colleagues performed a Cochrane systematic review on the effects of progressive resistance or strengthening exercise and endurance or aerobic exercise in people with amyotrophic lateral sclerosis (ALS)/motor neuron disease, which included two studies (Dal Bello-Haas 2013). The review found a significant increase on the ALS Functional Rating Scale when combining the data from

one study of undefined endurance exercise and one resistance exercise program. The certainty of evidence in the review was limited by small sample sizes and a high risk of bias in one of the studies. The findings of both reviews are consistent with those of our review.

## Authors' conclusions

### Implications for practice

It is uncertain whether combined strength and aerobic exercise training is beneficial or harmful in people with spinal muscular atrophy (SMA) type 3 in terms of walking distance, cardiopulmonary exercise capacity, fatigue, quality of life, functional performance, muscle strength, and adverse effects, as the quality of evidence is very low.



### Implications for research

We need more evidence, of greater certainty to be able to develop exercise guidelines for people with SMA type 3. National or international multicenter studies should be developed, which include sufficient participants and meet requirements for statistical power. The balance between feasibility and optimal dosing seems pivotal in the design of effective training programs for people with SMA type 3, especially with regards to aerobic exercise training. We need studies to determine the optimal dose of exercise, both for people with mild weakness and for those who are more severely affected, and when used as monotherapy or in combination with a drug. Training protocols should be clearly described with regards to the FITT-factors (Frequency, Intensity, Time, and Type). Blinding of outcome assessors is important and achievable, but adequate blinding of participants and personnel is rarely possible in exercise trials. Knowledge of group assignment can affect participant behaviour, but use of objective outcome measures, with monitoring of participant effort where this can influence outcome measurement, may improve the certainty of findings.

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the Department of Health. Cochrane Neuromuscular is also supported by the Queen Square Centre for Neuromuscular Disease.

We based the Methods section of the protocol on a template developed by Cochrane Neuromuscular from an original created by the Cochrane Airways Group.

The Information Specialist for Cochrane Neuromuscular, Angela Gunn, developed the search strategy in consultation with the protocol authors.

## Differences between protocol and review

We changed the title from that of the protocol, 'Skeletal muscle training for spinal muscular atrophy type 3' (Bartels 2016), to 'Physical exercise training for spinal muscular atrophy type 3', to better reflect the review topic.

To minimize bias in the review process, JdG substituted for JM in extracting data and performing 'Risk of bias' assessments for Montes 2015.

We did not report data from the questionnaires on fatigue and quality of life as MDs but as separate raw scores for children and adults because of the small subgroup sample sizes for adults (N = 8) and children (N = 4).

We did not assess performance bias separately for subjective outcomes (e.g. questionnaires and visual analogue scales) and objective outcomes (e.g. physiological outcomes), as the distinction was not important in the included trial.

Subgroup analyses by subtype and age were not possible. We moved sections of the protocol that were not applicable in practice to Appendix 12. We also updated the planned approach to heterogeneity, based on current Cochrane guidance.

We reported that we used the RevMan calculator tool to obtain missing standard deviations from P values for the differences between means in the two groups (RevMan 2014).

## Montes 2015

| | |
|---|---|
| Methods | Single-blind, randomized, controlled clinical trial |
| Participants | **Inclusion criteria**<br>Genetic confirmation of SMA diagnosis<br>SMA type 3<br>Aged between 8 and 50 years<br>Able to walk at least 25 m without assistance<br>Able to pedal the stationary cycle ergometer<br>In good health, based on the findings of a physical examination and the judgment of the clinical investigator at the time of screening assessment.<br>**Exclusion criteria**<br>Use of investigational medications intended for the treatment of SMA<br>A contraindication to exercise according to American College of Sports Medicine (ACSM) criteria<br>Pregnancy<br>Breastfeeding<br>Study sample<br>"At baseline, all participants had normal pulmonary function and substantially attenuated exercise capacity. On average, the exercise capacity was 35.3% of predicted for age and gender. Both groups were insufficiently active, spending on average 83.5% of waking hours in sedentary activities.<br>Intervention group (N = 7)<br>Age in years (SD): 27.0 (14.6), range 10 to 43<br>Sex (male/female): 6/1<br>SMA subtype: 3a (N = 0); 3b (N = 7)<br>Severity of illness (HFMSE score): 53.4 (8.9)<br>Control (usual care) group (N = 7)<br>Age in years (SD): 26.7 (17.7), range 10 to 48<br>Sex (male/female): 5/2<br>SMA subtype: 3a (N = 3); 3b (N = 4)<br>Severity of illness (HFMSE score): 54.0 (8.2) |

| | |
|---|---|
| Interventions | **Combined strength and aerobic exercise training or no training**<br>***Aerobic exercise training***<br>*Frequency:*<br>5 times weekly<br>*Intensity*<br>Moderate intensity (a score of 5 to 7 on the OMNI Scale of Perceived Exertion)<br>Participants were allowed to increase their workload once every 2 weeks, provided they maintained a submaximal level of intensity, as measured on the OMNI Scale of Perceived Exertion.<br>*Time*<br>Session: 30 minutes<br>Program: 6 months<br>*Type*<br>Recumbent cycle ergometry<br>***Strength training***<br>*Frequency*<br>3 times weekly<br>*Intensity*<br>60% to 80% of 1 repetition maximum<br>3 sets, each of 8 to 12 repetitions<br>2 to 3 minutes break between each set<br>*Time*<br>30 minutes<br>*Type*<br>5 to 6 exercises, concentric, isometric, or modified in a gravity-eliminated position for weaker muscles<br>Muscle groups: hip, ankle, and all shoulder muscles, and core muscles. Target muscles were individually selected based on most weakened muscles |
| Outcomes | **Primary outcome**<br>6-minute walk test (6-minute walking distance)<br>HFMSE<br>**Secondary outcomes**<br>Cardiopulmonary exercise capacity ($VO_{2max}$)<br>10-meter walk/run<br>Timed Up and Go Test<br>Muscle strength (manual muscle testing sum scores, hand-held dynamometry (kg))<br>Fatigue (questionnaire)<br>Quality of life (questionnaire)<br>Number of serious adverse events |
| Conflicts of interest among principal investigators | The authors report no conflict of interests. |
| Funding | Department of Defense; USAMRAA Grant/Cooperative award number: 09131005 (W81XWH-10-1-0127), and the Spinal Muscular Atrophy Foundation. "The sponsors had no role in the conduct of this study." |
| Notes | ClinicalTrials.gov id: NCT01166022<br>Start date: December 2010, completion date August 2014<br>Location: USA |

**Risk of bias table**

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote: "Participants were randomized to control and exercise cohorts."<br>Comment:<br>The author (Montes) informed us that a designated research coordinator blindly selected a group assignment slip (exercise or control) from a bin and placed it in an envelope labeled with the participant identification code. The envelopes were kept in a locked cabinet. |
| Allocation concealment (selection bias) | Low risk | Quote: "Participants were randomized to control and exercise cohorts."<br>Comment:<br>The author (Montes) informed us that a designated research coordinator blindly selected a group assignment slip (exercise or control) from a bin and placed it in an envelope labeled with the participant identification code. The envelopes were kept in a locked cabinet. |
| Blinding of participants and personnel (performance bias) | High risk | The primary evaluator was blinded but other study personnel and participants were not blinded. |
| Blinding of outcome assessment (detection bias) | Low risk | Quote: "To maintain the blind, study personnel as well as patients and families will be instructed not to discuss study design, group assignment, or exercise program with the primary evaluator."<br>Comments:<br>The primary evaluator was blinded. Other study personnel and participants were not blinded. |
| Incomplete outcome data (attrition bias) | Low risk | Quote: "The two participants who dropped out during the first 7 months of the study lived more than 1000 miles from the study site and found the travel too burdensome."<br>Comment:<br>2/14 participants (both in the training group) did not complete the controlled period of 6 months. Reasons for missing outcome data unlikely to be related to true outcome at six months (N = 2) because of random missing data (travel reasons) |
| Selective reporting (reporting bias) | Low risk | No evidence found for selective reporting<br>Report of both significant and non-significant differences in accordance with protocol (Montes 2014) |
| Other bias | Low risk | No risk of bias from other sources detected |

## Footnotes

HFMSE: Hammersmith Functional Motor Scale - Expanded; SD: standard deviation; SMA: spinal muscular atrophy;

## Characteristics of excluded studies

| | |
|---|---|
| Basoglu 2006 | |
| Reason for exclusion | Not a RCT (multi-case study) and insufficient description of the intervention |
| Cunha 1996 | |
| Reason for exclusion | Not a RCT (multi-case study) and insufficient description of the intervention |
| Dahl 2004 | |
| Reason for exclusion | Insufficient description of the intervention and participants |
| Lewelt 2015 | |
| Reason for exclusion | Not a RCT (multi-case study). Included in the Discussion |
| Madsen 2015 | |
| Reason for exclusion | Not a RCT (controlled trial in people with SMA versus healthy controls). Included in the Discussion |
| McCartney 1988 | |
| Reason for exclusion | Not a RCT (multi-case study) and inadequate exercise protocol |
| Milner-Brown 1988 | |
| Reason for exclusion | Not a RCT, and insufficient description of participants and the intervention |
| Salem 2010 | |
| Reason for exclusion | Not a RCT (case study) and insufficient description of the intervention |
| Vry 2014 | |
| Reason for exclusion | Not a RCT (multi-case study) and inadequate exercise protocol |

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## Data and analyses

1 Combined strength and aerobic exercise training versus usual care in SMA type 3

| Outcome or Subgroup | Studies | Participants | Statistical Method | Effect Estimate |
|---|---|---|---|---|
| 1.1 Walking distance: 6-minute walk test (m) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 9.54 [-83.04, 102.12] |
| 1.2 Functional performance: Hammersmith Functional Motor Scale Expanded | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 2.00 [-2.06, 6.06] |
| 1.3 Functional performance: 10-meter walk test (s) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | -0.65 [-1.84, 0.54] |
| 1.4 Functional performance: Timed Up and Go test (s) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 4.28 [-3.43, 11.99] |
| 1.5 Cardiopulmonary exercise capacity: $VO_{2ma}$x (mL/kg/min) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 1.22 [-2.16, 4.60] |
| 1.6 Muscle strength: total manual muscle testing score | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 11.94 [-3.44, 27.32] |
| 1.7 Muscle strength: arm manual muscle testing score | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 7.51 [-0.05, 15.07] |
| 1.8 Muscle strength: leg manual muscle testing score | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 4.43 [-5.64, 14.50] |
| 1.9 Muscle strength: hand-held dynamometry - knee extension (kg) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | -0.73 [-3.10, 1.64] |
| 1.10 Muscle strength: hand-held dynamometry - knee flexion (kg) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | -0.79 [-16.24, 14.66] |
| 1.11 Muscle strength: hand-held dynamometry - shoulder abduction (kg) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | -0.40 [-0.92, 0.12] |
| 1.12 Muscle strength: hand-held dynamometry - elbow extension (kg) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | 0.29 [-0.49, 1.07] |
| 1.13 Muscle strength: hand-held dynamometry - elbow flexion (kg) | 1 | 12 | Mean Difference (IV, Fixed, 95% CI) | -0.34 [-2.12, 1.44] |

Chapter 7

# Randomised, double-blind cross-over, phase II trial of pyridostigmine versus placebo in spinal muscular atrophy types 2, 3 and 4




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*Submitted*

## Abstract

### Background

Neuromuscular junction dysfunction is a characteristic of spinal muscular atrophy (SMA) and contributes to impaired motor function and increased fatigability. We studied safety and efficacy of the acetylcholinesterase inhibitor pyridostigmine on motor function and fatigability in SMA types 2-4.

### Methods

We conducted a phase II, monocenter, placebo-controlled, double-blind cross-over trial at the SMA Center in The Netherlands, in patients with SMA types 2-4. Each patient received 8 weeks of pyridostigmine and 8 weeks of placebo in random order. Randomisation in a permuted four-block design and masking was performed by an independent pharmacist. Primary outcomes were the Motor Function Measure (MFM) for motor function and the repeated nine-hole peg test (R9HPT) for fatigability. Secondary outcomes were the Endurance Shuttle Test Combined Score (ESTCS) for fatigability, patient-reported treatment effect and adverse events (AEs). Analyses were performed according to the intention-to-treat principle. Trial registration: SPACE trial, ClinicalTrials.gov number NCT02941328

### Findings

Between November 2015 and August 2017, 35 patients were randomised and included in analyses. Mean treatment difference on the MFM was 0.74% (95% CI: 0·00–1·49; p=0·050), favoring pyridostigmine. There was no difference in mean treatment effect for the R9HPT (0·17s/trial; 95% CI: -1·17-1·49; p=0·8) but there was a 70% reduced drop-out risk on the ESTCS under pyridostigmine (HR: 0·30; 95% CI: 0·15-0·58) and patients reported a beneficial effect of pyridostigmine on fatigability (OR: 6·9; 95% CI: 2·2-24·0). AEs, mostly mild and self-limiting, occurred significantly more frequently under pyridostigmine. Serious AEs were not related to study medication.

### Interpretation

There was no statistically significant effect of pyridostigmine on primary outcomes. Secondary outcomes did show an objective reduction of fatigability accompanied by a patient-reported beneficial effect, both with a large effect size, supporting a clinically meaningful effect of pyridostigmine on fatigability.



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# Introduction

Spinal muscular atrophy (SMA) is a leading genetic cause of childhood and adult disability. It is caused by homozygous loss-of function of the survival motor neuron 1 (*SMN1*) gene[1] and is characterized by degeneration of motor neurons in the anterior horn of the spinal cord, progressive muscle atrophy and weakness and abnormal anatomy and function of the neuromuscular junction (NMJ).[2-4] There is a wide range of disease severity, ranging from lethal infantile onset SMA type 1, chronic childhood variants type 2 and 3, to adult onset type 4.[5] The first genetic therapies that improve survival and gross motor development in infants with SMA type 1 and motor function in young children with SMA type 2 have recently become available.[6-8]

Muscle weakness is the best-known characteristic of SMA, but patients often complain of limited endurance of motor functions they still can perform. This increased fatigability is an additional dimension of incapacitating physical impairment in SMA. Abnormalities in development, maturation, and function of the neuromuscular junction (NMJ) in SMA mouse models[9-11] and patients with SMA types 1, 2 and 3[4,12,13] suggest that NMJ dysfunction significantly contributes to increased fatigability. Pyridostigmine, a widely used first line treatment of disorders affecting NMJ function (i.e. Myasthenia Gravis), inhibits the enzymatic breakdown of acetylcholine in the neuromuscular synapse, increasing its availability and enhancing neuromuscular transmission.[14] We therefore investigated the effect and efficacy of pyridostigmine on motor function and fatigability in patients with SMA.



# Methods

## Study design and monitoring

This investigator-initiated, phase II, monocenter, placebo-controlled, double-blind cross-over trial was conducted at the SMA Center at the University Medical Center Utrecht, a tertiary referral center for patients with SMA in The Netherlands. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht and the Central Committee on Research Involving Human Subjects. The trial was monitored for accuracy, integrity and safety by an external independent party (Julius Clinical, Zeist, The Netherlands). The trial design and procedures have been published previously[15] and the full protocol and statistical analysis plan are available as supplementary appendix with the full text of this article.

## Patients

Detailed inclusion and exclusion criteria are provided in the protocol[15] (supplementary appendix). In short, all patients had a genetically confirmed diagnosis of SMA type 2, 3, or 4, were 12 years or older, and had to meet predetermined minimum or maximum scores on the repeated nine-hole peg test (R9HPT)[16] and Motor Function Measure (MFM).[17] Main exclusion criteria were a known concomitant disorder of the NMJ, the use of drugs that alter NMJ function, or contra-indications for the use of pyridostigmine. Prior to study participation, all patients and parents when patients were <18 years old, gave written informed consent. Confidentiality was secured by assigning a random study ID after inclusion.

## Randomisation and masking

If eligible, patients were enrolled by the investigators. Patients were randomised in a permuted four-block design by an independent pharmacist of the University Medical Center Utrecht, who was not part of the study team, using a random order table, to receive either pyridostigmine or visually identical placebo tablets during the first 8-week treatment period. After a one-week washout period patients crossed-over to the other treatment for another 8-week period. Both patients and investigators were blinded for treatment allocation. Study medication was delivered by the pharmacist in identical jars to the investigator who handed it to the patient. The randomisation code was revealed to the investigators by the pharmacist after the database was closed in a two-step approach to reduce the risk of bias: first the two study drugs were named 'A' and 'B' to perform the main analysis, followed by further unblinding.

## Procedures

Production of both pyridostigmine and placebo was done by the qualified pharmacy of the Apotheek Haagse Ziekenhuizen, The Hague, The Netherlands, and subsequently sent to the pharmacy of the University Medical Center Utrecht, Utrecht, The Netherlands. Study medication was given four times daily and, in order to minimize side-effects, the dosage was increased from 2 mg/kg/day to 4 mg/kg/day, and finally to the targeted 6 mg/kg/day in the course of the first week of each treatment period. If side-effects occurred after a dosage increase that were not acceptable for the patient, the treatment period was continued with the highest dose that was tolerated. There were four study visits after the screening visit: one at the beginning and one at the end of each 8-week treatment period. During study visits all primary and

secondary outcomes were performed in identical order and study drug intake was standardized to one hour prior to assessment. Follow-up for adverse events after study participation was done by telephone during one week, or longer if indicated.

## Outcomes

A detailed description of all outcome measures was published previously.[15] We here report the primary and most relevant secondary outcome measures. Primary outcome measures were the Motor Function Measure (MFM) and repeated nine-hole peg test (R9HPT). The MFM is a 32-item scale developed to measure distal, proximal and axial motor function of patients with neuromuscular diseases, including SMA.[17] The R9HPT is a fatigability test in which patients have to perform five consecutive rounds of the 9HPT as fast as possible.[16,18] Secondary outcome measures were 'risk of drop-out' and 'time to drop-out' on the Endurance Shuttle Test Combined Score (ESTCS), the patient-reported perceived treatment effect (range: strong decline – strong improvement), and adverse events (AEs). The ESTCS has been validated to capture fatigability across the severity spectrum of SMA by having patients perform a repetitive task at a predetermined speed that mimics activities of daily living. The ESTCS combines individual scores of one of three Endurance Shuttle Test (EST) that reflects the individual's physical capability and allows combined analysis of (non)ambulant patients with SMA type 2-4. The three different ESTs were designed using the same construct, i.e. the endurance shuttle nine-hole peg test (ESNHPT) for patients with motor function of hand and forearm, the endurance shuttle box and block test (ESBBT) for patients with anti-gravity motor function of at least one arm, and a modified version of the endurance shuttle walk test (ESWT) for ambulatory patients. Drop-out was defined as the inability to sustain the maximum duration of 20 minutes on the appropriate EST.[19,20] Adverse events were assessed by patient inquiry and a physical examination during each study visit. Between visits patients were instructed to contact the study team if any complaints occurred and after study completion follow-up for adverse events was done by the investigators by phone for one week, or longer if indicated.

## Statistical analysis

We needed 40 patients to obtain 80% power to detect a treatment difference at a two-sided significance level of 5% (for details, see the study protocol). All efficacy analyses were performed according to the intention-to-treat principle and included data from all patients that had at least one efficacy measurement

(i.e. efficacy population). Primary outcomes were assessed individually (i.e. not co-primary) for statistical significance. Linear mixed effects models (LME) were used to account for the intra-individual clustering of observations. For the MFM, we used an LME with fixed effects for treatment period (1 or 2) and treatment (pyridostigmine or placebo). The random part was modelled with a random intercept per individual and an unstructured covariance matrix. For the R9HPT, we used a similar approach, with the addition of round (integer 1 to 5) as a fixed effect and random slope of round per individual. Treatment response was evaluated in interaction with round (i.e. does the duration of the 9HPT change differently over rounds under treatment A versus B?). For all models, we tested for a period-treatment interaction to assess potential carry-over effects. The likelihood ratio test was used to compare models; 95% confidence intervals were based on the profile likelihood. We performed sensitivity analyses that included both a complete-case and per-protocol analysis for both primary endpoints, and in an additional sensitivity analysis for the R9HPT, censoring patients after 100 seconds. Time to drop-out on the ESTCS (i.e. pooled analysis of ESTs, adjusted for test type) was analyzed using a parametric Weibull survival model with a Gaussian frailty term per subject and a maximum of 20 degrees of freedom. As a sensitivity analysis, we performed a Cox proportional hazards analysis with a Gaussian frailty term per subject. The patient-reported perceived treatment effect was analyzed with an LME similar to the MFM model. Safety analyses were carried out based on the treatment actually received (i.e. safety population). AEs were categorized according to the Common Terminology Criteria for AEs[21] and were summarized in frequency tables, including severity ratings and likelihood of association with study medication. We used R (v3.6.0 for Windows with RStudio v1.2.1335) for all statistical analyses. The LMEs were fitted using the lmer function of lme4 (v1.1-21).[22] Results were considered statistically significant when alpha was less than 0·05. Due to the exploratory nature of this phase II study, results were not adjusted for multiple testing. This trial was registered with ClinicalTrials.gov, NCT02941328.

## Results

We randomised 37 of 47 screened patients between November 24, 2015 and August 24, 2017. Two patients discontinued study participation prior to the first efficacy evaluation due to concomitant medical issues, not related to study medication. After the washout period, 35 patients started the second period and 32 patients completed all study visits (figure 1). Patient characteristics of the efficacy population are summarized in table 1. There were more patients with SMA type 2 (10 (59%) vs 5 (28%)) and more male patients (8 (47%) vs 5 (28%)) in the group that started with pyridostigmine and there were more patients with SMA type 3a in the group that started with placebo (9 (50%) vs 5 (29%)). Other characteristics were comparable between groups at baseline. Maximum study medication dose had to be reduced in 9 patients due to side-effects.



**Table 1.** Baseline characteristics

| **characteristic** | **pyridostigmine-placebo group** | **placebo-pyridostigmine group** |
|---|---|---|
| | **n=17** | **n=18** |
| SMA type, n (%) | | |
| 2 | 10 (59) | 5 (28) |
| 3a | 5 (29) | 9 (50) |
| 3b | 2 (12) | 3 (17) |
| 4 | 0 | 1 (6) |
| | | |
| Male gender, n (%) | 8 (47) | 5 (28) |
| Age, years, mean (SD; range) | 34 (12; 13-53) | 38 (14; 13-59) |
| Disease duration, years, mean (SD; range) | 33 (12; 11-51) | 34 (14; 11-59) |
| Ambulant, n (%) | 2 (12) | 2 (11) |
| MFM score, median (range) | 37·5 (15·6-75) | 36·5 (20·8-76) |
| ***SMN2*** copies, n (%) | | |
| 2 | 1 (6)* | 0 |
| 3 | 7 (41) | 8 (44) |
| 4 | 9 (53) | 10 (56) |

Abbreviations: n=number; SMA= spinal muscular atrophy; SD=standard deviation; MFM=Motor Function Measure; SMN2=Survival Motor Neuron 2. *patient with SMA type 2 and SMN2 c.859G>C pointmutation23

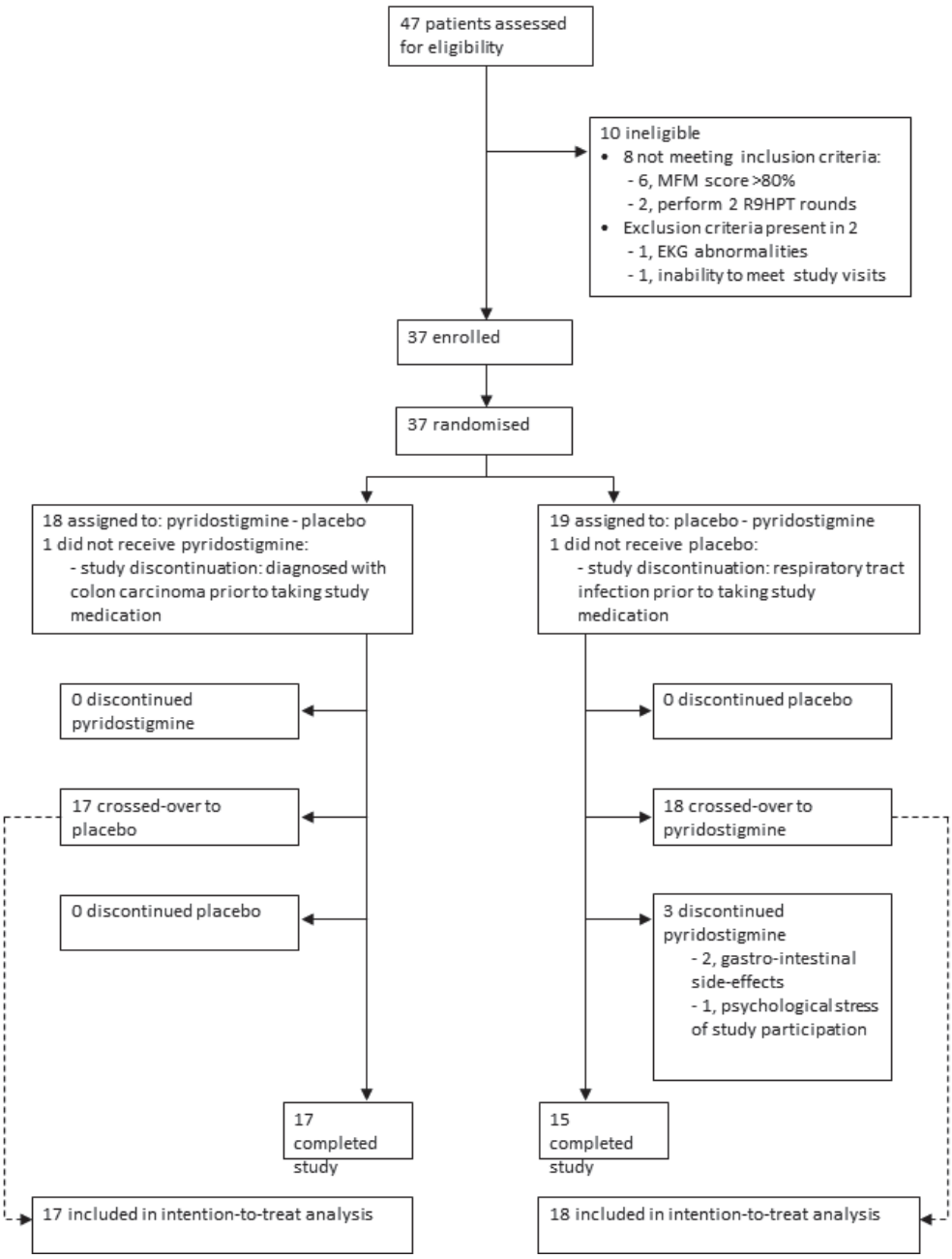


**Figure 1.** Patient flow diagram
Abbreviations: MFM=Motor Function Measure; R9HPT=repeated nine-hole peg test; EKG=electrocardiogram

Efficacy results are summarized in table 2. The MFM score was on average 41·6% (95% CI: 35·95 – 47·26) under placebo and 42·4% (95% CI: 35·95 – 48·75) under pyridostigmine, resulting in a mean difference of 0·74% (95% CI: 0·00 – 1·49; p=0·050). For the R9HPT the mean rate of increase in time needed to complete one round was 0·70 s/trial (95% CI: -0·42 – 1·81) under placebo and 0·86 s/trial (95% CI: -0·25 – 1·98) under pyridostigmine, resulting in a mean difference of 0·17 s/trial (95% CI: -1·17 to 1·49; p=0·81). There was a treatment-independent learning effect for the R9HPT, as the mean time to complete the test decreased with 2·89s (95% CI: 0·98 - 4·81) between the first and second efficacy assessment. Results were similar in sensitivity analyses that included both a complete-case and per-protocol analysis for both primary endpoints, and in an additional sensitivity analysis for the R9HPT, censoring patients after 100 seconds.

Sixteen patients performed the ESNHPT, 15 performed the ESBBT and 4 patients performed the ESWT. Pyridostigmine reduced the risk of drop-out during the 20-minute ESTCS by 70% compared to placebo (HR: 0·30; 95% CI: 0·15-0·58; figure 2).



**Table 2:** Summary of outcome measures

| **Primary outcomes** | **placebo, mean (95% CI)** | **pyridostigmine, mean (95% CI)** | **Mean difference (95% CI)** | **p-value** |
|---|---|---|---|---|
| MFM Total score | 41·60 (35·95 – 47·26) | 42·4 (35·95 – 47·26) | 0·74 (0-1·49) | 0·050 |
| R9HPT, s/trial | 0·70 ( -0·42 – 1·81) | 0·86 ( -0·25 – 1·98) | 0·17 (-1·17-1·49) | 0·81 |
| **Secondary outcomes** | | | | |
| ESTCS; | | | | |
| risk of drop-out under pyridostigmine, HR (95% CI) | | 0·30 (0·15 - 0·58) | | |
| time to drop-out, s, mean difference (95% C) (pyridostigmine – placebo) | | 154 (48-216) | | |
| Perceived treatment effect; odds of favorable effect under pyridostigmine, OR (95% CI) | | 6·9 (2·2-24·0) | | |

Abbreviations: CI=Confidence Interval; MFM=Motor Function Measure; R9HPT= repeated nine-hole peg test; s=seconds;
ESTCS=Endurance Shuttle Test Combined Score; HR=Hazard Ratio; OR=Odds Ratio

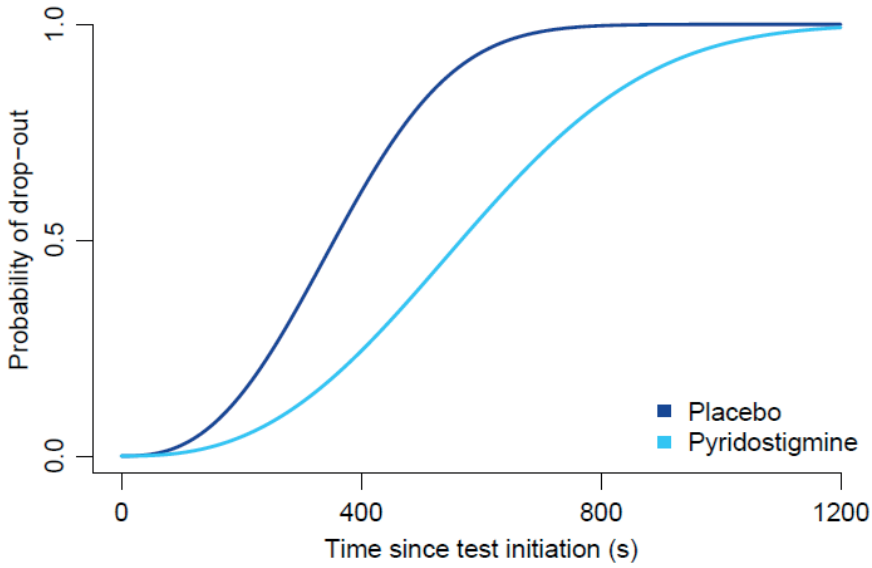


**Figure 2.** Probability of drop-out on the Endurance Shuttle Test Combined Score

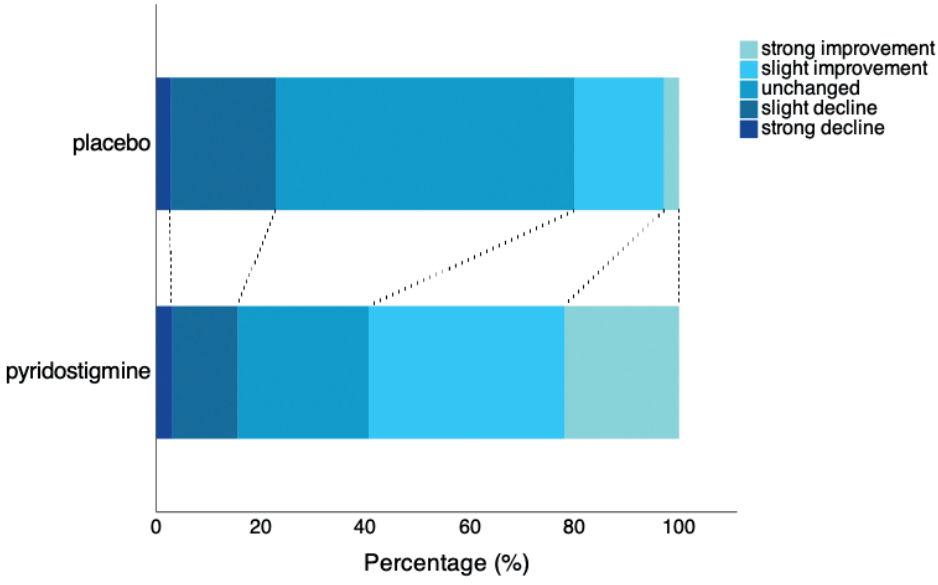


**Figure 3.** Patient-reported perceived treatment effect on fatigability
Patient-reported change in endurance at the end of each treatment period.

The probability of drop-out on the Endurance Shuttle Test Combined Score, assessed using a Weibull parametric survival model with a Gaussian frailty term. The lines represent the drop-out probability of the average patient under placebo or pyridostigmine treatment. Note that a relative increase in endurance is depicted and therefore, this figure should not be interpreted as depicting absolute data.

Mean difference in 'time to drop-out' was 154 seconds (95% CI: 48-216), favoring pyridostigmine. Patients reported a beneficial effect of treatment with pyridostigmine on fatigability (74·4%, compared to 29·7% under placebo). The odds of having a favorable effect (i.e. a slight or strong improvement in endurance) was higher under pyridostigmine compared to placebo: OR: 6·9 (95% CI 2·2-24·0; figure 3).



**Table 3.** Adverse Events

**Summary of adverse events**

| AE | All | Placebo | Treatment | Follow-up |
|---|---|---|---|---|
| Cardiac disorders | 2 | 0 | 2 | 0 |
| Eye disorders | 9 | 1 | 8 | 0 |
| General disorders and administration site conditions | 9 | 6 | 3 | 0 |
| Infections and infestations | 3 | 0 | 3 | 0 |
| Metabolism and nutrition disorders | 1 | 1 | 0 | 0 |
| Psychiatric disorders | 2 | 1 | 1 | 0 |
| Reproductive system and breast disorders | 2 | 1 | 1 | 0 |
| Skin and subcutaneous tissue disorders | 10 | 1 | 9 | 0 |
| Vascular disorders | 2 | 0 | 2 | 0 |
| Gastrointestinal disorders | 78 | 19 | 59 | 0 |
| Musculoskeletal and connective tissue disorders | 24 | 3 | 21 | 0 |
| Nervous system disorders | 14 | 8 | 6 | 0 |
| Other | 1 | 1 | 0 | 0 |
| Renal and urinary disorders | 17 | 4 | 13 | 0 |
| Respiratory, thoracic and mediastinal disorders | 20 | 13 | 6 | 1 |

**Seriousness of adverse events**

| AE | All | Placebo | Treatment | Follow-up |
|---|---|---|---|---|
| Serious | 5 | 2 | 2 | 1 |
| Intermediate | 18 | 4 | 14 | 0 |
| Mild | 171 | 53 | 118 | 0 |

Abbreviations: AE=Adverse event

Adverse events (AEs) are summarized in table 3, a detailed description of all AE's is available as a supplementary table (table S1, Supplementary Appendix). Overall, there were more AEs under pyridostigmine compared to placebo. Most AEs were mild, self-limiting and acceptable for patients. The most common AEs were gastro-intestinal (GI) complaints. Other related side-effects included increased saliva production and blurry sight. Participants reported muscle cramps and pain after study visits. There were four serious adverse events (SAEs), all considered to be unrelated to study medication or procedures. Two SAEs occurred in the pyridostigmine treatment period. One patient had surgery after study inclusion, but prior to the start of study medication, because of a diagnosis of colon carcinoma. Another patient was admitted to hospital because of head trauma from falling of the stairs during a game. One patient with an extensive medical history of upper GI tract bleedings was admitted to hospital because of two upper GI tract bleedings shortly after each other during the placebo period. Finally, during follow-up, one patient developed a severe respiratory tract infection several weeks after study completion. In this patient there were no signs of infection in the initial one-week follow-up period.

## Discussion

In this phase II study, we show that pyridostigmine is a safe and well-tolerated drug that reduces fatigability in patients with SMA types 2-4. Although there was no effect on R9HPT performance and the beneficial effect on the MFM score did not reach statistical significance, there was a 70% reduction in drop-out risk on the Endurance Shuttle Test Combined Score (ESTCS) and a large self-reported beneficial effect of pyridostigmine on fatigability.

Scales that can detect changes in motor function have been used as primary outcome measure in most SMA trials.[6-8] We used the MFM, a validated outcome measure for patients with SMA of 6 years and older that has been used previously in a phase II trial.[17,24,25] The difference in MFM scores between the treatment arms favored pyridostigmine but this did not achieve conventional threshold levels of statistical significance. We cannot exclude that treatment duration was too short or patients were too old to detect meaningful changes, since the reported meaningful changes in motor function of genetic SMA therapies were observed after longer treatment periods and were more often reported in younger children.[6-8] At the same time, motor function scales such as the MFM have not been designed to document fatigability, which is the most likely dimension of motor

function that pyridostigmine may improve. We therefore included the endurance tests that can be used across the severity spectrum of SMA, that were newly available when we designed this trial, as secondary outcomes,[15] i.e. a combination of the R9HPT[16] and the complementary ESTs. The ESTs do not have the ceiling effects of the R9HPT for ambulant and non-ambulant patients with remaining upper arm function. Since the ESTs share the same construct, combined analysis (ESTCS) allows inclusion of patients with different levels of motor function.[19] The results suggest that the use of the R9HPT is indeed limited by its ceiling effects, whilst the ESTCS revealed the beneficial effect of pyridostigmine on fatigability (figure 2). This objective improvement of the ESTCS is supported by the self-reported improvement by participants under pyridostigmine (figure 3).

Our results indicate that neuromuscular junction dysfunction is an important target for SMA therapies. Pyridostigmine improved endurance in an important fraction of patients with incapacitating muscle weakness. Patients for example reported that pyridostigmine helped them to finish meals, drive their (power driven) wheelchair, or walk longer distances without pause. It is likely that such improvements will contribute to patients' independence and quality of life.[26]



It remains to be established whether the recently introduced SMN-augmenting therapies (i.e. nusinersen and onasemnogene abeparvovec) that were tested in babies with SMA type 1[6,8] and children with SMA type 2[7,27] improve NMJ function and reduce fatigability. Because it has a different mode of action, pyridostigmine potentially has important value as add-on therapy to genetic therapies. Since use of pyridostigmine is less restricted by costs (less than $5,- per day) and safety concerns are few, it is also an alternative for patients without access to the new genetic SMN-augmenting treatments.

The cross-over design of this study is useful for a rare disease like SMA. Because patients are their own controls, unsystematic variance is reduced and systematic variance can be detected with smaller sample sizes. To decrease the risk of selection bias we invited all patients with SMA types 2-4 listed in the Dutch National SMA registry[28] (more than 300 patients) to participate. Patients were initially reluctant to participate and not all patients screened for eligibility met in- and exclusion criteria (figure 1). As a result, our sample size falls short of the original design which precludes subgroup analyses and is only suitable to detect relatively large changes. Therefore, clinically relevant differences may still have been missed.

The vast majority of side-effects that we encountered were known side-effects of pyridostigmine and were mostly mild. Since we used a standardized dose in this study, it is possible that further tailoring of the dose could result in an improved balance between efficacy and side-effects. This could be

investigated in an additional study in addition to confirming these phase II study results.

## Acknowledgments

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Chapter 8

# General discussion

When I started the work summarized in this thesis, SMA was still a disorder that could not be treated. This has changed dramatically. Many children and adults with SMA and varying clinical severity are now being treated with SMN protein augmenting therapies. It is expected that the number of therapies with market access in the *European Union* will increase up to 3 in the course of 2020 and the first trial on combinatorial therapy is underway. Treatment will probably change and expand the SMA phenotypes that we know[1]. Severely affect infants with SMA type 1 survive and may develop motor milestones but will not be cured completely. Moderately affected children with SMA type 2 may learn to walk and improve their functional skills. For many of them, the focus of treatment will shift towards the meaningful use of these newly acquired motor functions in daily life. Sufficient stamina and mental resilience will be more required than ever.

Within the currently fast changing context of clinical trials of pharmaceutical interventions, the purpose of this thesis was to first quantify and characterize fatigability in patients with SMA using newly developed outcome measures and secondly to evaluate the role of pharmaceutical and non-pharmaceutical interventions. Given the lack of reliable and valid outcome measures to assess fatigability in patients with SMA, often reported as a limiting factor in daily activities, an important aim was to develop tools to assess fatigability. In this general discussion I will summarize and reflect on the results of our fatigability studies in SMA and look forward in the context of this rapidly evolving field. Finally, I will summarize what we hopefully have contributed to research and treatment of fatigability in patients with SMA.

## Quantification of fatigability in SMA: the need for customized outcome measures

Systematic data collection of children and adults with SMA in the Netherlands started in 2010. We were struck by the fact that many patients did not only mention weakness but also and explicitly their lack of endurance during motor activities they were able to perform. An initial pilot study in which patients repetitively placed 9 pins in 9 holes indicated that this lack of endurance, or fatigability, was real and could be quantified[2]. It also showed that we would need to develop a multi-level test that could be used by patients across the severity spectrum.

## Development of a set of endurance tests

We initiated this study to meet a direct need for fatigability outcome measures especially in non-ambulatory patients with SMA despite the fact that the development of new outcome measures is generally discouraged - if reasonable alternatives are available[3]. Besides the fact that measurement development is complex and time consuming, an abundance of tests complicates the comparison of cohorts of patients and efficacy of different clinical trials. We therefore used the methodological steps as recommended by the Consensus-based Standards for the selection of health Measurement Instruments (COSMIN) to systematically select or, in the absence of an appropriate test, develop a set of endurance tests for patients with SMA (**Chapter 2**)[4,5]. The choice of an outcome measure depends on many factors such as the proposed use, the concept to be measured, the feasibility of assessment, the requirements and costs associated with the use, the perceived burden of subjects and the measurement properties[6].



Since there was evidence that fatigability was at least partially the result of neuromuscular transmission failure[7], we developed the Endurance Shuttle Tests to detect fatigability as a result of repetitive action with the thought that it should be useful for clinical practice and trials. With these aims in mind, we set a number of important test criteria: endurance tasks should mimic daily life activities, so that changes in test performance will most likely lead to changes in daily life as well; outcome measures should be easy accessible to physical therapists, clinical evaluators and industry, so they can be included in clinical practice and pharmaceutical trials; and outcome measures should use one methodology for a broad clinical spectrum that will enable clinical trial design of small heterogenous samples. We used the tests as an outcome measure in the protocol for an investigator-initiated clinical trial on the effects of the acetylcholine esterase inhibitor pyridostigmine (**Chapter 7**).[8,9] The Endurance Shuttle Nine Hole Peg Test (ESNHPT) and the Endurance Shuttle Box and Block Test (ESBBT) have also been included in an international phase 2 study on the efficacy and safety of myostatin inhibition in patients with later-onset SMA[10], and in an open-label, observational prospective study on the clinical efficacy of Nusinersen (Spinraza©) in older children and adults (SMANL_1901).

Successful outcomes of the Endurance Shuttle Tests (EST's) in SMA does not automatically transfer to validity for other patient populations without prior consideration about the purpose and the construct of fatigability. In our study on the validity of the EST's, we compared patients with SMA with patients with Duchenne Muscular Dystrophy, Becker Muscular Dystrophy and Limb

Girdle Muscular Dystrophy (**Chapter 3**). These patient groups with similar levels of muscle weakness and motor impairment to SMA, showed normal endurance capacity on the EST's. That does not rule out the possibility that they may experience fatigability during certain physical efforts. As mentioned previously, the extent of fatigability may vary according to the method of testing[11]. Increased use of muscle capacity and impaired vasodilation due to deficiency of neuronal nitric oxide synthase (nNOS) have been suggested to cause fatigability in DMD and LGMD but this has not been confirmed with appropriate outcome measures[12-15]. The EST´s may be of added value to the assessment of patients with Myasthenic syndromes as they experience similar complaints of fatigability.

## Clinical relevant endpoints

Clinically relevant endpoints are a prerequisite for the regulatory authorities that grant market access to new drugs[16]. However, this proves to be a flexible concept in practice as clinical relevance has not been properly assessed for most outcome measures in SMA. For example, in the CHERISH-study (Nusinersen versus sham control in later-onset SMA) a change in the Hammersmith Functional Motor Scale Expanded (HFMSE) score of at least 3 points was considered to be clinically meaningful[17]. This was based on a previous phase 2 open-label study on the effect of valproic acid in SMA type 2, which reported a standard deviation of 3.19 for the Modified Hammersmith Functional Motor Scale (MHFMS-SMA). This distribution-based approach relates the observed change to the sample variability and therefore does not provide a good indication of the clinical importance of the observed change. Moreover, 88% of caregivers of children with SMA would consider taking part in clinical trial if the treatment effect was to only slow down deterioration rather than improvement[18]. To assure the clinical relevance of endurance tests we integrated a number of steps in the development of our test protocol (**Chapter 2**). We took questionnaires from patients with SMA to determine which activities of daily living provoked fatigability and clustered them intro three functional domains namely leg function, upper arm function and hand function. Consecutively, we modified three functional tests (Endurance Shuttle Walk Test, Box and Block Test, Nine Hole Peg Test) that already had been validated in other neuromuscular, neurological an pulmonary diseases and corresponded each with one functional domain for patients with SMA[19-25]. Consecutively, we compiled the Endurance Shuttle Test Combined Score (ESTCS) which allows comparison of patients with varying severity using data from the individual's most challenging endurance test (**Chapter 3**). To verify

the clinical relevance of the EST's we took two different approaches. In our validation study (**Chapter 3**), we asked subjects after each test whether it corresponded with activities causing fatigability in daily life. Interestingly, most patients indicated that it was recognizable but more intense, as they would normally not go on for so long in everyday life to prevent loss of important motor functions. This implies that other methods that are proposed to detect and quantify fatigability, such as questionnaires and activity registration may underestimate fatigability in SMA and it underlines the importance of endurance performance tests to determine true endurance capacity[26,27]. Secondly, in our clinical trial we included a patient reported outcome measure to determine patient reported changes in endurance at the end of each treatment period (**Chapter 7**). We found both a large objective effect (70% reduced drop-out risk on the ESTCS) and a large subjective effect (OR: 6.9) of pyridostigmine on fatigability in patients with SMA. Taken together, these data do not only demonstrate the clinical relevance of the EST's but also the important dimension of fatigability in SMA related motor dysfunction.



### *Capturing real-life impact of interventions*

To assess the efficacy of interventions it is important that outcomes, besides being clinically relevant, also capture, as much as possible, the real-life impact of disease on daily life functioning of an individual[28 29]. Children and adults with SMA that participated in our trial (**Chapter 7**), spontaneously mentioned that they could swim longer, drive longer distance with their power driven wheelchair, increase their repetitions during weight lifting and finish their make-up without breaks. This clearly indicates that reductions in fatigability improved quality of life and truly represent real-life impact of treatment on an individual level[29]. In rare disease drug trials, like in SMA, many outcome measures have threshold or ceiling effects and measure constructs that are not relevant or sensitive for all patients. This is certainly challenging in older and more severely affected patients for whom it is more likely that treatment efficacy is reflected by improvement in endurance during specific activities such as driving their power driven wheelchair, rather than acquiring new gross motor skills. The inclusion of endurance in future studies could ultimately reduce the percentage of patients being defined as non-responders.

Goal Attainment Scaling (GAS) is an outcome measure that can potentially solve the issue of small, heterogenous populations and has been used in approximately 40 drug studies of mainly neurological, geriatric, psychiatric and hematologic conditions to tap changes in the areas that are not covered by standardized outcome measures[28,30-32]. Together with their practitioner,

patients set individual treatment goals that meet the SMART principles (Specific, Measurable, Agreed upon, Realistic and Time-related). The levels of the goal attainment are quantified on a 3-5-point scale and attainment of these goals is measured in standardized manner that is comparable for each patient. The GAS is not a novel instrument but it has regained interest due to drug development in rare diseases. Advanced methodology and statistical analysis have been recently developed to validate GAS as a primary endpoint in drug trials for specific conditions[33,34]. To determine whether GAS is capable of capturing real-life impact of treatment in specific patient populations, construct validity must first be evaluated[33]. This can be done by comparing the change scores on the personal goals with change scores on other outcome measures in the same domain. Now that we have a panel of endurance tests at our disposal that represent multiple functional domains, it may be of interest to validate GAS within a clinical trial aimed at reducing fatigability in SMA (table 3).

**Table 3.** examples of Goal Attainment Scaling (GAS) in SMA

| Goals | -2 score | 0 score | +2 score | Comparator |
|---|---|---|---|---|
| *Being able to walk longer distances* | Able to walk 50 meter without a rest | Able to walk 100 meter without a rest | Able to walk 150 meter without a rest | ESWT Time to limitation |
| *Being able to eat a meal without breaks* | Able to eat a meal with 4 breaks | Able to eat a meal with 2 breaks | Able to eat a meal without breaks | ESBBT Time to limitation |
| *Being able to work on a laptop for a longer period of time* | Being able to work on a laptop during 5 minutes | Being able to work on a laptop during 10 minutes | Being able to work on a laptop during 15 minutes | ESNHPT Time to limitation |

## Characterization of fatigability: towards a better understanding

At the start of this project we knew very little about the mechanisms underlying fatigability in patients with SMA. Unlike muscle weakness and hypotonia, fatigability had never been well characterized, partly due to a lack of appropriate outcome measures. Studies of small cohorts of ambulatory patients did suggest altered gait patterns and muscle activation as measured by surface EMG during the 6MWT. Studies that included non-ambulatory patients were lacking[35,36]. As a consequence, the concept of fatigability in SMA has long remained unclear and has often been confused with perceived

fatigue by both clinicians and researchers[37]. Endurance performance is regulated by an interaction between fatigability and perceived fatigue and is influenced by central factors, peripheral factors and psychological factors[37]. In patients with SMA, the motor unit in particular appears to be vulnerable to fatigability, as abnormalities of the motor neuron, neuromuscular junction and muscle have been reported[7,27,38-40]. To reach a better understanding of fatigability in SMA we studied motor unit recruitment during endurance performance with surface EMG (**Chapter 4**) and determined correlations between fatigability, neuromuscular junction function, muscle function and perceived fatigue (**Chapter 5**).

## Motor unit recruitment

The hierarchy of motor units dictates that small, easily recruited, fatigue resistant motor units are activated first, followed by large, fast and rapidly fatigable motor units during progressive exercise intensity. This has become known as Henneman's size principle[41]. This recruitment pattern has a great economic advantage in that the most frequently used motor units are fatigue resistant and thus can provide enough force for most every day activities that require small forces over prolonged periods[11]. Fatigability in healthy persons during dynamic tasks is characterized by a shift towards lower median frequencies due to slower muscle fiber conduction velocities and towards higher amplitudes due to increased firing rates, recruitment and synchronization of motor units followed by a decrease[42-44]. Whether SMA motor units respond in a similar way can be questioned because the number of motor neurons is reduced, neuromuscular transmission failure has been detected in over 50% of patients and fiber type composition is characterized by a greater atrophy of fast-twitch type 2 muscle fibers and enlarged slow-twitch type 1 fibers[7,45,46]. It is important to increase our knowledge on motor unit recruitment in SMA as it may help to design effective treatment strategies. A recent study on the 6MWT in ambulatory patients did report a decrease in amplitude but unfortunately motor unit recruitment patterns could not be determined[35].

In our study on motor unit recruitment during the endurance shuttle tests, we made two important observations (**Chapter 4**). Patients with SMA demonstrated increased fatigability at submaximal intensity levels which further substantiates the assumption that SMA is associated with submaximal exercise intolerance. Secondly, we revealed motor unit reserve capacity in a subgroup of patients. This heterogeneity in muscle activation is probably partly explained by the methodology we used in our study and by the

degree of involvement of different parts of the motor unit. To closely mimic performance of daily life activities, we accepted certain movement strategies and compensatory mechanisms. This implies that muscle activation patterns we observed with surface EMG is above all a representation of movement behavior instead of isolated muscle capacity.

To explore isolated muscle activation during voluntary fatiguing exercise in more depth, a laboratory setting is probably better than the clinical setting we used in this study. Isokinetic dynamometry is considered the gold standard in muscle function assessment and has a number of advantages compared to functional tests, manual muscle testing and hand-held myometry. Isokinetic contraction is the muscular contraction that accompanies constant velocity limb movements around a joint[47]. Isokinetic muscle testing allows measurement during dynamic activity, quantifies multiple dimensions of muscle function such as peak torque (N-M), work (Joule), power (watts) and is appropriate for assessing strength and endurance in combination with surface EMG registration[48]. Although mostly applied in athlete sports and orthopedic rehabilitation, isokinetic muscle testing is an interesting approach for neuromuscular diseases. It has proven valid, reliable and feasible, even in weak patients[49,50]. Studies using isokinetic dynamometry to evaluate fatigability in neuromuscular disease are scarce and mainly consist of methods quantifying decline during maximal dynamic or maximal static contractions. Eeken et al. developed a submaximal repetitions-to-fatigue protocol using the isotonic mode of the isokinetic dynamometer to study endurance of the quadriceps in adolescents with spastic cerebral palsy. This innovative protocol which shares the same methodology as the endurance shuttle tests could potentially serve as a set-up to delve deeper into muscle activation during fatiguing submaximal exercise in SMA.

## Correlates of fatigability

An important reason for conducting the study on fatigability in SMA was the fact that many patients with SMA spontaneously reported having difficulty with sustaining activities of daily living. These complaints are reminiscent of problems encountered by patients with myasthenic syndromes which are caused by neuromuscular transmission failure, but are far less present in patient with other neuromuscular diseases with a similar pattern of muscle weakness and motor impairments. We therefore hypothesized that fatigability in SMA is associated with neuromuscular junction dysfunction and represents a separate dimension of physical impairment, in addition to muscle weakness. The results of our studies on the correlates of fatigability (**Chapter 5**) and on the effect of pyridostigmine on fatigability (**Chapter 7**) support these hypotheses and

provide novel insights into the associations between endurance, muscle strength, neuromuscular junction function and perceived fatigue (figure 1).

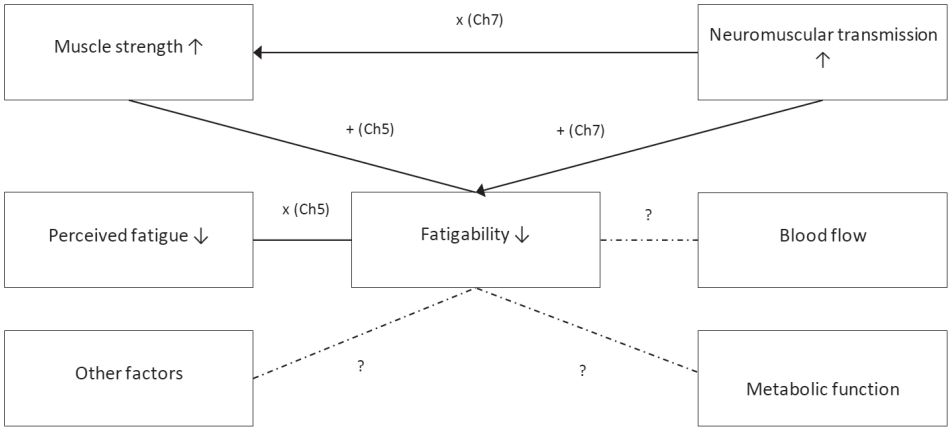


**Figure 1.** Correlates of fatigability in SMA



Lower levels of muscle strength were generally associated with increased fatigability but not in all patients. We have detected subgroups of patients with either relatively spared muscle strength but poor endurance and vice versa implying that fatigability and weakness represent different concepts. This assumption was further substantiated by the fact that patients with SMA treated with pyridostigmine improved their endurance performance while muscle strength remained unchanged. The question then is how fatigability can be explained in these relatively mild patients, in whom the prevalence of neuromuscular transmission failure was much lower than in more severely affected patients (**Chapter 5**)[7,51]. In addition to the possibility of undetected neuromuscular junction dysfunction, metabolic abnormalities could be part of SMA[52-57]. These abnormalities may play a greater role in relative mildly affected patients who have greater muscle mass and typically perform physical activities that require more aerobic capacity, such as walking, climbing stairs, cycling and swimming. To investigate the contribution of impaired energy metabolism to fatigability and exercise intolerance in patients with SMA type 3-4, we are currently conducting a study of supine dynamic exercise testing combined with in vivo Magnetic Resonance Spectroscopy (MRS)[58].

Where we managed to reduce fatigability with pyridostigmine, a first line treatment of disorders affecting NMJ function, it proved challenging to demonstrate the association between fatigability and neuromuscular junction function (**Chapter 5**). The nerve conduction studies were complicated by technical difficulties and low Compound Muscle Action Potential (CMAP) amplitudes leading to a large number

of missing values, particularly in patients with SMA type 2 in whom neuromuscular transmission failure is more common. This may have led to underestimation of the association between fatigability and neuromuscular junction. Repetitive nerve stimulation is used for diagnostics in patients suspected of Myasthenia Gravis but in patients with SMA it may be unfit for screening or evaluative purposes during clinical trials targeting neuromuscular junction dysfunction and fatigability[59]. Until sensitive and feasible alternatives are available, there is an important role to play for patient reported outcome measures and fatigability tests in the selection an evaluation of patients being treated.

## Physical exercise training in SMA: closing the gap

Physical exercise training is strongly advocated for in neuromuscular disorders including patients with SMA[60,61]. Training may improve functional muscle strength and exercise capacity by optimizing metabolic function in available muscle tissue and counteract further deterioration due to sedentary behavior[62]. Preclinical studies in SMA mouse models have reported neuroprotective effects of exercise by means of improved maturation and resistance of motor neurons to neuronal cell death[63-66]. Since patients with SMA may now benefit from the first approved disease-modifying therapies and other compounds that target skeletal muscle are being investigated, it is very likely that training will finally become possible for more patients. It may eventually enhance the effects of pharmacological interventions. The current international standards of care for SMA state the following on exercise: 'exercise programs and activities that encourage muscle activation should be encouraged since it can have an effect on maintaining and improving function, strength, range of motion, endurance, balance, activities of daily living, and participation in school, social activities and occupation´. Recommended exercise for sitters includes aquatic therapy, concentric and eccentric exercise and aerobic and general conditioning exercise with and without resistance. Recommended exercise for walkers include aerobic and general conditioning exercises such as swimming, walking, cycling, yoga, hippotherapy, rowing, cross-trainers. Aerobic exercises should at least last for 30 minutes. The practical applicability of these recommendations is however very limited due to the lack of specific guidelines on the FITT factors (Frequency, Intensity, Time and Type of exercise), which are required to design and execute an individual training program[67]. This is not surprising when you consider the paucity of well-designed studies on physical exercise training in patients with SMA (figure 2).

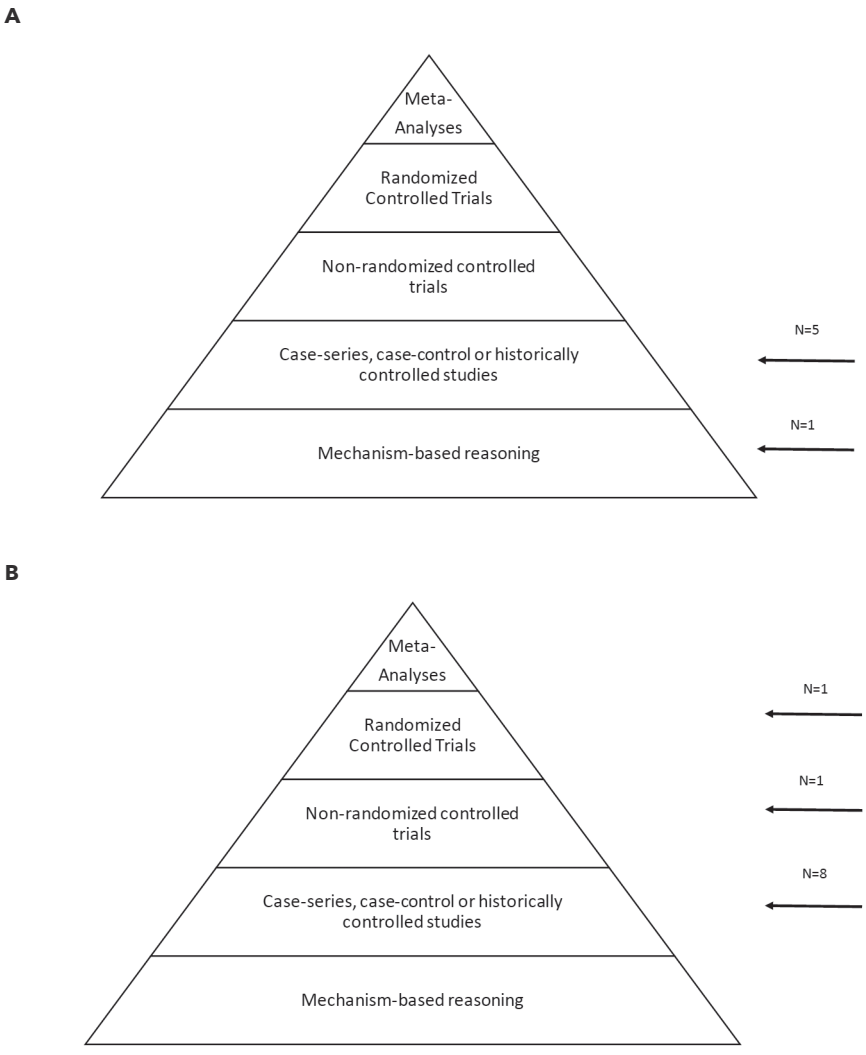


**Figure 2.** Quality of evidence for physical exercise training in SMA type 2 (A) and type 3 (B) (2019)

## Current evidence regarding exercise in SMA

To determine the current body of knowledge on exercise training in patients with SMA and uncover knowledge gaps complicating the design of effective exercise programs, we performed a Cochrane systematic review on physical exercise training in patients with SMA type 3 (**Chapter 6**). We identified 10 studies including one randomized controlled trial (RCT), one controlled trial, seven multi-case studies and one multi case study. We included one RCT[68] for quantitative analysis and additionally included two non-RCT's[69,70] in our discussion section that fulfilled the same standards regarding diagnostic

criteria, description of intervention and outcome measures. All other studies demonstrated either incomplete description or inadequate prescription of training programs[71-77]. We concluded that it is uncertain whether strength training and aerobic exercise are beneficial or harmful since the quality of evidence was very low.

## Exercise dosing

The three studies did provide interesting new insights into dose-response relationship during aerobic exercise training and strengthening training in patients with SMA. The feasibility and effect of aerobic exercise training was significantly different between studies[69,78]. Whereas Montes et al. found no significant improvement in aerobic capacity in the training group versus the usual care group, Madsen et al. reported an improvement of 27% in peak oxygen uptake. Montes et al. reported no significant difference in adverse events between groups, while fatigue was a major complaint in the study by Madsen causing one patient to drop out, increased need for sleep in three patients and training modifications in two patients. Optimal dosing of exercise (frequency x duration x intensity) is crucial for a balanced training program that is both feasible and effective. Exercise prescription in both studies was based on the ACSM guidelines for prescribing exercise in healthy adults[79]. Exercise intensity was set et either a level of 60-75% of peak oxygen uptake or a score of 5-7 on a subjective scale of perceived exertion, both parameters frequently used in cardiopulmonary rehabilitation. Whether these parameters, developed for cardiac and pulmonary disease, truly reflect exercise intensity in neuromuscular patients can be questioned. We recently studied cardiopulmonary exercise capacity in patients with Duchenne Muscular Dystrophy and Becker Muscular Dystrophy, both dystrophinopathies characterized by proximal weakness, and detected high peak Respiratory Exchange Rate ($RER_{peak}$) values indicating maximal effort, despite low peak heart rate values and low perceived fatigue score[80]. Both studies on aerobic exercise training in patients with SMA did not reach optimal training frequency within the predetermined period due to the inability to increment exercise intensity during the program, because of exercise induced fatigue, overexertion and fall incidents. Strength training was better tolerated reflected by high completion rates without an increase in adverse events[68,70]. Existing guidelines for (aerobic) exercise in healthy persons and patients with cardiopulmonary disease seem inappropriate for patients with SMA. Therefore, we have to expand our knowledge of exercise dosing in SMA before efficacy of training programs can be investigated and exercise guidelines can be developed.

### *High intensity versus low intensity training*

Recent insights from studies using different training modalities in the SMA mouse model may contribute to the development of a new exercise paradigm in patients with SMA [63-66,81]. Cahli et al. compared the efficacy of high intensity and amplitude swimming and low intensity and amplitude running for 20 min per day and 5 days per week for 10 consecutive months in a mild type 3 SMA-like mouse model compared to sedentary and trained controls. Remarkably , the high intensity swimming protocol demonstrated superior exercise improvements with respect to muscle fatigue and motor neuron protection (in particular medial and large motor neurons) compared to the low intensity training while similar positive results were seen in preservation of neuromuscular junction structure and neuromuscular excitability compared to sedentary SMA-like mice. An exercise specific motor neuron protection and hypertrophy was observed with fast motor neurons and large myofibers preferentially affected by high intensity training and slow motor neurons and intermediate muscle fibers by low intensity training. Additionally, Houdebine et al. demonstrated that SMA related systemic metabolic defects, such as glucose intolerance and lipid overload due to impaired fatty acid metabolism significantly improved by these exercise types, in particularly high intensity exercise[81] High intensity exercise in SMA may seem counterintuitive because of reports of exercise induced fatigue but these complaints usually occurred during endurance training. High intensity Interval Training, better known as HIT-training is a training modality that incorporates brief, intermittent bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise[82]. Efficacy and feasibility of HIT-training has been studied in few other neuromuscular diseases than SMA. In sedentary adults with facioscapulohumeral muscular dystrophy type 1, an eight week of supervised HIT-training was compared to usual care followed by an unsupervised training period of 8 weeks for both groups [83]. FSHD is a slowly progressive hereditary myopathy typically presenting during the second or third decades of life characterized by facial weakness, scapular winging and gradual involvement of the trunk, hip girdle and lower extremities muscles[84]. Patients trained three times weekly on a cycle ergometer. Training sessions lasted 21 min including an 8-minute warming-up and two sets of 5 minutes separated by a 3-min low intensity cycling. Each minute of HIT included 30 sec at low intensity (<30% peak workload), 20 seconds at middle high intensity (<60% peak workload) and 10 seconds of maximal intensity (90-100% peak workload)[85]. Both supervised and supervised HIT improved fitness (increase in oxygen uptake) without adverse events (unaffected plasma-CK levels and pain scores). Equally

important, patients preferred HIT over conventional aerobic exercise training. A similar training study in patients with Spinal and Bulbar Muscular Atrophy, a slowly progressive X-linked disease of the lower motor neuron and muscle based on a defect androgen receptor, showed similar positive results on fitness and feasibility[86]. SBMA is characterized by proximal limb, distal limb and bulbar muscle weakness, tremor and cramping with an average disease onset in the mid-40s[87]. Low and moderate exercise training studies in patients with SBMA have demonstrated a similar blunted exercise response and exercise induced fatigue comparable to SMA[69]. HIT-training could potentially serve as an effective time-efficient alternative to endurance exercise training in SMA as well[82,88]. The precise intensity and minimal volume of high intensity interval training to potentiate effect in neuromuscular disease is probably the most challenging part and key to a successful outcome. Andersen et al. studied the effect of different levels of intensity (65-95% of maximal oxygen uptake) on plasma creatine kinase (CK) in patients with Becker Muscular Dystrophy (BMD), FSHD, Limb Girdle Muscular Dystrophy type 2 (LGMD) and healthy subjects and showed that high-intensity was well tolerated in patients with FSHD and LGMD while patients with BMD were prone to exercise-induced damage[89]. Similarly in patients with SMA, a series of studies is needed to systematically titrate exercise dosing and determine subsequent beneficial and detrimental physiological and functional responses, before effective and feasible (HIT) training studies can be developed.

## Treatment in SMA: a motor unit based approach

While SMA is traditionally categorized as a disease of motor neurons, dysregulation of synaptogenesis including neuromuscular junction dysfunction appears to play a pivotal role in early disease pathogenesis as well as intrinsic abnormalities in SMA skeletal muscle[90-94]. The randomized double blinded cross-over trial of pyridostigmine we performed in otherwise untreated patients with SMA type 2-4 is the first study to effectively target fatigability in SMA through the pathway of neuromuscular transmission (**Chapter 7**) (figure 3). It showed that patients with SMA can be treated effectively through enhancement of neuromuscular transmission without upregulation of SMN protein which further substantiates the important role of the neuromuscular junction in SMA. Patients demonstrated improved endurance capacity without associated increases in strength or motor function suggesting that these are partly independent symptoms that may co-exist

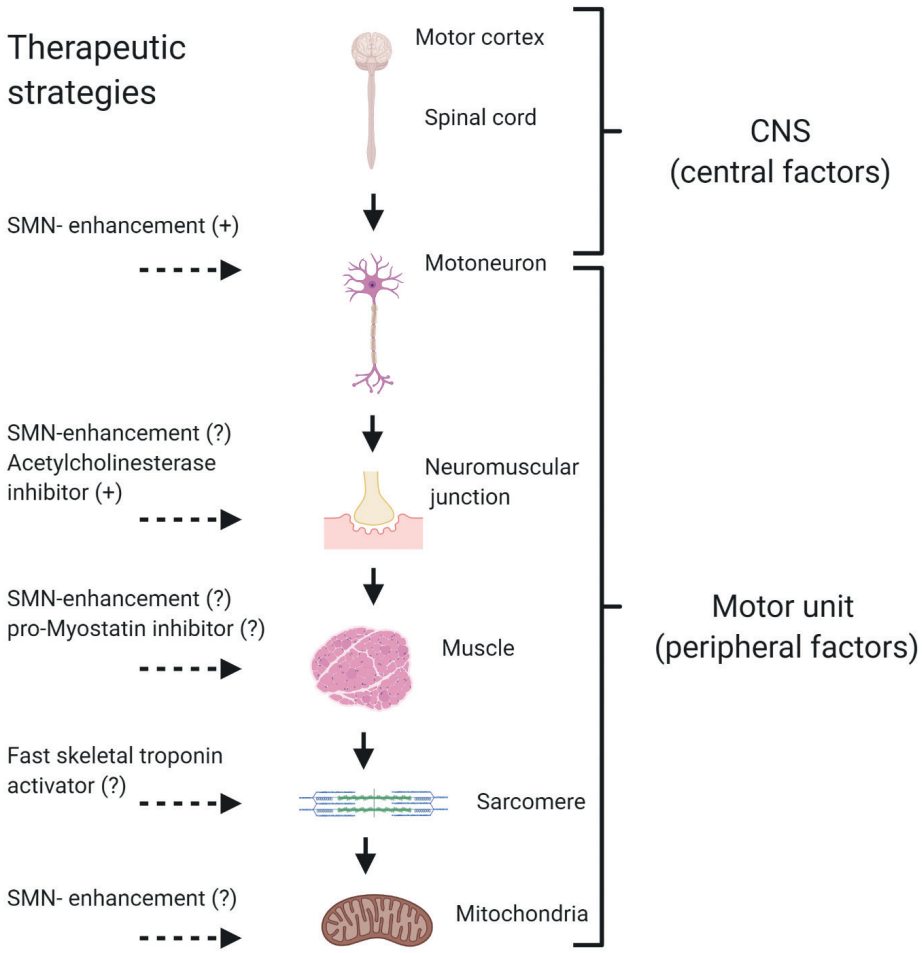


**Figure 3.** Therapeutic strategies

and require other treatment strategies (figure 1). The therapeutic window for fatigability is not limited to the period of neuromuscular development and maturation during early childhood but extends into adulthood. This is possibly explained by the fact that synaptic function plays an important role in the long-term maintenance of motor neurons which is at risk at (early) adulthood due to neurodegeneration[91,95].

We were able to reduce fatigability in most but not all patients with SMA. Respectively 40% and 20% of subjects reported slight and strong improvement at the end of the treatment period with pyridostigmine while

40% of patients did not perceive benefit from this treatment. This could have several reasons including suboptimal dosing, varying involvement of neuromuscular transmission failure between patients, involvement of other causes of fatigability and insufficient treatment duration. Fatigability is a complex phenomenon which can be influenced by many factors. Besides neuromuscular transmission failure, fatigability may be compromised by reduced motor neuron capacity, metabolic dysfunction, muscle weakness and impaired blood flow[39,40,52,96-99]. Complementary therapy strategies are probably required to reduce fatigability in patients who respond insufficiently to pyridostigmine.

## SMN-enhancing therapy

The effect of SMN enhancing therapy on fatigability in SMA has not been systematically studied. While the exact working mechanism of SMN protein has not been elucidated, increased levels of SMN protein might directly or indirectly improve fatigability through increased motor neuron capacity, improved neuromuscular junction and mitochondrial function. None of the pivotal phase 3 clinical trials on Nusinersen (Spinraza©) and gene therapy or ongoing trials on systematic delivery of ASO included fatigability outcome measures[17,100-102]. Post hoc analysis of a phase 2 study in ambulatory children and adolescents with SMA type 3 do suggest moderate reduction or stabilization of fatigability during walking on the 6MWT [103]. To further elucidate the effect of SMN protein enhancing therapy on fatigability we have included the endurance shuttle tests in an open-label, observational prospective study on the clinical efficacy of Nusinersen (Spinraza©) in children above 9.5 years of age and adults that has recently started in the Netherlands.

## Muscle-directed therapy

There are currently two muscle directed approaches in development to treat SMA that use additional pathways to enhance muscle function (figure 3). Cytokinetics Inc. developed a small molecule fast skeletal troponin activator (CK-2017357), that increases calcium sensitivity by slowing the rates of calcium release from troponin C, allowing submaximal forces to be produced at lower energic cost [95,104]. A recent study in rodent skeletal muscle, demonstrated improved muscle endurance during either rotarod or treadmill running accompanied by decreased ATP demand and reduced glycogen usage[105]. A phase 2, double blind, randomized, placebo-controlled clinical study in 70 patients 12 years and older with SMA type 2-3 demonstrated a moderate increase in six minute walk distance and maximal expiratory mouth

pressure[106]. Scholar Rock Inc. has developed a fully human anti-proMyostatin monoclonal antibody (SRK-015) that promotes muscle growth, preferentially of fast twitch muscle fibers, by inhibition of myostatin, a negative regulator of muscle mass[45,107]. While inhibition of myostatin pathways gives only small or no effect in severe SMA mice likely due to low levels of myostatin, the combination of myostatin inhibition with either a high dose or low dose SMN-restoring antisense therapy demonstrated an improvement in weight, muscle mass, muscle fiber size and anaerobic performance in the high dose group and improvement in survival, functional strength, neuromuscular junction development and neuromuscular circuitry in the low dose group of a severe SMA mouse model[108]. Remarkably, physical endurance of the high dose group significantly increased in the first 45 days but declined steeply from then. This may be explained by a combination of increasing oxygen cost of hypertrophic muscles and a shift of muscle towards anaerobic energy metabolism[109]. Thus, the inhibition of myostatin may potentially lead to an increase in strength but at the expense of endurance capacity. A phase 2 study is currently underway in which endurance capacity is measured with the ESNHPT and ESBTT which will likely provide more insight into the influence of myostatin inhibition on fatigability in SMA [10].



## Combinatorial therapies

Muscle function is subject to a complex interaction between the motor neuron, neuromuscular junction and muscle. Deficits at each particular level have been found in SMA and can potentially lead to increased weakness and fatigability. Our clinical trial has demonstrated proof of concept that fatigability can be treated through improved neuromuscular transmission. The fact that a single approach is not enough is strikingly illustrated by one of the subjects in our trial who noticed improved endurance but did experience exercise induced muscle pain for the first time. A motor unit approach in which different treatment strategies are combined seems the way forward to optimally treat both weakness and fatigability in SMA. It goes without saying that the selection of clinimetric robust and meaningful outcome measures for muscle strength, muscle endurance and muscle power are an essential part of this approach.

## Concluding remarks

Fatigability is frequently mentioned and considered as a highly debilitating impairment of daily life by many patients with SMA, but has nevertheless been given little priority for a long time. Until recently, clinical trials have been mainly focused on survival and early development of young infants with little understanding or sense of urgency with respect to fatigability despite this is an important problem for older patients. The results of these studies on fatigability in SMA will hopefully contribute to research and treatment of fatigability on three different levels. First, outcome measures are now available that can be used in clinical trials to assess efficacy in small heterogenous samples of children and adults with SMA. Secondly, we have demonstrated that fatigability is a highly prevalent additional dimension of physical impairment that requires attention and treatment. Third, we have demonstrated efficacy of a safe and low-cost treatment which can be used as an add-on therapy to genetic therapies or as an alternative for children and adults without access to expensive SMN-augmenting treatments. Fatigability in SMA is, however, a complex phenomenon of which the pathophysiological background, the impact on daily life and the optimal treatment will vary between patients. It is important to aim for a personalized approach in the near future, aiming to identify the main limiting factor, the most relevant outcome measures and the best treatment for each individual patient.

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# Nederlandse samenvatting

Spinale musculaire atrofie (SMA) is een ernstige, autosomaal recessieve neuromusculaire aandoening en één van de belangrijkste genetische oorzaken van sterfte op de kinderleeftijd. SMA wordt gekarakteriseerd door degeneratie van alfa-motoneuronen in het ruggenmerg, ernstige progressieve spierzwakte en spieratrofie. SMA wordt gekenmerkt door een grote variabiliteit in ziekte-ernst variërend van neonatale respiratoire insufficiëntie en sterfte tot relatief milde beperkingen op de volwassen leeftijd. SMA wordt traditioneel ingedeeld in drie klinische subtypen (SMA type 1-3) op basis van het debuut van de klachten en het behalen van specifieke motorische mijlpalen. Dit proefschrift richt zich op kinderen en volwassen met SMA type 2 (1ste klachten tussen de 6-18 maanden en (ooit) zelfstandig kunnen zitten) en SMA type 3 (1ste klachten > 18 maanden en (ooit) zelfstandig kunnen lopen). Sinds de start van deze studie in 2014 is het vooruitzicht voor veel kinderen met SMA en hun ouders, en volwassenen met SMA drastisch veranderd. In 2017 werd de eerste effectieve behandeling met de intrathecale toediening van de antisense-oligonucleotide (ASO) therapie Nusinersen (Spinraza®) goedgekeurd door de European Medicines Agency (EMA). Naar verwachting zal in 2020 ook de eerste gentherapie voor SMA worden goedgekeurd. Alhoewel behandeling met ASO- en gentherapie leidt tot significante verbeteringen in overleving en motorische functie in voornamelijk jonge kinderen met SMA, is het een reële verwachting dat er bij de meeste patiënten veel restklachten aanwezig blijven. De combinatie van behandelingen gericht op het behoud en verbetering van de motor unit (motor neuron, de neuromusculaire overgang en de spier) gedurende de verschillende fases van de ziekte zal hoogstwaarschijnlijk de volgende stap zijn in de behandeling van patiënten met SMA. De core-set van fysieke uitkomstmaten voor SMA bestaat voornamelijk uit functionele schalen. De verwachting is dat deze uitkomstmaten minder sensitief zijn in het meten van vooruitgang bij oudere kinderen, volwassenen en patiënten die mildere aangedaan zijn of niet de dimensies van fysieke beperkingen meten die relevant zijn voor deze patiëntcategorieën. Spierzwakte en spieratrofie worden gezien als de belangrijkste kenmerkende symptomen van de aandoening. Alhoewel vermoeibaarheid al in de jaren tachtig werd gerelateerd aan SMA, heeft het lang geduurd totdat het erkend werd als een belangrijke additionele beperking van het dagelijks functioneren. Vermoeibaarheid wordt gedefinieerd als de achteruitgang in prestaties en werd spontaan genoemd door ongeveer 40% van de patiënten met SMA die in de periode van 2010-2014 werden geïncludeerd in de Nederlandse SMA database. Op het moment dat een bepaalde klacht gekwantificeerd moet worden zal er bij

voorkeur gebruik gemaakt worden van een bestaand meetinstrument dat gevalideerd is voor de doelgroep. Wanneer dit niet mogelijk is zal een bestaand meetinstrument aangepast of een nieuw meetinstrument ontwikkeld en gevalideerd moeten worden. Voordat een meetinstrument gebruikt kan worden in klinische trials moeten een aantal psychometrische eigenschappen beoordeeld worden. Hierbij wordt onderscheid gemaakt tussen de validiteit (meet ik wat ik wil weten?) en de betrouwbaarheid (zijn de metingen reproduceerbaar?). In de ontwikkeling van een meetinstrument wordt bij voorkeur de content validiteit als eerste bepaald. Content validiteit betreft de mate waarin het meetinstrument een adequate reflectie geeft van het construct wat gemeten moet worden. In **hoofdstuk 2** wordt het uitgebreide proces van de ontwikkeling en het bepalen van de content validiteit van een nieuwe set van uitkomstmaten voor vermoeibaarheid bij zowel milde als ernstige patiënten met SMA beschreven. Er is hierbij gebruik gemaakt van de vijf methodologische stappen volgens COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments) en input vanuit een scoping review, vragenlijsten ingevuld door patiënten, klinische expert panels en verschillende pilot studies. Dit heeft geleid tot de ontwikkeling van de Endurance Shuttle Walk test (ESWT) voor ambulante patiënten, de Endurance Shuttle Box and Block Test (ESBBT) voor non-ambulante patiënten met proximale armfunctie en de Endurance Shuttle Nine Hole Peg Test (ESNHPT) voor non-ambulante patiënten met alleen nog handfunctie. Deze Endurance Shuttle Tests (EST's) werden vervolgens in drie verschillende pilot studies onderzocht op begrijpelijkheid, haalbaarheid en sensitiviteit in gezonde proefpersonen, patiënten met neuromusculaire ziekten en andere chronische zieke kinderen en patiënten met SMA . Dit heeft uiteindelijk geresulteerd in een set van goed toepasbare uitkomstmaten die het uithoudingsvermogen tijdens relevante activiteiten bij zowel ernstige als relatief milde patiënten kwantificeert. Op het moment dat een uitkomstmaat een goede content validiteit heeft is de volgende stap het bepalen van de betrouwbaarheid en de constructvaliditeit in een grotere groep patiënten. In **hoofdstuk 3** onderzochten we de constructvaliditeit en de test-hertest betrouwbaarheid bij 61 patiënten met SMA types 2-4, 25 gezonde controles en 15 ziektecontroles bestaande uit andere neuromusculaire ziekten met een vergelijkbaar fenotype. Daarnaast werd de Endurance Shuttle Test Combined Score (ESTCS) ontwikkeld, wat het mogelijk maakt om milde en ernstige patiënten met elkaar te vergelijk op hun individuele meest relevante EST. Er bleek een hoge prevalentie van vermoeibaarheid bij zowel ernstige als relatief mild aangedane patiënten. In overeenstemming met de hypothese a priori zien we dat het uitvoeren van de EST's gepaard ging met een toename van subjectieve lokale spiervermoeidheid, toename in compensa-

toire bewegingen en afname in lokale spierkracht. Alle EST's lieten een duidelijk onderscheid zien tussen snel en sterk vermoeibare patiënten met SMA en nauwelijks vermoeibare gezonde controles. De ESNHPT bleek ook in staat om een duidelijk onderscheid te maken te maken tussen sterke vermoeibare patiënten met SMA en minimaal vermoeibare ziektecontroles ondanks het feit dat de spierkracht vergelijkbaar was. Hieruit kan worden afgeleid dat naast spierzwakte, vermoeibaarheid een aparte dimensie is van fysieke beperkingen bij patiënten met SMA. Ook werd het duidelijk dat de ESNHPT en ESBBT met name sensitieve uitkomstmaten zijn voor patiënten met SMA type 2 en 3a terwijl de ESWT geschikt is voor ambulante patiënten. De betrouwbaarheid en de mate van overeen-stemming tussen test en hertest was redelijk en substantieel bij de ESNHPT, hoog en excellent bij de ESBBT en ESWT. Het gebruik van de ESTCS resulteerde in een toename in sensitiviteit en grotere sample terwijl de betrouwbaarheid enigszins afnam. Dit betekent dat afhankelijk van de samenstelling en grootte van de studie sample en de acceptabele meetfout gekozen kan worden voor de ESTCS of een individuele EST. Vermoeibaarheid is een ernstige functionele beperkingen bij SMA maar de precieze oorzaak is nog onbekend. Nu er valide en betrouwbare uitkomstmaten voor vermoeibaarheid beschikbaar zijn is het mogelijk om vermoeibaarheid uitgebreider te bestuderen. Oppervlakte EMG registratie tijdens inspanning is een methode die spieractivatie tijdens inspanning en vermoeibaarheid inzichtelijk maakt. In **hoofdstuk 4** hebben we spieractivatie met oppervlakte EMG bij patiënten met SMA tijdens de uitvoering van de EST's bestudeerd. We onderzochten de intensiteit waarop de testen werden uitgevoerd in vergelijking met gezonde controles en we onderzochten of patiënten met SMA de mogelijkheid hadden om motor unit reserve capaciteit aan te spreken tijdens vermoeibaarheid. Spieractivatie werd gemeten bij 70 patiënten met SMA en 19 gezonde controles tijdens uitvoering van de ESNHPT, ESBBT en ESWT. Patiënten met SMA voerden alle EST's uit op een hogere sub-maximale intensiteit dan gezonde proefpersonen. Hiermee werd het sub-maximale karakter van de EST's bevestigd en hebben we laten zien dat er bij patiënten met SMA sprake is van inspanningsintolerantie tijdens sub-maximale inspanning. Een deel van de patiënten met SMA lieten een toename in amplitude zien tijdens uitvoering van de verschillende EST's. Dit suggereert dat sommige maar niet alle patiënten de beschikking hebben over reservecapaciteit aan motor neuronen die ingezet kunnen worden tijdens het optreden van vermoeibaarheid. Het behouden of vergroten van deze motor unit reservecapaciteit is een potentieel therapeutisch doel voor klinische trials en klinische behandeling. Het is nog onbekend wat de oorzaken van vermoeibaarheid bij SMA zijn. Eén van de mogelijke hypotheses is dat vermoeibaarheid wordt

veroorzaakt door een dysfunctie van de neuro-musculaire overgang. De vermoeibaarheid die patiënten met SMA ervaren tijdens repeterende activiteiten lijkt sterk op de vermoeibaarheidsklachten die patiënten met Myasthenia Gravis, een ziekte gekenmerkt door dysfunctie van de neuromusculaire overgang, en uit onderzoek blijkt dat zowel de anatomie als de fysiologie van de neuromusculaire overgang bij SMA afwijkend is. Vermoeibaarheid kan echter ook gerelateerd zijn aan andere factoren zoals spierzwakte, beperkte motoriek en ervaren vermoeidheid. In **hoofdstuk 5** onderzochten we de associatie tussen vermoeibaarheid, spierkracht, motorische functie, functie van de neuromusculaire overgang en ervaren vermoeidheid bij 61 patiënten met SMA type 2-4. Uit deze studie bleek dat sterkere vermoeibaarheid geassocieerd is met spierzwakte en een beperkte motoriek. We vonden echter ook subgroepen van patiënten met een goede spierkracht en motorische functie maar sterke vermoeibaarheid en vice versa. Dit laat zien dat vermoeibaarheid niet equivalent is aan spierzwakte en er andere factoren betrokken zijn. Het onderzoek naar een associatie tussen vermoeibaarheid en functie van de neuromusculaire overgang werd bemoeilijkt door veel missende EMG-waarden bij voornamelijk ernstige patiënten. De trend die we vonden in deze studie komt overeen met andere studies en de resultaten uit **hoofdstuk 7** die een relatie tussen vermoeibaarheid en dysfunctie van de neuromusculaire overgang laten zien. Vermoeibaarheid bij SMA kan mogelijk verminderd worden door zowel farmacologische- als fysieke interventies. Fysieke training kan mogelijk de spierfunctie en -cardiorespiratoire fitheid bij SMA verbeteren. Optimalisatie van de aerobe capaciteit of andere bronnen in resterend spierweefsel door middel van inspanning kan mogelijk achteruitgang in spierfunctie ten gevolge van het verlies van motorneuronen en inactiviteit tegengaan. Alhoewel er enkele gecontroleerde studies gedaan zijn naar het effect van training bij patiënten met SMA type 3 was er geen synthese beschikbaar van de beschikbare evidentie op basis waarop geconcludeerd kan worden of training veilig en effectief is. In **hoofdstuk 6** beschrijven we een systematische Cochrane review naar het effect en mogelijk bijwerkingen van fysieke training bij kinderen en volwassenen met SMA type 3. We vonden tien studies naar het effect van training bij SMA type 3 waarvan slecht één enkel geblindeerde gerandomiseerde gecontroleerde studie naar het effect van een gecombineerde spierkracht en aerobe training gedurende zes maanden geïncludeerd werd. De andere negen studies werden geëxcludeerd op basis van een inadequaat design waarbij veel studies ook een incomplete beschrijving van de interventie gaven. De geïncludeerde studie liet geen significant verschil in effect zien van training op de loopafstand, functionele vaardigheden, spierkrachten inspanningscapaciteit en bijwerkingen tussen de interventiegroep en de controlegroep. Het bewijs op

basis van deze studie is tevens erg onzeker door een aantal methodologische beperkingen zoals de kleine groepsgrootte, onvoldoende trainingsdosis en enkele blindering. Samenvattend levert de synthese van de beschikbare evidentie onvoldoende bewijs voor of tegen fysieke training bij patiënten met SMA type 3. Er is meer evidentie van hoogstaande kwaliteit nodig om effectieve en veilige trainingsrichtlijnen en -adviezen te kunnen ontwikkelen voor patiënten met SMA type 3. De balans tussen haalbaarheid en optimale dosering van training lijkt cruciaal bij de ontwikkeling van effectieve training interventies, met name t.a.v. aerobe training. Pyridostigmine is een acetylcholinerestase inhibitor wat bij patiënten met Myasthenia gebruik wordt om de functie van de neuromusculaire overgang en daarmee de vermoeibaarheid te verbeteren. Vanwege het feit dat patiënten met SMA vergelijkbare klachten en afwijkingen laten zien onderzochten we in een fase 2, mono center, placebo gecontroleerde, dubbelblinde cross-over trial het effect en de veiligheid van Pyridostigmine op motorische functie en vermoeibaarheid bij SMA type 2-4 (**Hoofdstuk 7**). Elke patiënt werd in willekeurige volgorde acht weken behandeld met Pyridostigmine en achten weken behandeld met placebo. Vijfendertig patiënten werden gerandomiseerd en geïncludeerd in de analyses. We vonden een rand significant gemiddeld verschil in effect op de MFM van 0.74% ter faveure van Pryidostigmine. Er was geen verschil in effect op de andere primaire uitkomstmaat (RNHPT) maar we vonden wel een 70% afname in het risico op uitval op de ESTCS in de groep die Pyridostigmine kreeg en een sterk subjectief ervaren verbetering in vermoeibaarheid. In de groep die behandeld werd met Pyridostigmine kwam meer milde bijwerkingen voor dan de placebogroep maar geen ernstige bijwerkingen.

## Samenvattend zijn de belangrijkste bevinden van dit proefschrift:

- Vermoeibaarheid is een frequent voorkomende functionele beperking tijden het uitvoeren van dagelijkse activiteiten bij zowel milde als ernstige patiënten met SMA.

- De Endurance Shuttle Walk Test, Endurance Shuttle Box and Block Test en de Endurance Shuttle Nine Hole Peg Test zijn valide en betrouwbare uitkomstmaten voor vermoeibaarheid bij zowel ernstig als relatief mild aangedane kinderen en volwassenen met SMA.

- Het gebruik van de Endurance Shuttle Test Combined Score maakt het mogelijk om patiënten met verschillende ziekte-ernst met elkaar te vergelijken en kan daardoor toegepast worden in kleine heterogene trials.

- Een deel van de patiënten met SMA heeft de beschikking over een reservecapaciteit van motoneuronen die ingezet kan worden tijdens vermoeibaarheid.

- Vermoeibaarheid is geassocieerd maar niet equivalent aan spierzwakte. Er bestaan subgroepen van patiënten met een goede spierkracht en motorische functie maar sterke vermoeibaarheid en vice versa.

- De associatie van vermoeibaarheid met dysfunctie van de neuromusculaire overgang is mogelijk aanwezig maar onderzoek hierna wordt gecompliceerd door veel technische beperkingen van het EMG-onderzoek bij zwakke patiënten met SMA.

- De behandeling met Pyridostgimine leidt tot klinisch relevante verbeteringen in vermoeibaarheid met relatief milde bijwerkingen.

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# Curriculum Vitae

Bart Bartels was born on November 14, 1980 in Nijmegen, The Netherlands. After graduating from Canisius College Mater Dei in Nijmegen in 1998, he studied physiotherapy at the HU University of Applied Sciences Utrecht between 1999-2003. In 2004, he worked for 6 months as a physiotherapist in the pediatric ward of 'Obras Sociales Hermano Pedro', a small hospital in Antigua, Guatemala. In 2008 he obtained a professional master in pediatric physiotherapy at Breda University of Applied Sciences. Between 2004-2009 he worked in several rehabilitation centers and the Sophia Children's Hospital in Rotterdam as a pediatric physical therapist and obtained his first junior research appointment at the department of rehabilitation medicine of the Erasmus Medical Center Utrecht investigating young adults with Duchenne Muscular Dystrophy (dr. Pangalila, dr. Roebroeck). In 2009 he continued his career as a pediatric physiotherapist in the Wilhelmina Children's Hospital University Medical Center Utrecht at the Child Development and Exercise Center (prof dr. Helders). In 2012 he obtained his Master in Sciences in Clinical Health Sciences at Utrecht University. Between 2012-2014, he worked as a junior researcher investigating exercise response in children with Duchenne and Becker Muscular Dystrophy (dr. J.F. de Groot). In 2014 he started his PhD-training at the SMA center of expertise in close collaboration with the Child Development and Exercise Center (Prof. dr. W.L. van der Pol and dr. J.F. de Groot) which resulted in this thesis. During this doctoral project, he initiated and collaborated in several other research projects, including two projects using dynamic in vivo $^{31}$p magnetic resonance spectroscopy to explore metabolic function and muscle fiber type recruitment in patients with Spinal Muscular Atrophy (dr. J. Jeneson, prof. dr. W.L. van der Pol). He is part of the TULIPS (Training Upcoming Leaders in Pediatric Science) PhD curriculum 2016-2017. In 2019, he initiated the Spieren voor Spieren Inspanningslab, funded by Stichting Spieren voor Spieren, with the aim of developing safe and effective training guidelines for children with muscle diseases. Currently he combines his clinical and research activities with a teaching position at the pre-master Clinical Health Sciences of Utrecht University. Bart is married to Marina Samayoa Marroquín and they have two children: Sara (2015) and Lucas (2019).

# List of publications

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Marloes Stam*, Camiel A. Wijngaarde*, **Bart Bartels***, Fay-Lynn Asselman, Louise A.M. Otto, Laura E. Habets, Ruben P.A. van Eijk, Bas M. Middelkoop, H. Stephan Goedee, Janke F. de Groot, Kit. C. B. Roes, Marja A.G.C. Schoenmakers, Edward E.S. Nieuwenhuis, Inge Cuppen, Leonard H. van den Berg, Renske I. Wadman#, W. Ludo van der Pol#. Randomised, double-blind, cross-over, phase 2 trialof pyridostigmine versus placebo in spinal muscular atrophy types 2,3 and 4 (submitted)