UNIVERSITY OF COPENHAGEN
FACULTY OF HEALTH AND MEDICAL SCIENCE

PhD Thesis

Linda Kahr Andersen

FATIGUE AND PHYSICAL ACTIVITY IN PATIENTS WITH MYASTHENIA GRAVIS

This thesis has been submitted to the Graduate School of the Faculty of Health and Medical Sciences, University of Copenhagen, 1 October 2021

PH.D. THESIS

Linda Kahr Andersen, PT, MSc

Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark.

SUPERVISOR

John Vissing, MD, DMSc, Professor, Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark.

CO-SUPERVISOR

Nanna Witting, MD, Ph.D., Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark.

EXTERNAL ASSESSOR

Mette Aadahl, PT, Ph.D., Professor, Center for Clinical Research and Prevention, Copenhagen University Hospital - Bispebjerg and Frederiksberg, Denmark.

ASSESSMENT COMMITTEE

Finn Sellebjerg, (chairman of the committee), MD, DMSc, Professor, Department of Neurology, Copenhagen University Hospital - Rigshospitalet, Glostrup, Denmark

Henning Andersen, MD, DMSc, Professor, Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

Anna Rostedt Punga, MD, Ph.D., Professor, Department of Neuroscience, Clinical Neurophysiology, Uppsala University Hospital, Uppsala, Sweden

The photo on the front page is from Colourbox.com

CONTENT

ACKNOWLEDGEMENT

First, thanks to all the patients with myasthenia gravis that participated in the studies. This thesis would never have been possible without your enthusiasm, interest, willingness, time, and dedication. Thanks for sharing your thoughts, ideas, and experiences of life with MG. I sincerely hope that the findings of the studies will benefit you and other patients in the future.

This project was only possible with the support from lots of people. From my heart, thanks to each of you: To principal supervisor John Vissing for always believing in my ideas and supporting me when needed. To co-supervisor Nanna Witting and external assessor Mette Aadahl for exciting discussions and constructive feedback on the papers. To my colleagues at Copenhagen Neuromuscular Center for laughing, coffee breaks, and inspiring conversations. To Kirsten Knak, my fellow from the very beginning in 2014. Thanks for this long journey into the neuromuscular field and research. Thanks for always being cheerful, smiling, calm, and supporting in challenging times (statistic courses) and sunny days. To Anna Sofie Jakobsson for finding the anchor in PASS, for co-authorship and drinks in the cold. To Karoline Lolk Revsbech for your friendly and professional test of patients. To Pia Hynne for keeping track of the funding and Thomas Krag for kindly help with the illustrations.

Thanks to Bernt, my children, my parents, family, and friends for your faith in me and for supporting me in any way. Thanks to all of you for showing interest in my work.

This Ph.D. thesis is based on studies executed at Copenhagen Neuromuscular Center, Copenhagen University Hospital – Rigshospitalet from March 2014 to June 2020. The thesis was generously funded by:

- Copenhagen University Hospital – Rigshospitalet
- Argenx (study III) (the company did not influence study design, analyses, or writing of the manuscript)
- Grosserer L.F. Foghts Fond

Linda Kahr Andersen, 2021

ABBREVIATIONS

2MWT = 2-minute walk test

6MWT = 6-minute walk test

AChR = acetylcholine receptor

BMI = body mass index

CCI = Charlson Comorbidity Index

CI = confidence interval

DNRP = Danish National Registry of Patients

gMG = generalized myasthenia gravis

IPAQ = International Physical Activity Questionnaire

ISI = Insomnia Severity Index

IQR = inter-quartile range

MDI = Major Depression Inventory

MFI-20 = Multidimensional Fatigue Inventory

MG = myasthenia gravis

MG-ADL = Myasthenia Gravis Activities of Daily Living profile

MGC = Myasthenia Gravis Composite scale

MGFA = Myasthenia Gravis Foundation of America

MG-QoL15 = MG-specific Quality-of-Life instrument

MuSK = muscle-specific kinase

MVPA = moderate-vigorous physical activity

PA = physical activity

PASS = Patient Acceptable Symptom State

QMG = Quantitative Myasthenia Gravis score

RAS = rhythmic auditory stimulation

SD = standard deviation

WT = walking-related fatiguability

LIST OF PAPERS

PAPER I

L.K. Andersen, M. Aadahl, J. Vissing, Fatigue, physical activity and associated factors in 779 patients with Myasthenia Gravis, Neuromuscular Disorders, 2021-08-01, Volume 31, Issue 8, p. 716-725

PAPER II

L.K. Andersen, J. Vissing. Habitual physical activity in patients with myasthenia gravis assessed by accelerometry and questionnaire, Journal of Neuromuscular Diseases, 2021. Jul 28. DOI: 10.3233/JND-210693. Epub ahead of print.

PAPER III

L.K. Andersen, A.S. Jakobsson, K.L. Revsbech, J. Vissing. Causes of symptom dissatisfaction in patients with generalized myasthenia gravis. Journal of Neurology, 2021. DOI: 10.1007/s00415-021-10902-1. Epub ahead of print.

PAPER IV

L.K. Andersen, K.L. Knak, N. Witting, J. Vissing. Two- and 6-minute walk tests assess walking capability equally in neuromuscular diseases. Neurology, 2016-02-02, Vol.86 (5), p.442-445.

PAPER V

Linda Kahr Andersen, Nanna Witting & John Vissing (2021) Effects of rhythmic auditory stimulation on walking during the 6-minute walk test in patients with generalised Myasthenia Gravis, European Journal of Physiotherapy, DOI: 10.1080/21679169.2021.1876760

ENGLISH SUMMARY

Myasthenia gravis (MG) is a chronic, autoimmune disease, where degeneration of the components of the postsynaptic membrane in the neuromuscular junction results in fluctuating strength and distinct fatigue in skeletal muscles.

This thesis aimed to measure fatigue in patients with MG, based on the five fatigue domains from the Multidimensional Fatigue Inventory (MFI-20); physical-, general- and mental fatigue, reduced activity and reduced motivation, and to identify associations of severe fatigue. Moreover, to measure the level of habitual physical activity, either by self-report or by accelerometry, and to estimate the association of physical activity (PA) and fatigue, taking relevant disease- and patient factors into consideration. Finally, to measure gait parameters in a heterogeneous group of patients with neuromuscular diseases and explore if rhythmic auditory stimulation (RAS) could influence the 6-min walk test (6MWT) in patients with MG.

Patients were either recruited from the Danish National Registry of Patients (DNRP) or the outpatient clinic at Copenhagen Neuromuscular Center from 2014 to 2020.

We found that the level of patient-reported fatigue was high in patients with MG, with the highest scores in physical and general fatigue (MFI-20, median 13). Around half of the included patients had low levels of PA, measured by self-reports and accelerometry. Higher levels of fatigue were strongly associated with lower levels of PA. Factors associated with severe fatigue were severe MG, insomnia, comorbidity, overweight, job status, and living alone. Moreover, severe MG and high age were associated with more time spent on sedentary behavior and less time spent on PA. One-third of the included patients reported dissatisfaction with their current MG symptom state. Factors associated with dissatisfaction were severe MG and fatigue, depression, low MG-related quality of life, and short disease duration. The 6MWT measured walking-related fatiguability in a heterogeneous group of patients with neuromuscular diseases. RAS increased walking distance and gait speed in patients with MG without additional exertion compared to standard 6MWT.

DANSK RESUMÉ

Myasthenia gravis (MG) er en kronisk autoimmun sygdom, hvor degeneration af komponenter i den postsynaptiske membran resulterer i varierende styrke og udtrætning i skeletal muskulatur. (Udtrætning anvendes i dette resumé som det danske ord for fatigue).

Formålet med denne afhandling var at måle udtrætning hos patienter med MG med udgangspunkt i de fem dimensioner i Multidimensional Fatigue Inventory (MFI-20); fysisk-, generel- og mental udtrætning, reduceret aktivitet og reduceret motivation, og at identificere faktorer associeret med svær udtrætning. Derudover at måle niveauet af selv-rapporteret og målt habituel fysisk aktivitet, og vurdere sammenhængen mellem fysisk aktivitet og udtrætning med inddragelse af relevante sygdoms- og patientfaktorer. Endeligt at undersøge gangparametre i en heterogen gruppe af patienter med neuromuskulære sygdomme, og at undersøge om musik påvirker disse gangparametre i en 6-minuters gangtest hos patienter med MG.

Patienterne blev rekrutteret enten fra Landspatientregisteret eller fra Klinik for Nerve- og Muskelsygdomme, hvor de gennemførte forskellige tests og spørgeskemaer. Patienterne blev rekrutteret i perioden 2014 til ultimo 2020.

Vi fandt, at niveauet af patient-rapporteret udtrætning var højt hos patienter med MG med de højeste niveauer målt for fysisk- og generel udtrætning (MFI-20, median 13). Omkring halvdelen af patienterne havde et lavt fysisk aktivitetsniveau målt ved selv-rapportering og accelerometer. Højere niveauer af udtrætning var stærkt associeret med lavere niveauer af fysisk aktivitet, men også med svær MG, søvnproblemer, ko-morbiditet, overvægt, job-status og at bo alene. Desuden var svær MG og høj alder associeret med mere stillesiddende tid og mindre fysisk aktivitet. En tredjedel af de inkluderede patienter rapporterede utilfredshed med nuværende MG sygdomstilstand. Faktorer associeret med utilfredshed var svær MG, svær udtrætning, depression, lav MG-relateret livskvalitet og kort sygdomslængde. Seks-minutters gang test var brugbar til at måle gang-relateret udtrætning i en heterogen gruppe af patienter med neuromuskulære sygdomme, og musik øgede gangdistance og ganghastighed uden yderligere anstrengelse hos patienter med MG.

INTRODUCTION

MYASTHENIA GRAVIS

Myasthenia gravis (MG) is a rare, chronic, autoimmune disease caused by dysfunction of the neuromuscular junction. The patient's immune system produces pathogenic antibodies directed against the postsynaptic muscle endplate, primarily against the acetylcholine receptors (AChR) or muscle-specific kinase (MuSK). Blocking signal transduction and destruction of the synaptic components result in fluctuating strength in ocular, bulbar, limb, and axial muscles, and distinct muscular fatigue occurs with repeated muscle contractions. The disease is restricted to ocular symptoms in around 10-15% of the patients, whereas approximately 90% experience a more generalized disease. Patients with MG are classified into different sub-groups depending on serum antibody status (such as AChR, MuSK, seronegative), phenotype (ocular, generalized), and disease debut (such as early-onset <50 years, late-onset ≥50 years)[1]. Also, patients are classified according to the Myasthenia Gravis Foundation of America (MGFA) clinical classification (I-V)[2]. The MG diagnosis is verified by a typical clinical history, symptom improvement with acetylcholinesterase inhibitor, a blood test confirming antibodies, or significant decrement/increased jitter on electromyography.

The Danish prevalence of MG is estimated to be 180 per million people[3], with an annual incidence of 9.2 per million[4]. This prevalence corresponds to estimates of around 1000 patients registered with an MG diagnosis in Denmark who are followed at a neurological department[5]. The worldwide prevalence of MG is estimated to be 40-180 per million with an annual incidence of 4-12 per million[1], indicating a considerable variation in regional occurrence, which is explained by genetic and environmental factors and differences in study designs[6]. Recent estimates tend to be higher than older ones due to more widespread autoantibody testing and improved registration of cases. MG has a bimodal age pattern of incidence, with a peak around 30 years (with a high frequency in women) and a steady increase in incidence above the age of 50 (with a slightly higher frequency in men)[1].

Thymic hyperplasia is present in most patients with early-onset MG, and thymoma in 10-15% of patients, often in antibody positive, generalized MG[1]. The frequency of other autoimmune

diseases, e.g., thyroiditis, is increased, especially in ocular and early-onset subgroups[1,7]. Besides thymoma and non-melanoma skin cancer, MG patients do not have an increased risk of cancer compared to non-MG individuals[8]. A Danish population-based register study found an increased mortality rate ratio in AChR-positive patients compared to non-MG individuals[5], explained by severe, non-compensated myasthenic conditions with respiratory problems at the time for diagnosis. Also, treatment-induced conditions, such as sepsis, or other associated autoimmune disorders, have increased MG mortality[5].

The first-line drug for MG is pyridostigmine, which is a symptomatic treatment. Patients not responding sufficiently to pyridostigmine will start supplemental immunosuppressive therapy. Corticosteroid treatment is given in shorter periods, but this treatment is typically replaced early on with steroid-sparing agents due to significant adverse effects. For refractory patients, several novel therapies are currently in clinical development[9]. MG is nearly always lifelong, but most patients with mild to moderate symptoms will obtain complete remission or substantial improvement with medical treatment.

Recently, disease satisfaction has been explored in patients with MG. Two studies have used a simple yes/no question to determine the “Patient Acceptable Symptom State” (PASS) in a group of patients with MG. These studies found that around 30% of the included patients reported dissatisfaction with their current symptom state[10,11]. PASS is used in study III to identify factors associated with the dissatisfaction of the current symptom state in a group of patients from Copenhagen Neuromuscular Center.

FATIGUE

Muscular fatigue is a core symptom of MG and is evidenced by clinical muscle testing and neurophysiological examination. However, many patients with MG also report a different, more constant, and overwhelming fatigue that is perceived differently from the well-described muscular fatigue and is separate from sleepiness. This fatigue is common in many chronic diseases[12–14], but the underlying etiology remains unknown.

The prevalence of patient-reported fatigue in MG varies between 42 and 82%[15] and is associated with MG severity, depressive symptoms, and female sex[15]. Fatigue has been challenging to treat, but promising results are reported for novel drug therapy[16].

To date, no standard definition of fatigue exists, even though many authors have made suggestions. In one attempt, Chaudhuri et al.[12] categorizes fatigue in neurological diseases into "peripheral" and "central" fatigue. "Peripheral fatigue" is a term for muscular fatiguability, as seen in MG. "Central fatigue" is a subjective sense of fatigue perceived at the central nerve system (CNS) level, including cognitive and mental fatigue.

Kluger et al.[17] differentiates fatigue in neurological diseases into "performance fatiguability" and "perceived fatigue." "Performance fatiguability" is defined as a decline in performance in either the motor domain (e.g., in muscular peak torque) or the cognitive domain (e.g., reaction time). "Perceived fatigue" is the subjective sensation of fatigue and is mainly measured by patient-reported outcome scales[17].

Research on patient-reported fatigue in MG has been published since 1998[18], and the multifactorial nature of fatigue in MG is evidenced[15]. Patient-reported fatigue is throughout the years measured by a variety of questionnaires, both generic questionnaires, questionnaires developed for neurological diseases, and MG-specific questionnaires[15]. This variation in instruments hamper a valid comparison of findings across studies[15], and the previously used questionnaires have several disadvantages. These questionnaires; 1) only measure few fatigue domains, e.g., cognitive and/or physical domains, 2) focus on mental/cognitive fatigue instead of the more overall general fatigue, 3) do not have separate scores for each fatigue domain, 4) include generic items not relevant for MG, or 5) are too lengthy[15].

It is not clear if disease-specific questionnaires offer advantages over generic scales[17] if the generic instrument includes relevant items for the specific disease and the purpose of the study. To overcome some of the disadvantages of previously used questionnaires, the chosen tool for measuring fatigue in studies I and III was the Multidimensional Fatigue Inventory (MFI-20)[19]. This instrument includes five fatigue domains: physical-, general-, and mental fatigue, reduced activity, and reduced motivation. As compared to the definitions of Chaudhuri[12] and Kluger[17], the MFI-20 term "physical fatigue" corresponds to the terms "peripheral fatigue" and "performance fatiguability." The remaining MFI-20 domains correspond to different aspects of the terms "central

fatigue" and "perceived fatigue." In this thesis, the definition of fatigue is multidimensional, including the five fatigue domains from the MFI-20.

PHYSICAL ACTIVITY

The health benefits of an active lifestyle have been extensively documented since the early nineties, including exercise as prevention and treatment for chronic diseases[20]. Following the international recommendations, physical activity (PA) reduces mortality and chronic conditions by at least 20-30% compared to physical inactivity[21].

The World Health Organization (WHO) recommends adults ≥18 years to undertake regular PA at least 150 minutes per week at moderate intensity or at least 75 minutes per week at a vigorous intensity or an equivalent combination of the two[22]. Also, research-directed guidelines recommend a minimum of 10,000 steps per day[23,24].

No official recommendations of PA for MG exist, and an early study indicates a previous caution and reluctance towards recommending PA[18]. To date, this reluctance, or lack of knowledge, still exists among patients (and clinicians). This might be based on fear of overuse muscle damage, even though this is not documented in these patients.

The first articles of exercise and MG are from 2000, 2007, and 2012[25–28]. These articles are all case stories of patients performing demanding aerobic- or resistance exercise despite an MG diagnosis without any adverse effects. Apart from a study from 1993[29], which found improvements in muscle strength in 11 patients with MG during a supervised 10-week resistance training period, the first exercise intervention studies were published from 2017[30–33]. These studies of mostly supervised aerobic and/or resistance training for 8-12 weeks found that exercise is safe and well-tolerated and positively influences muscle strength and performance-based outcome measures. Also, interventional exercise studies have been published, focusing on, e.g., balance training[34], postoperative rehabilitation[35], and respiratory training[36,37].

Habitual physical activity means PA integrated throughout the patient's everyday life, including supervised exercise sessions. Habitual physical activity is a relevant outcome measure, as lifelong daily activity is essential to obtain the health benefits of being active. However, only a few studies have examined habitual physical activity in patients with MG either by accelerometry[38,39] or

with a combination of accelerometry and questionnaires[31,32]. These studies included 33, 27, 10, and 11 patients with ocular or generalized MG with disease severity of class I-III on the MGFA classification scale. The studies included patients with juvenile (<18 years), early- and late-onset MG, and patients with the antibody-status, AChR, MuSK, or seronegative. These studies found that patients with MG were less active than controls. The control data was obtained from an accelerometer manufacturer database[39] or a WHO database[38]. The minor studies[31,32] examined baseline PA before a 12-week aerobic- and resistance training intervention without comparing it with any controls. The authors only briefly discussed the baseline PA in these studies, but a large proportion of the patients reported primarily sedentary activities.

Research on the relationship between PA levels and fatigue is sparse. Ruiter et al. assessed fatigue in 420 Dutch patients and found significantly lower fatigue scores in physically active patients[40]. Farrugia et al. found minor but un-sustained improvements in fatigue after a 10-week program with physical activity, relaxation, patient education, and psychological interventions[41]. Rahbek et al. found no improvements in fatigue after eight weeks of aerobic and resistance training[30].

The definitions of physical activity, exercise, and inactivity differ. In this thesis, we used the following definitions:

Physical activity: "Any bodily movement produced by skeletal muscles that require energy expenditure"[42].

Exercise: "A subcategory of physical activity that is planned, structured, repetitive, and purposeful in the sense that the improvement or maintenance of one or more components of physical fitness is an objective"[42].

Physical inactivity: "An insufficient physical activity level to meet present physical activity recommendations"[43].

These definitions are consistent with the WHO guidelines on physical activity and sedentary behavior from 2020[22].

WALKING-RELATED FATIGUABILITY

Walk tests are widely used in the clinic to measure disease progression and treatment efficacy, e.g., the 6-min walk test (6MWT). However, the 6MWT is time-consuming and exhausting for patients with severe weakness in the lower limbs. A shorter and less demanding alternative might be the 2-min walk test (2MWT). Previous studies have found that the 2MWT is a valid alternative to the 6MWT in selected neurological diseases[42–44]. Still, to our knowledge, no previous studies have examined this in a heterogenous group of adult patients with neuromuscular diseases. In patients with MG, the 2MWT and the 6MWT are found reliable and valid[44].

In MG, muscle strength and -fatiguability are measured by dynamometry[45–47]. However, it is preferable to assess fatiguability in functional tests reflecting the patient's daily life for some purposes. A walk test is a method for measuring daily life mobility. Fatigue during walking is named walking-related fatiguability (WT). Timed walk tests have previously been used to measure WT in healthy and clinical populations[48]. In MG, WT has been measured by the 2MWT and the 6MWT[44,49], finding higher levels of WT in patients compared to healthy controls[49].

Rhythmic auditory stimulation (RAS) is the concept of synchronizing auditory stimuli and movement, e.g., music rhythm and steps. It relies on connections between the auditory and motor systems in the brain. It is based on entrainment, which means a locking process where signal frequency in one system affects the frequency of another system[50]. RAS has improved gait parameters (such as walking distance and gait speed) in patients with neurological diseases in repeated walking interventions[51–53] and one-time walking tests[54,55]. RAS has never been used in patients with MG, and the influence of RAS on gait parameters and WT is unknown.

STUDY OBJECTIVES

Based on the existing knowledge described in the introduction section, we conducted five studies whose purposes I have stated below:

STUDY 1 To describe the level of patient-reported fatigue and physical activity in a national cohort of patients with myasthenia gravis and examine the association between fatigue and physical activity, including relevant disease - and patient-related factors.

STUDY II To describe the habitual physical activity level in a sample of patients with myasthenia gravis, measured by accelerometry (ActiGraph) and questionnaire (the International Physical Activity Questionnaire), and identify factors associated with physical activity and inactivity.

STUDY III To determine MG-specific and generic factors related to PASS status, including fatigue, depression, and comorbidity.

STUDY IV To examine if the 2- and 6-min walk test assesses walking capability equally well in different neuromuscular diseases with a wide range of disease severity.

STUDY V To compare gait speed and distance walked in patients with generalized myasthenia gravis when performing a 6-min walk test with rhythmic auditory stimulation versus a standard 6-min walk test.

METHODS

PATIENTS AND PROCEDURES

We recruited patients through the Danish National Registry of Patients (DNRP) (studies I+II) and from the outpatient clinic at the Copenhagen Neuromuscular Center (studies II, III, IV, V). All patients were residents of Denmark and a minimum of 18 years old. For studies III, IV, and V, we did not invite patients >80 years.

Patients from the DNPR are coded with an MG diagnosis according to the International Classification of Diseases eighth edition (ICD-8: 733.09, 1971- 1993) or tenth edition (ICD-10; G.70.0, from 1994) from 1977 to the end of 2018.

For patients with MG, recruited from the outpatient clinic (studies II, III, V), the MG diagnosis was verified by at least 2 out of 3 criteria: symptom improvement with acetylcholinesterase inhibitors, positive acetylcholine receptor antibodies, or significant decrement/increased jitter on electromyography. Exclusion criteria were 1) comorbidity that would interfere with the completion of tests, 2) current participation in a clinical trial, 3) pregnancy, 4) communication issues. For patients on pyridostigmine treatment, the tests were, if possible scheduled 1½-2 hours after the drug intake.

Study I

In this cross-sectional study, we measured the levels of fatigue and PA in a national web-based survey. The patients were identified from the DNRP and received an invitation to the survey in their e-Boks (a personal, digital and secure mailbox). With a link for the survey, we send the invitation using the patient's unique civil registration number, which all Danish citizens are assigned at birth or upon immigration. The software REDCap administered the survey and stored all responses in a secure database. We sent the invitation in June 2019 and to non-responders in July and November 2019. Individuals not subscribing to e-Boks did not receive an invitation. Aside from demographics and disease-related background questions, the survey included six standardized and validated questionnaires of fatigue (Multidimensional Fatigue Inventory, MFI-20), PA (Saltin-Grimby Physical Activity Level Scale, SGPALS and the International Physical Activity Questionnaire, IPAQ short form), MG-severity (Myasthenia Gravis Activities of Daily Living profile, MG-ADL), MG-related quality of life (MG-specific quality-of-life instrument, MG-QoL15) and insomnia

(Insomnia Severity Index, ISI). We used the data from the SGPALS for study I and the data from the IPAQ for study II.

Study II

This cross-sectional study measured PA levels in patients with gMG in two ways; 1) by the IPAQ from the national web-based survey (described in study I) and 2) by accelerometry in patients recruited from the outpatient clinic.

We consecutively recruited patients from the clinic from November 2018 - February 2020. The patients were informed about the study when they arrived for their regular follow-up in the outpatient clinic. We instructed patients who met the inclusion criteria and accepted participating to wear an accelerometer (ActiGraph) at home, 24 hours a day for seven days unless showering and swimming. The patients wore the accelerometer in an elastic belt on the right side of the waist, and the measuring started when the patient left the clinic. The patients completed the MG-ADL while still in the clinic, and background information (age, sex, height, weight, MG treatment, and duration) was registered.

Study III

In this cross-sectional study, patients with gMG followed at the outpatient clinic were recruited from October 2019-June 2020. Patients, meeting the inclusion criteria and accepting to participate, completed (semi) clinician-derived tests of MG-severity (Quantitative Myasthenia Gravis score**,** QMG, and the Myasthenia Gravis Composite Scale, MGC), and several patient-reported questionnaires regarding MG (MG-ADL, MG-QoL15), fatigue (MFI-20), depression (Major Depression Inventory, MDI), overall health state (EQ-5D-3L), comorbidity (Charlson Comorbidity Index, CCI) and Patient Acceptable Symptom State (PASS). The tests were completed during one test-meeting of 1½-2 hours by three experienced investigators, who had trained the tests together beforehand. As this study also worked as a quality assessment study of our outpatient clinic, we decided a priori to include 100 patients. This number corresponds to more than a fourth of the patients with gMG followed in our clinic.

Study IV

This cross-sectional, randomized, and controlled study assessed walking capability by the 2- and 6-min walk test in a heterogeneous group of patients with neuromuscular diseases (no patients with MG). The patients participated in two test days, each consisting of one 2MWT and one 6MWT. The patients were recruited from the outpatient clinic from March 2014 to January 2015. Inclusion criteria were a genetic- or biopsy-verified neuromuscular diagnosis and the ability to walk ≥60 meters in 6 minutes. The exclusion criteria were any comorbidity that could interfere with walking capability. The order of the tests was randomly assigned via sealed envelopes and separated by a minimum of 30 minutes of rest. We measured muscle weakness by manual muscle testing (Medical Research Council scale, MRC). We recruited age- and sex-matched controls without conditions interfering with walking among colleagues. Controls only participated in one test day. Part of the data from this study has been published in two other studies[56,57].

Study V

In this cross-sectional, randomized study, we recruited patients with gMG from the outpatient clinic from May 2018 to December 2019. Patients that met the inclusion criteria and accepted to participate completed two 6MWTs in one test meeting. One standard 6MWT and one 6MWT added RAS with a frequency of 100% or 110% of the patient's fastest gait speed. The fastest gait speed was determined (before any of the 6MWTs) by one short 60-meter walk test. In this test, we recorded average steps per minute by an app (BPM, version 3.04, developed by CHEEBOW, iPhone App Store). The average steps per minute corresponded to the beats per minute in RAS. We randomly assigned the 6MWTs via sealed envelopes and separated the tests by 30 minutes of rest. The auditory stimuli were vocal pop or rock with a distinct rhythm. We instructed the patients to follow the rhythm of the music. The rhythm was indicated to the patients before the test by clapping or stepping.

A pulse watch (Suunto Quest, model SS018153000) measured the patient's heart rate before, during, and after the 6MWT. We measured exertion right after each of the 6MWTs by the Borg Scale of Perceived Exertion, where the patients rated their exhaustion from 6-20 (20=very, very exhausting)[53]. MG-severity was measured before the 6MWTs by the MGC.

Table 1 provides an overview of the methods used in studies I-V.

Table 1. Methods used in studies I-V

	Studies				
	I	II	III	IV	V
Questionnaires					
Multidimensional Fatigue Inventory	X		X		
Saltin-Grimby Physical Activity Level Scale	X				
International Physical Activity Questionnaire		X			
MG Activities of Daily Living profile	X	X	X		
MG-specific QoL instrument	X		X		
Insomnia Severity Index	X				
Major Depression Inventory			X		
EQ-5D-3L			X		
Charlson Comorbidity Index			X		
Patient Acceptable Symptom State			X		
Functional test					
2-min walk test				X	
6-min walk test				X	X
MG Composite Scale			X		X
Quantitative MG score			X		
Manual muscle testing				X	
Accelerometer		X			

QUESTIONNAIRES

We measured fatigue by the **Multidimensional Fatigue Inventory (MFI-20)**[19]. The MFI-20 is a generic, patient-reported questionnaire that measures fatigue severity. It was developed in 1995[58] with a Cronbach's alpha of 0.84[19]. The MFI-20 consist of five fatigue domains: physical-, general-, mental fatigue, reduced activity, and reduced motivation. Each domain has a score of 4 to 20, where 20 indicates severe fatigue. The MFI-20 has been validated and/or used in several patients- and healthy populations since 1995, e.g., multiple sclerosis, stroke, polio[59–64], and spinal muscular atrophy[65]. The MFI-20 was validated and translated into Danish in 2000[66,67].

We measured patient-reported PA levels by the **Saltin-Grimby Physical Activity Level Scale (SGPALS)**[68] and by the **International Physical Activity Questionnaire (IPAQ)** short form[69].

The SGPALS was developed in 1968 and has been slightly modified over the years[70,71]. It is an overall score of PA and is used in several Nordic population studies[72–76]. SGPALS has previously been used in clinical populations, e.g., patients with stroke[77–80]. The original questionnaire is used in study I, but with a slightly modified wording: "During the last year, how physically active have you been, including leisure-time physical activities and transportation? Please categorize yourself into one of four levels of physical activity":

I. Mainly sedentary or engaged in light physical activity less than 2 hours per week (e.g., reading books, watching television, or going to the cinema).
II. Light-to-moderate physical activity 2-4 hours per week (e.g., walking, cycling for pleasure, gardening, housework, light exercise).
III. Moderate physical activity more than 4 hours per week, or more strenuous activities 2-4 hours per week (e.g., brisk walking, fast bicycling, heavy gardening, or exercises that makes you short of breath).
IV. More strenuous physical activities more than 4 hours per week, or regular vigorous exercise (e.g., competitive sport) several times a week.

IPAQ short form measures patient-reported time spent on PA across a comprehensive set of domains; 1) activity in leisure time, 2) domestic and gardening activities, 3) activity related to work, and 4) transportation, in four specific types of activity; sitting, walking, moderate- and vigorous-intensity activities. Patients report the number of days, hours, and minutes (of a minimum of 10

minutes) spent on these activities during the last seven days. The IPAQ short form is previously used in patients with disorders in the neuromuscular junction[81].

MG severity and MG-related quality of life were measured by the **Myasthenia Gravis Activities of Daily Living profile (MG-ADL)**[82] and the **MG-specific quality-of-life instrument (MG-QoL15)**[83]. The MG-ADL is a patient-reported questionnaire with scores from 0-24, where 24 indicates severe MG. The scale consists of 8 items, including common MG symptoms. The MG-QoL15 is a patient-reported questionnaire assessing MG-related quality of life. It consists of 15 items with a total score of 0-60 points (60=low quality of life). A revised version of the MG-QoL15 exists (MG-QoL15r)[84], but as the original version is still widely used, we used the original version in the thesis.

The **Insomnia Severity Index** (ISI)[85] is a patient-reported questionnaire assessing insomnia, sleep quality, and sleep disturbances during the previous two weeks. ISI consists of seven items, each rated on a five-point scale with a total score of 0-28 (most severe insomnia=28). A total score of ≥8 indicates clinical insomnia. One previous MG study[86] has used the ISI.

The **EQ-5D-3L**[87] provides a generic classification system for measuring overall health status. It compromises five dimensions; mobility, self-care, usual activities, pain/discomfort, and depression/anxiety. The index score is derived from three different questions in each dimension, giving an index ranging from 0 to 1; 0 meaning death, 1 meaning complete health[88]. We also included the **EQ-VAS** scale ranging from 0-100 (100= the best health you can imagine), indicating the patient's self-rated overall health status[87]. EQ-5D has previously been used in patients with MG[11].

The **Major Depression Inventory (MDI)**[89] is a patient-reported rating scale for depression (range 0-50, 50=severe clinical depression), used to either rate depression severity or to diagnose existing depression. It is compatible with both the ICD-10 and DSM-IV diagnostic criteria for clinical depression[90]. MDI has previously been used in patients with MG[30,91].

The **Charlson Comorbidity Index (CCI)**[92] is a tool for measuring the long-term prognosis of lethality in comorbid patients. The point-score ranges from 0-37 and is accumulated according to associated diseases and age ranges: the higher score, the less predicted 10-year survival. One previous study of MG[93] has used the CCI.

The **Patient Acceptable Symptom State (PASS)**[94] measures patient satisfaction with the current symptom state. In one dichotomous question, patients report satisfaction (yes/no). In our thesis, we used a PASS question inspired by a previous study[11] "*Consider all the ways you are affected by myasthenia gravis. If you had to stay in your current symptom state for the next months, would you say that your current disease state is satisfactory?"* PASS has previously been used in clinical studies[95,96], also in MG[10,11].

No easy-to-use instrument exists for measuring satisfaction with **adverse effects** of current MG medical treatment. Therefore, we asked the patients one question: "*How satisfied are you with the adverse effects of current MG medical treatment?"* with a score ranging from 1-10 (1=not at all satisfied to 10=very satisfied).

FUNCTIONAL TESTS

We measured MG severity by the clinician-derived **Quantitative Myasthenia Gravis score (QMG**)[97] and the clinician-derived/patient-reported **Myasthenia Gravis Composite Scale (MGC)**[98]. The QMG consists of 13 items and examines the patient's muscle strength and endurance with a summary score from 0-39 (most severe MG=39). The MGC consists of 10 items with a summary score from 0-50 (most severe=50). Both instruments assess common MG symptoms.

We used the **2-minute walk test (2MWT)** and the **6-minute walk test (6MWT**) to measure walking-related fatiguability in studies IV and V. The tests were administered according to the American Thoracic Society Guidelines[99].

In Study IV, bilateral muscle strength was assessed in the lower limb by the manual muscle testing **Medical Research Council scale (MRC)**[100], ranging muscle strength from 0-5 (normal strength=5).

ACCELEROMETRY

We used the **ActiGraph** wGT3X-BT accelerometer (ActiGraph, LLC, Pensacola, FL) to measure habitual physical activity levels. The dimensions of the accelerometer are 4.6 x 3.3 x 1.5 cm, weight 19 g. Accelerometry is previously used in studies of MG patients [31,32,38,39]. The ActiGraph monitors were set to activity counts in triaxial mode, using a 10-second epoch. The Actigraph monitors acceleration intensity and duration of hip motion in three axes. The number of counts increased with the frequency and intensity of movement.

ETHICS

The studies were approved by the ethics committee of the Capital Region of Denmark (H-18031231) (study IV: H-4-2014-FSP) and registered at the Danish Data Protection Agency (VD-2018-440, I-Suite nr.6694). The Danish Health Data Authority approved the extract from the DNRP. Informed consent was obtained from all participants, even though this was not required for respondents to the survey.

ANALYSES

We presented continuous variables by means and standard deviation (SD), non-parametric continuous variables by medians and inter-quartile ranges (IQR), and categoric variables by numbers and percentages. We assessed normality visually by histograms and boxplots. We examined differences between groups by unpaired *t*-test for continuous data, Mann-Whitney test for non-normal continuous data, and Fisher's exact test for categorical data.

We estimated associations by general linear regression analyses (studies I+II) or logistic regression analyses (study III). We performed the regression analyses as complete-case analyses and checked for the following model assumptions: independence of observations, linearity of covariates, homogeneous and normally distributed residuals. In case of violation, we conducted the analyses on log-10 transformed data. The convergence criterion was satisfied in all studies.

A linear mixed model was used to estimate the effect of RAS (study V). We used an unstructured covariance pattern to account for the correlation in the repeated measurements and possible changes in variance.

Covariates were selected a priori in all studies and determined from known evidence or experiences from the clinic. We included covariates in the statistical analyses as either continuous or categorical variables.

For analyses, a *p*-value of ≤ 0.05 (2-tailed testing) was considered significant. We used SAS enterprise guide 7.1 in all statistical analyses.

Study I

We grouped the SGPALS classifications into low PA levels (SGPALS levels I and II) and regular PA levels (SGPALS levels III and IV). We fitted the regression models with each of the five MFI-20 domains as continuous outcomes. As some respondents in the survey confirmed their MG status twice but were not on medical treatment for MG and/or regularly seen by a neurologist, all analyses were executed on a sup-sample of patients on MG medical treatment AND regularly followed by a neurologist.

Study II

Wear time was defined as 24 hours subtracted non-wear time and sleeping time. We defined intervals of a minimum of 60 consecutive minutes of zero counts as non-wear time[101] and sleeping time between 12:00 a.m. and 6:00 a.m.[102,103]. We included patients with seven days of at least 10 hours of recording during the daytime in the analyses.

We defined PA intensities as; light= 100–1951 counts per minute (CPM); moderate= 1952–5724 CPM; vigorous≥ 5725 CPM in bouts of a minimum of 10 minutes[104]. CPM<100 was defined as sedentary time. We converted CPMs to minutes per week (m/w) for each PA intensity and sedentary time to minutes per day (m/d).

We fitted linear regressions models to investigate associations of inactivity and PA intensities. Minutes spent on moderate and vigorous activities were merged into one variable; moderate-vigorous physical activity (MVPA), as many patients reported zero minutes on vigorous activities. The outcomes of the regression analyses were time spent in sedentary- and light activities, MVPA, and steps per day measured by accelerometer. For the IPAQ, the outcomes were time spent on sitting, walking, and MVPA. For analyses of raw accelerometer data, we used the commercial software ActiLife v6.11.6.

Study III

We used PASS-status as an anchor for the descriptive analyses. Factors associated with PASS-negative status (patients not satisfied with current MG symptom state) were identified by logistic regression analysis, using the PASS answer as a dichotomous (yes/no) outcome. We included the following co-variates; MG-severity (MG-ADL, QMG), MG-related quality of life (MG-QoL15), depression (MDI), physical- and general fatigue (MFI-20), MG disease duration, BMI, Comorbidity (CCI), MG medical treatment, sex, and age.

Studies IV+V

Results were expressed as mean and ranges. We used the Pearson correlation coefficient to examine the relationship between the 2MWT and the 6MWT. In study IV, we used data from the first test day, but we used the data from the second test day to assess the influence of a learning effect with repeated tests. The continuous outcomes of study V were walking distance and gait speed.

Table 2 provides an overview of outcomes, covariates, and analyses in the studies I-V.

Table 2. Outcomes, covariates, and analyses in studies I-V

Studies	Outcomes	Covariates		Statistical analyses
		Continuous	Categorical	
I	MFI Physical fatigue General fatigue Reduced activity Reduced motivation Mental fatigue	SGPALS Age BMI MG-duration MG-ADL ISI	Sex Job-status Cohabitation Comorbidity Depression Medication, other	General linear regression
II	IPAQ Sitting Walking MVPA Accelerometer Sedentary Light MVPA Steps	Age BMI MG-duration MG-ADL	Sex	General linear regression
III	PASS	Age MG-duration QMG MDI	Sex	Logistic regression
IV	2MWT 6MWT			Descriptive
V	6MWT 6MWT + RAS	Age BMI MGC	Sex Order of tests	Linear mixed model

RESULTS

The total number of included participants was 1149. Of these, 996 had a diagnosis of MG, 115 had another neuromuscular diagnosis, and 38 were healthy controls. N=779 patients with MG responded to the survey (study 1), and we included a total of 217 patients with MG from the outpatient clinic. Some patients participated in more than one study. Table 3 shows age, sex, BMI, MG disease duration, MG severity, and MG-related quality of life for included patients with MG across studies.

Table 3. Characteristics of included patients with MG across studies I, II, III, V

	Study I (n=779)	Study II (n=69)	Study III (n=100)	Study V (n=48)
Age, years	60.8 (15.5)	58.9 (17.3)	60.2 (15.4)	55.0 (16.8)
Sex, female	413 (53%)	44 (64%)	57 (57%)	34 (71%)
BMI, kg/m^2	27.2 (5.7)	27.5 (6.5)	28.1 (6.1)	27.0 (6.6)
MG duration, years	13.6 (12.4)	8.0 (8.9)	6.0 (3-13)	9.4 (10.1)
MG-ADL, total score	3.0 (1.0-5.0)	2.0 (0-6.0)	3.0 (0-5.0)	N/A
MG-QoL15, total score	9.0 (2.0-19.0)	N/A	8.0 (3.0-21.0)	N/A
MGC, total score	N/A	6.0 (3.0-13.0) [#]	5.0 (3.0-10.0)	7.5 (3.5-13.5)
QMG, total score	N/A	8.0 (5.0-11.0) [#]	9.0 (5.0-11.0)	N/A

Table 3. We present age, BMI, and MG disease duration as means and standard deviations (SD) and sex as numbers and percentages. We present MG-ADL, MG-QoL15, MGC, and QMG total scores as medians and inter-quartile ranges (IQR). Abbreviations: MG-ADL=MG Activities of Daily Living profile, MG-QoL15= MG-specific QoL instrument, MGC= MG Composite scale, QMG= Quantitative MG score. Missing data: study I (age, sex=1, BMI=6, disease duration=7, MG-ADL=72, MG-QoL15=73), study II (MG-ADL=5). N/A=is not available. [#] These measures are not included in the published paper of study II.

Study I

A total of 1745 persons were registered in the DNPR with an MG diagnosis. N= 282 persons (164 women [58.2%]; mean [SD] age, 78.0 [12.6] years) did not subscribe to e-Boks and, therefore, did not receive an invitation for the survey. The remaining 1463 persons received an information letter in their e-Boks, including a link for the survey. We excluded 294 persons (173 women [58.8%]; mean [SD] age, 62.2 [15.1] years) as they were uncertain about the MG diagnosis, e.g., due to absence of symptoms for years despite no MG medical treatment. N=390 persons (226 women [57.9%]; mean [SD] age, 55.0 [16.5] years) never responded to the survey. The remaining

779 patients completed the survey either completely (n=662) or partially (n=117). In- and exclusions of the survey (studies I and II) are illustrated in Figure 1 and by age in Figure 2.

Figure 1. Flowchart of in- and exclusions of the survey

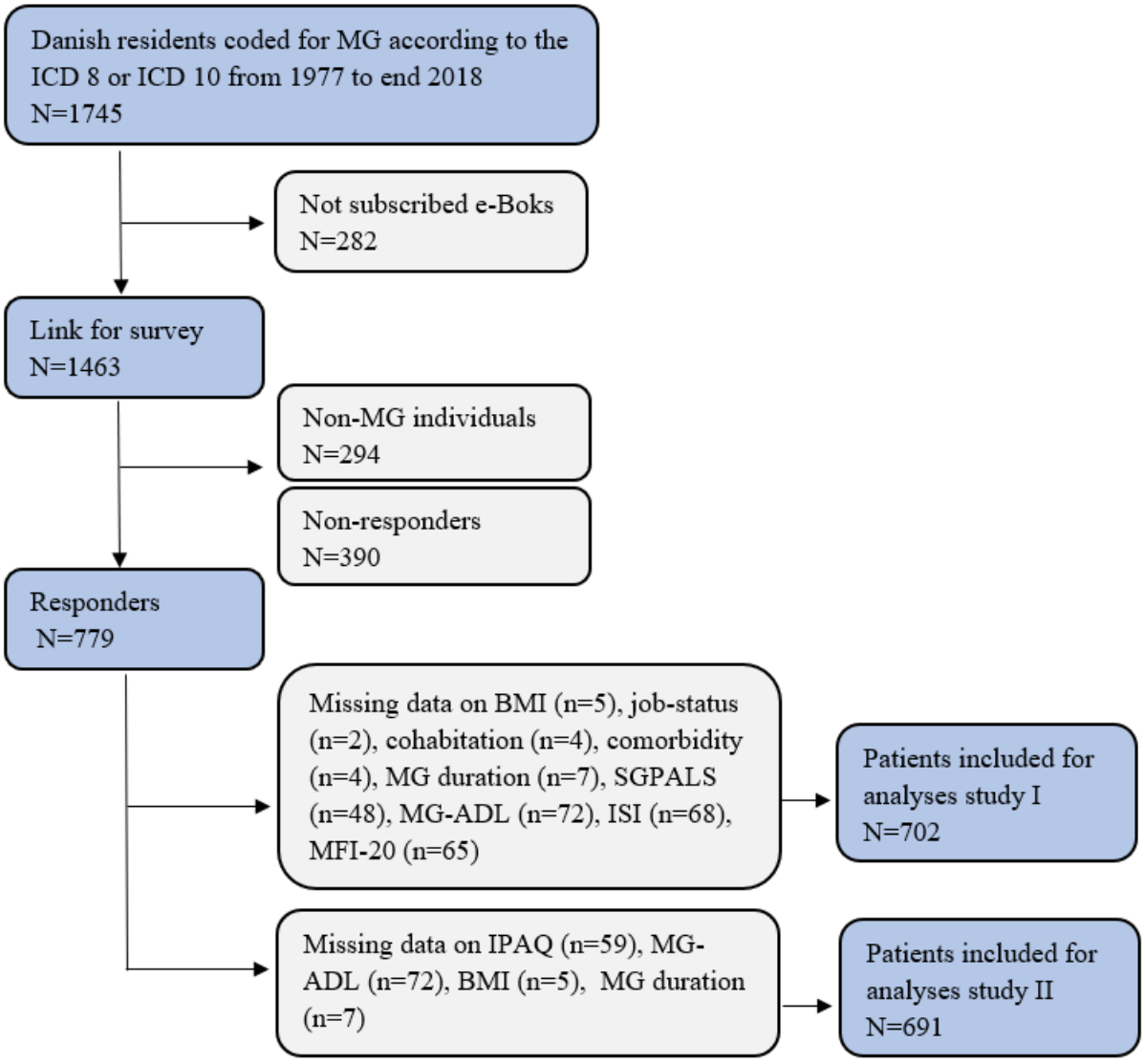


Figure 2. Age of responders, non-responders, and persons not subscribing e-Boks

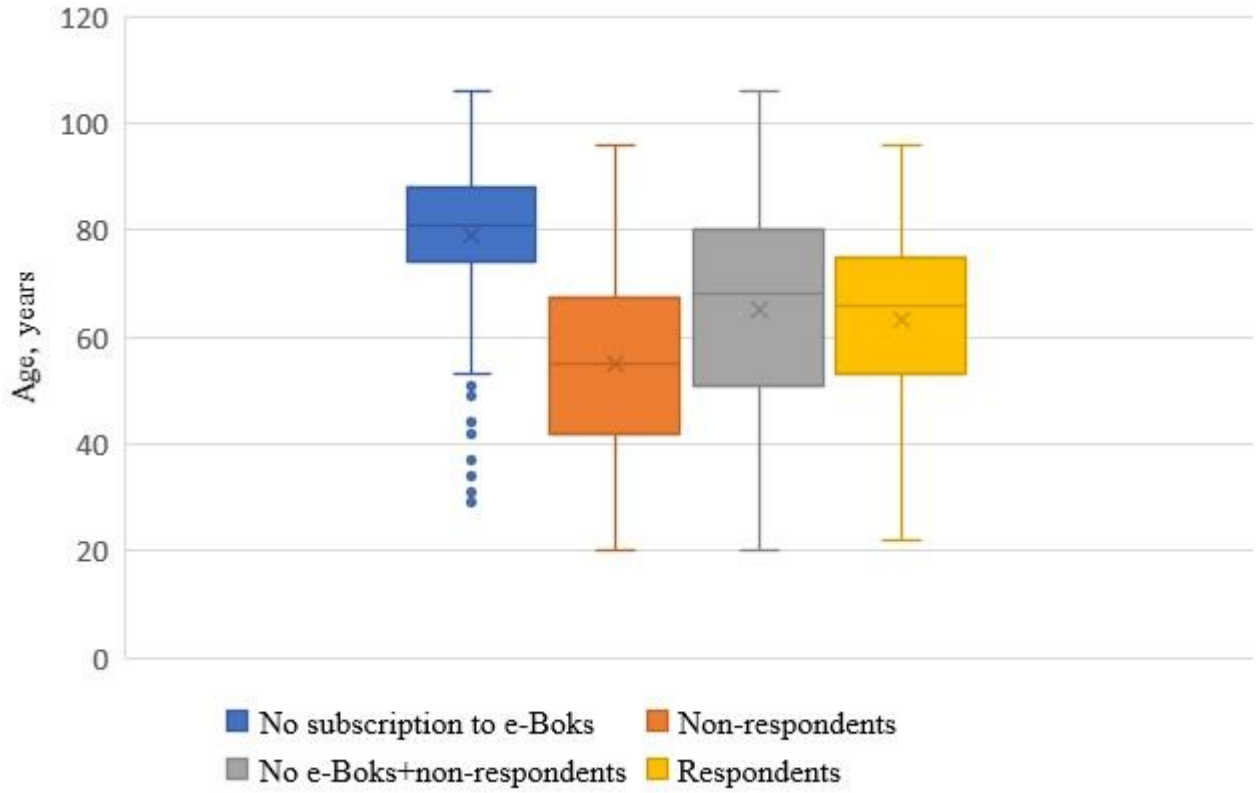


Figure 2. In- and exclusions of the survey illustrated by age. The box illustrates the interquartile range (between the 25- and 75 quartiles), and the line that divides the box illustrates the median. The cross inside the box illustrates the mean, and the whiskers illustrate ages outside the interquartile range. Dots illustrate outliers. No subscription to e-Boks (n=282), non-respondents (n=390), respondents (n=779).

The sub-sample of patients on active MG treatment AND regularly followed by a neurologist included 486 patients. Table 4 shows the baseline characteristics of the overall- and the sub-sample.

Table 4. Characteristics of patients included in study I

	Overall sample (n=779)	Sub-sample (n=486)
Age, years	60.8 (15.5)	62.5 (14.9)
Sex, female	413 (53%)	245 (50%)
BMI, kg/m^2	27.2 (5.7)	27.6 (6.1)
Disease duration, years	13.6 (12.4)	11.6 (11.0)
Job-status		
Retired due to age	328 (42%)	229 (47%)
Working full- or part-time	300 (39%)	173 (36%)
Retired due to sickness	82 (11%)	46 (9%)
Out of work [#]	50 (6%)	28 (6%)
Student	17 (2%)	9 (2%)
Regularly follow-up by neurologist	528 (68%)	486 (100%)
Thymectomy, yes	235 (30%)	130 (27%)
MG Treatment [¤]		
Pyridostigmine	448 (56%)	359 (74%)
Glucocorticoids	92 (12%)	87 (18%)
Immunosuppressive drugs, other	290 (37%)	266 (55%)
Immunoglobulin	19 (2%)	17 (4%)
Plasmapheresis	8 (1%)	3 (1%)
No treatment	187 (24%)	0
Comorbidity [¶]	494 (64%)	319 (66%)
SGPALS		
I: Sedentary	81 (11%)	52 (11%)
II: Light to moderate	305 (42%)	178 (39%)
III: Regular moderate	290 (40%)	201 (44%)
IV: Regular vigorous	55 (7%)	28 (6%)
MFI-20, total		
General fatigue	13 (10-16)	13 (10-15)
Physical fatigue	13 (9-15)	13 (10-15)
Reduced activity	12 (9-15)	12 (9-15)
Reduced motivation	9 (6-11)	9 (7-12)
Mental fatigue	9 (6-12)	10 (6-12)
MG-ADL, total	3 (1-5)	3 (1-5)
MG-QoL15, total	9 (2-19)	11 (4-21)
ISI, total	8 (4-13)	8 (4-13)

Table 4. We present variables as mean (standard deviation, SD), median (Inter-quartile ranges, IQR), or number (n) and percentages (%). Abbreviations: BMI (Body mass index), MFI-20 (Multidimensional Fatigue Inventory), MG-ADL (MG Activities of Daily Living profile), MG-QoL15 (MG-specific quality-of-life instrument), ISI (Insomnia Severity Index), SGPALS (Saltin-Grimby Physical Activity Level Scale). [#] Out of work= unemployed, sick-leave, non-classified. [¤] As some patients received more than one drug the percentages do not match 100 [¶] Comorbidity: hypertension/high cholesterol, chronic pain, autoimmune disease, diabetes type 2, depression/anxiety, chronic fatigue syndrome, chronic obstructive lung disease, severe cardiovascular disorder. There was missing data in the overall sample: age, sex, follow up at neurologist (n=1), job-status, thymectomy (n=2), BMI (n=6), MG-duration (n=7), SGPALS (n=48), MFI-20 (n=65), MG-ADL (n=72), MG-QoL15 (n=73), ISI (n=68). Missing data in the sub-sample: Job-status (n=1), thymectomy (n=1), BMI (n=4), MG-duration (n=2), SGPALS (n=27), MFI-20 (n=36), MG-ADL (n=39), MG-QoL15 (n=40), ISI (n=37).

The highest levels of fatigue were seen for the MFI-20 domains; general fatigue (median [IQR], 13 [10-16]), physical fatigue (median [IQR], 13 [9-15]), and reduced activity (median [IQR], 12 [9-15]). N=386 (53%) patients reported low levels of PA (SGPALS, levels I+II) and 345 (47%) patients reported regular levels of PA (SGPALS, levels III+IV). Almost similar distribution of fatigue and PA levels was seen for the sub-sample (Table 4). Patients in remission (MG-ADL=0, n=142) still reported fatigue, however, at lower levels: general fatigue (median [IQR], 9 [6-12]), physical fatigue (median [IQR], 8 [6-12]), reduced activity (median [IQR], 9 [6-12]), reduced motivation (median [IQR], 7 [5-9]), and mental fatigue (median [IQR], 7 [4-9]).

For general fatigue, patients with a high level of fatigue (≥13, the sample median, MFI-20, n=358) were often women (55 vs. 45%, $p<.01$), retired due to sickness (70 vs. 30%, $p<.01$), comorbid (55 vs. 45%, $p<.01$), and living alone (62 vs. 39%, $p<.01$). These patients reported a higher level of disease severity (MG-ADL, median 4 vs. 1, $p<.01$), lower quality of life (MG-QoL15, median 17 vs. 3, $p<.01$), more sleeping problems (ISI, median 12 vs. 5, $p<.01$), and medical treatment for non-MG diseases (54% vs. 46%, $p=.01$) compared to patients with low levels of general fatigue (MFI-20 score <13). This distribution pattern was similar for the other MFI-20 domains, also for the sub-sample. Patients with another autoimmune disease besides MG and patients treated with steroids had higher fatigues scores than the remaining MG patients ($p<.01$).

Higher levels of fatigue were strongly associated ($p<.0001$) with lower levels of PA (unless for mental fatigue in the sub-sample, $p>.05$), severe MG symptoms (high score of the MG-ADL), sleeping problems (high score of the ISI), high BMI, and depression (yes/no). Job-status (e.g.,

retired due to sickness) and comorbidity (yes/no) were associated with fatigue in the overall sample. Cohabitation was associated with fatigue in the sub-sample. Figure 3 shows the associations of fatigue in the overall sample.

Figure 3. Factors associated with fatigue in the overall sample, study I

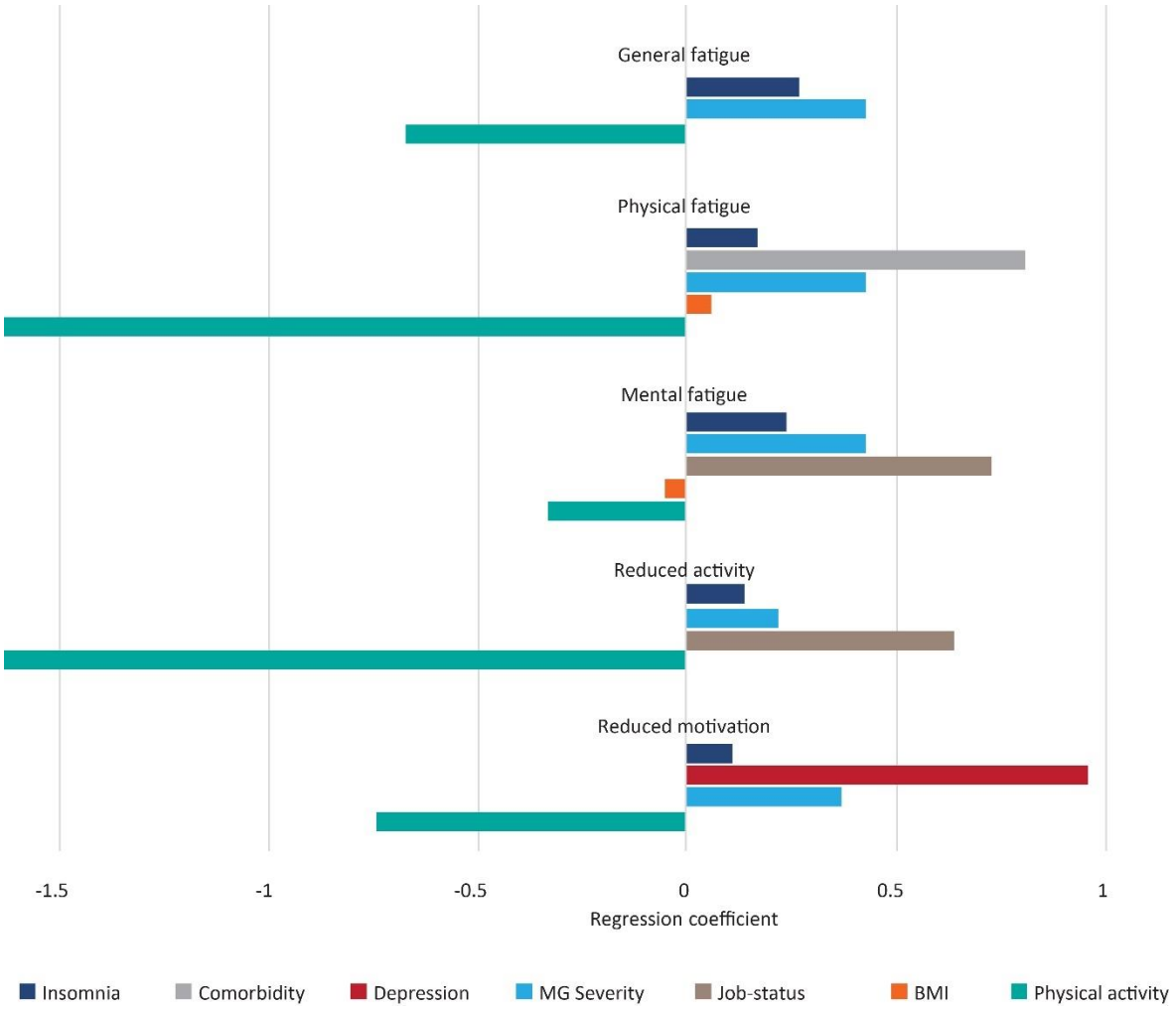


Figure 3. Statistic significant ($p \leq .05$) associations of fatigue illustrated for each of the MFI-20 domains in the overall sample. The x-axis: the regression coefficients. The y-axis: the MFI-20 fatigue domains. The colored bars illustrate the covariates. The regression coefficients must be interpreted in the following way (an example); when the SGPALS (physical activity) increases by one step (higher levels of physical activity), the MFI-20 score of general fatigue decreases by 0.67 steps.

Study II

A total of 69 patients were included from the outpatient clinic to evaluate habitual physical activity by accelerometers. The average (mean± SD) wear time of the accelerometers was 16±1.4 hours/day. A total of 691 patients completed the IPAQ short form as part of the survey (described in study I). Table 5 shows the patient characteristics and PA measurements for study II.

Table 5. Patient characteristics and measurements of PA, study II

Characteristics	Clinic sample (n=69)	Survey sample (n=691)
Age, years	58.9 (17.3)	60.5 (15.5)
Body mass index, BMI	27.5 (6.5)	27.1 (5.9)
Sex, female	44 (64%)	365 (53%)
MG disease duration, years [#]	8.0 (8.9)	13.8 (12.6)
MG-ADL, total score	2.0 (0-6) [¶]	3.0 (1-5)
Pyridostigmine treatment [¤]	50 (73%)	399 (58%)
Immunosuppressive treatment [¤]	53 (77%)	351 (51%)
ActiGraph		
Sedentary, m/d	654 (596-694)	
Light, m/w	2037 (1643-2371)	
Moderate, m/w	157 (91-292)	
Vigorous, m/w	0 (0-3)	
Steps per day	9299 (5562-13,255)	
IPAQ		
Sitting, m/d		420 (300-600)
Walking, m/w		225 (90-525)
Moderate, m/w		150 (15-360)
Vigorous, m/w		60 (0-210)

Table 5. Age, BMI, and MG disease duration are presented as means and standard deviations (SD). Sex is presented as numbers and percentages. MG-ADL scores and PA levels are presented as medians and inter-quartile ranges (IQR). Abbreviations: MG-ADL=MG Activities of Daily Living profile, m=minute, d=day, w=week, IPAQ= the International Physical Activity Questionnaires. [#] Significant (p<.001) difference between the two samples. [¶] Missing data on five patients. [¤] Some patients were in a combined treatment of pyridostigmine and immunosuppressive drugs. Immunosuppressive drugs: prednisolone, Azathioprine, Methotrexate, Mycophenolic acid, Tacrolimus, Rituximab.

Measured by the accelerometer, 46% of the included patients did not meet the international recommendations[105] of ≥150 min/week at moderate intensity in bouts of at least 10 minutes. Measured by IPAQ, 48% of the patients did not meet these recommendations. Also, 57% of the patients walked less than the recommended10,000 steps per day[23] measured by the accelerometer. Factors associated with PA intensities ($p \leq .05$) were MG severity (measured by the MG-ADL) and age (Table 6). Sex, BMI, and MG disease duration were not significantly associated with PA ($p > .05$).

Table 6. Factors associated with physical activity and inactivity, study II

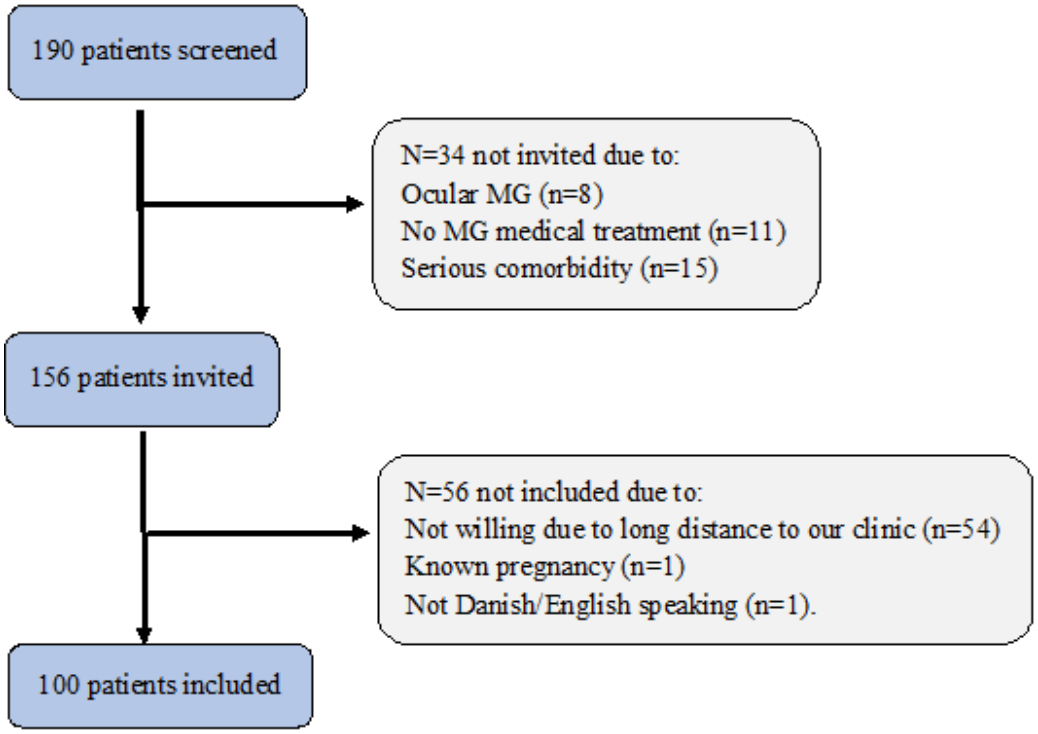
	MG-ADL		Age	
ActiGraph (n=64¶)	β	95%CI$_\beta$	β	95%CI$_\beta$
Sedentary, m/d	6.1	0.0; 12.1		
MVPA, m/w			-4.0	-6.3; -1.6
Steps per day			-90.8	-168.0; -13.5
IPAQ (n=691)				
Sitting time, m/d	2.5	0.9; 4.0	-0.5	-0.8; -0.2
Walking, m/w	-3.6	-6.5; -0.4		
MVPA, m/w			0.7	0.1; 1.3

Table 6. Factors associated with ($p < .05$) PA intensities and inactivity measured by ActiGraph and IPAQ. β = regression coefficient, CI $_\beta$ = confidence intervals of the regression coefficient. Abbreviations: MVPA= moderate-vigorous physical activity, MG-ADL=MG Activities of Daily Living profile, m=minute, d=day, w=week. ¶ Analyses were performed on 64 patients, as five patients did not complete the MG-ADL.

Study III

To reach the desired number of 100 patients (see the methods section), we consecutively screened 190 patients for eligibility (by checking their medical records) when they arrived in the outpatient clinic for their regular follow-up at the neurologist. N=156 patients met the inclusion criteria and were informed about the study, but 56 were not interested in participating, primarily due to practical issues. Figure 4 illustrates the inclusion of patients. Of the included patients, the mean± SD age was 60.2± 15.4 years, 57 were women, 94 were AChR positive, and 41 were thymectomized.

Figure 4. Flowchart of inclusions in study III



One-third of the patients (n=33) reported dissatisfaction (PASS-negative status) with their current symptom state. Compared to the satisfied patients (n=67), these patients had shorter MG disease duration (median 3 vs. 8 years, p=.001) and reported higher scores on almost all outcome measures (p<.05). We did not find any differences regarding age (p=.409), sex (p=.831), and comorbidity (p=.767) compared to the satisfied patients (Table 7). The MG medical treatment (including all drugs) was unequally distributed (p=.028); however, the distribution of patients on prednisolone treatment was just borderline significant (p=.050), and there were no dose differences of prednisolone between PASS-negative/positive patients (p=.120).

The patients had a median score of 8 (IQR 5-9) regarding satisfaction with current adverse effects. Of the PASS-negative patients, 64% reported low satisfaction (score<8) with adverse effects, whereas this was 30% of the PASS-positive patients (p=.002).

Table 7. Distribution of variables according to PASS status, study III

Characteristics	PASS negative (n=33)	PASS positive (n=67)	*p*-value
Age, years	58.3 (14.0)	61.1 (16.1)	.409
Sex, women	18 (55%)	39 (58%)	.831
MG disease duration, years	3 (1-6)	8 (4-15)	.001
MG-ADL	5 (4-7)	1 (0-3)	<.0001
QMG	11 (9-13)	8 (5-10)	.0001
MGC	9 (4-13)	5 (2-8)	.001
MG-QoL15	25 (12-32)	6 (1-12)	<.0001
MFI-20 general fatigue	17 (12-18)	10 (7-14)	<.0001
MFI-20 physical fatigue	17 (14-18)	11 (9-14)	<.0001
MFI-20 reduced activity	13 (12-16)	9 (6-12)	<.0001
MFI-20 reduced motivation	9 (7-13)	7 (5-10)	.001
MFI-20 mental fatigue	11 (7-13)	8 (5-11)	.003
MDI	17 (12-25)	8 (3-14)	<.0001
EQ-5D-3L	0.71 (0.66-0.78)	0.83 (0.78-1.00)	<.0001
EQ-VAS	60 (50-75)	80 (70-85)	<.0001
CCI	3 (1-5)	3 (2-5)	.767

Table 7. Values are presented as mean and standard deviation (SD), median and interquartile ranges (IQR), or as numbers and percentages (%). Abbreviations; CCI= Charlson Comorbidity Index, MG-ADL= MG Activities of Daily Living profile, MG-QoL15= MG-specific QoL instrument, MDI= Major Depression Inventory, MGC= MG Composite scale, QMG= Quantitative MG score, MFI-20= Multidimensional Fatigue Inventory.

Figure 5 illustrates the associations of PASS-negative status. Higher levels of MG severity (MG-ADL, QMG) were associated with higher odds for reporting PASS-negative status (OR 1.65, *p*=.001 and OR 1.23, *p*=.007). Also, lower quality of life (MG-QoL15, OR 1.14, *p*=.0006), higher levels of fatigue (MFI-20; examined for general – and physical fatigue, OR 1.25, *p*=.007, and OR 1.28, *p*=.003), depression symptoms (MDI, OR 1.10, *p*=.003), and shorter MG disease duration (OR 0.92, *p*=.016) were associated with higher odds for reporting PASS-negative status. Age, sex, BMI, comorbidity, and MG medical treatment (also analyzed separately for prednisolone) were not associated with PASS-negative status in the adjusted analyses.

Figure 5. Factors associated with PASS-negative status, study III

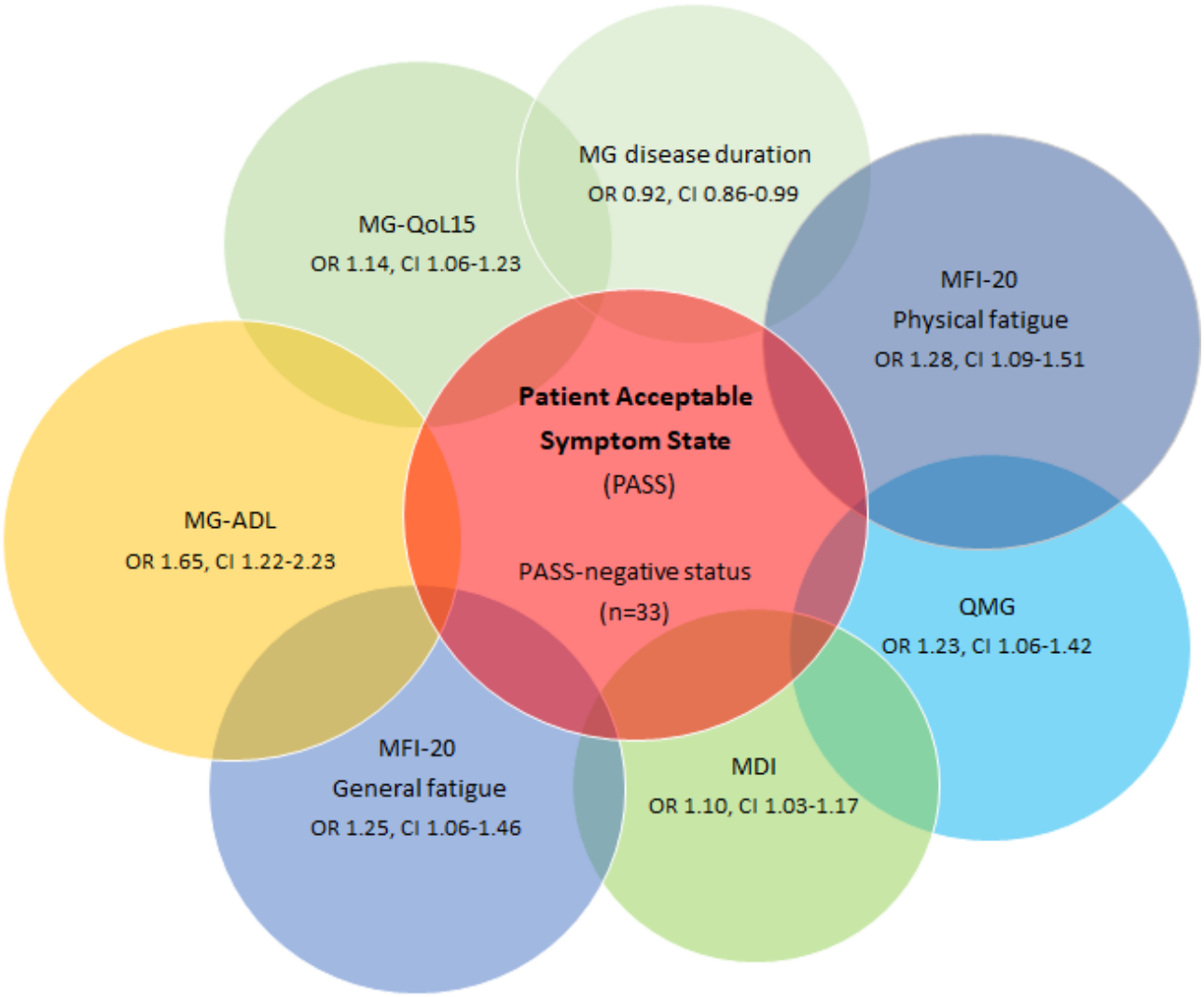


Figure 5. Factors significantly associated with PASS negative status ($p \leq .05$). Logistic regression analyses adjusted for age and sex. Abbreviations; MG-ADL= MG Activities of Daily Living profile, MG-QoL15= MG-specific QoL instrument, MDI= Major Depression Inventory, QMG= Quantitative MG score, MFI-20= Multidimensional Fatigue Inventory.

Study IV

We included 115 patients with the following diagnoses; myotonic dystrophy type 1 (n=20), limb-girdle muscular dystrophy (types 1B, 2A, 2I, 2L) (n=18), facioscapulohumeral dystrophy (n=15), Kennedy disease (n=14), Charcot-Marie-Tooth neuropathy (CMT1A and CMTX) (n=14), mitochondrial myopathy (n=10), sporadic inclusion body myositis (n=10), Becker muscular dystrophy (n=8), and a mixed group of myopathies (n=6). The patients completed one 2MWT and one 6MWT in a randomized order, and 38 healthy controls completed one 6MWT. For patients, the mean walking distance in the 2MWT was 143 meters (range 30-258 meters) and 405 meters (range 65-750 meters) in the 6MWT (Table 8). The distances walked in the tests correlated highly (r=0.99, p<.001) (Figure 6), and we found that the 2MWT was a valid alternative to the 6MWT to describe walking capability in these patients.

Table 8: Demographics, walking distance and gait speed, study IV

	Patients (n=115)	Healthy controls (n=38)
Age, years	52.6 (22-83)	47.6 (25-76)
Sex, female	40 (35%)	19 (50%)
BMI	25.2 (16.1-44.1)	25.1 (19.6-33.5)
Height	173.9 (138-198)	175.6 (155-192)
Walking distance		
2MWT	142.8 (30-258)	-
6MWT	405.3 (65-750)	685.1 (545-863)
Gait speed, 2MWT		
First minute	1.21 m/s	-
Second minute	1.19 m/s	-
Gait speed, 6MWT		
First minute	1.18 m/s	1.93 m/s
Sixth minute	1.13 m/s	1.90 m/s

Table 8. Values are mean (range) or number (percentages). Abbreviations: 2MWT= 2-min walk test, 6MWT = 6-min walk test, BMI= body mass index, m=minute, s=second.

Figure 6. Correlation of the walked distances in the 2- and 6-min walk test, study IV

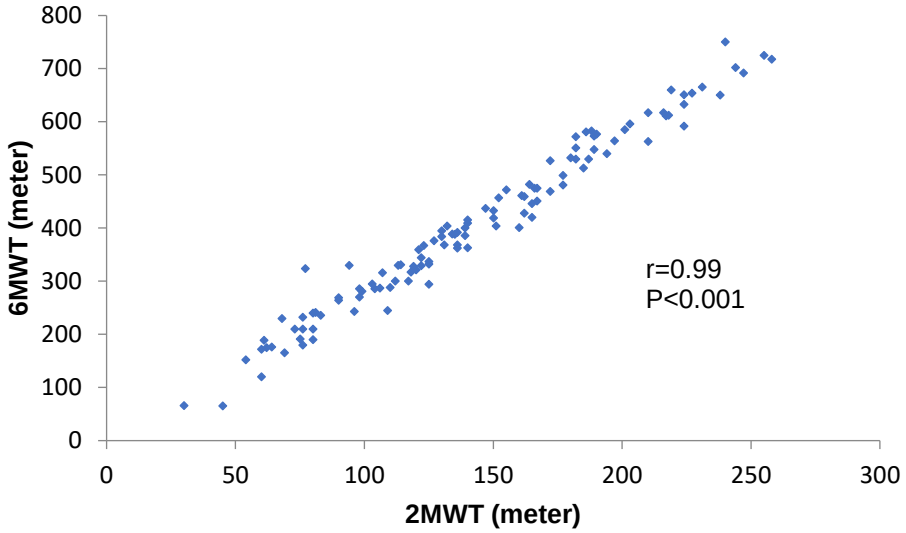


Figure 6. Abbreviations: 2MWT= 2-min walk test, 6MWT = 6-min walk test, BMI= body mass index, r=correlation coefficient, *p*=p-value.

When comparing the mean walked distance in the performed 6MWT with the predicted 6MWT (the distance walked in the 2MWT multiplied by 3), the walked distance in the performed 6MWT was shorter (*p*=.001), indicating walking-related fatiguability. Also, there was a decrease (4.6%) in gait speed in the sixth minute (1.18 m/s) compared to the first minute (1.13 m/s) (*p* =.001) in the performed 6MWT. This decrease was minimal (0.02 m/s, *p*=.001) in the 2MWT and was eliminated by the learning effect by repeated 2MWTs. We found a decline in gait speed of 1.4% (*p*=.01) in the healthy controls from the sixth minute (1.93 m/s) to the first minute (1.90m/s) of the 6MWT. The absolute fall in gait speed did not differ between patients and healthy controls (*p*=.10), but the percentage drop was higher in the patients (*p*=.002).

Study V

To examine the effect of RAS on walking distance and gait speed, a total of 48 patients with gMG completed two 6MWTs. One standard 6MWT and one 6MWT added RAS with a frequency of 100% (group 'FAST', n= 18) or 110% (group 'FAST10', n=30) of the patient's fastest gait speed. The walked distance increased by 8.3 meters (CI: 1.82-14.78, p=.01) in the 6MWT with RAS compared to standard 6MWT when the frequency was 110%. The order of tests (p=.36), MG severity (MGC, p=.07) and sex (p=.56) did not influence on walked distance, whereas increasing age and BMI decreased the distance (age, estimate -2.10, CI: -3.29; -0.91, p<.01, BMI: estimate -3.15, CI: -5.75; -0.55, p=.02). The increased walk distance did not result in additional exertion measured by heart rate or Borg scale. We did not find any statistically significant increase in walked distance (4.5 meters, CI: -7.91–16.91, p=.45) when the frequency of RAS was 100% of the patient's fastest gait speed. We found a decrease in gait speed (p<.05) in all tests, indicating walking-related fatiguability when comparing the first and sixth minutes. Table 9 shows patient characteristics and gait parameters.

Table 9. Patient characteristics and gait parameters

	FAST (n=18)	*P*-value	FAST10 (n=30)	*P*-value
Sex	5 men (28%) 13 women (72%)		9 men (30%) 21 women (70%)	1.00
Age, years	57 (17.9)		54 (16.3)	0.59
BMI, kg/m^2	27 (5.6)		28 (7.2)	0.70
MGC, total score	6 (3-15)		9 (4-13)	0.69
Walking distance, meter				
Standard 6MWT	544 (77.2)	0.45	567 (61.2)	0.01
RAS 6MWT	548 (85.3)		575 (63.7)	
Gait speed, standard 6MWT, m/s				
First minute	1.54 (0.22)	0.02	1.61 (0.16)	<.01
Sixth minute	1.51 (0.21)		1.57 (0.17)	
Gait speed, RAS 6MWT, m/s				
First minute	1.58 (0.25)	<.01	1.62 (0.18)	<.01
Sixth minute	1.52 (0.24)		1.59 (0.18)	
HR average, BPM				
Standard 6MWT	118 (18.7)	0.06	115 (16.9)	0.44
RAS 6MWT	109 (18.5)		113 (14.3)	
Borg, score				
Standard 6MWT	11.5 (10-13)	0.88	13.0 (11-14)	0.90
RAS 6MWT	11.0 (10-13)		13.0 (11-15)	

Table 9. Abbreviations: 6MWT = 6-min walk test, BMI= body mass index, m=meter, s=second. Values are mean and standard deviation (SD), number and percentage (%), or median and inter-quartile ranges (IQR).

SUMMARY OF MAIN FINDINGS

STUDY I Fatigue was increased in patients with MG compared to non-MG individuals. We found the highest fatigue scores in physical- and general fatigue and these domains' levels were similar. Around half of the patients reported low levels of PA, and higher levels of fatigue were associated with lower levels of PA. Other factors associated with severe fatigue were severe MG, insomnia, overweight, comorbidity, job status, and living alone.

STUDY II Around half of the included patients reported low levels of PA, which the accelerometer measurements confirmed. Severe MG and increasing age were associated with increasing time spent on sedentary activities and decreasing time spent on PA.

STUDY III One-third of the included patients reported dissatisfaction with their current symptom state. Severe MG, severe fatigue, insomnia, symptoms of depression, low levels of MG-related quality of life, and short MG disease duration were associated with higher odds for reporting dissatisfaction (PASS-negative status).

STUDY IV The 2MWT was a valid alternative to the longer 6MWT to measure walking capability in a heterogeneous group of patients with neuromuscular diseases. The 6MWT was useful to measure walking-related fatiguability in these patients.

STUDY V RAS improved walked distance and gait speed without additional exertion in patients with gMG when the frequency was 110% of the patient’s fastest gait speed.

DISCUSSION

This thesis is the first to unveil multiple fatigue domains in a substantial cohort of patients with MG and explicitly address the association between fatigue and PA levels. The combination of patient-reported/clinician-derived and MG-specific/generic outcomes gave a multi-faceted insight into the lives and well-being of patients with MG. This insight was not exclusively targeting the disease but also to symptoms indirectly related to the MG, e.g., depression, sleep, comorbidity, and symptoms acceptance.

FATIGUE

Fatigue in MG is widespread, complex, and increased compared to non-MG individuals. The highest fatigue scores were observed for the MFI-20 domains general- and physical fatigue, followed by reduced activity, and lowest, reduced motivation and mental fatigue. The fact that the included patients reported the same elevated level of general- and physical fatigue, despite that only physical fatigue can be explained by the disease, emphasizes the varied and complex nature of fatigue in MG.

In all of the MFI-20 domains, the median score was higher (2-4 "points") than found in the Danish background population[106] (4964 Danish adults, aged 49-63 years). Previous studies comparing MG fatigue levels to healthy controls (measured by other tools than the MFI-20)[107,108] also found elevated fatigue levels in patients with MG. Compared to other diseases, measured by the MFI-20, the fatigue level in MG was either slightly higher (compared to spinal muscular atrophy)[109]), similar (compared to post-stroke and other autoimmune diseases[60,110]), or slightly lower (compared to Parkinson disease and multiple sclerosis[64,111]).

Patients in complete remission still reported fatigue (study I), which was in line with a previous study of 200 patients with MG, where 32% of the patients in pharmacological remission still reported relevant fatigue (defined as a score of $\geq$4 points at the Chalder Fatigue Scale)[86]. However, we found that the fatigue scores in patients in complete remission were at the same level as scores in the background population[106]. This is similar to findings in two previous MG studies[40,107] and suggests that high fatigue is associated with active, non-controlled MG disease.

Severe MG is associated with fatigue in multiple papers[15], and this association was also evident in our findings.

Other factors associated with severe fatigue were comorbidity, low sleep quality, high BMI, living alone, and current job status, e.g., being retired due to sickness. In a Danish background population[67], lower social class, living alone, somatic diseases, and depression is associated with higher fatigue scores.

For MG, the associations between severe fatigue and 1) depression and 2) overall comorbidity (including depression) were significant for the overall sample but only significant for depression and reduced motivation in the sub-sample. Even though previous studies have found that depression is a strong predictor for severe fatigue in MG[15], our findings were not clear. However, due to the survey's design, we could not verify the patient reports of comorbidity, and these reports might be biased (see study limitation).

Insomnia was highly associated with severe fatigue in our findings, both in the overall- and the sub-sample. Results from previous studies vary[15], which might be due to different outcomes of sleep; insomnia, sleep quality, daytime sleepiness, and thereby other questionnaires used. In one study[86], using the ISI to examine fatigue in patients with MG, insomnia was associated with fatigue in the univariate analyses but not in the adjusted analyses. This study used an ISI score of ≥10 as the cut-off point for insomnia, even though the manual of the ISI recommends a score of ≥8. Also, this study adjusted for positive MuSK-ab status, which we didn’t. Further studies are needed to unravel the relation between sleep and fatigue in patients with MG.

We found an association between BMI and severe fatigue, not previously examined. However, previous cohorts[112] report increased BMI in patients with MG, which corresponds to our findings. Being overweight might be due to inactivity and treatment with steroids, which again leads to fatigue. The female gender was previously associated with severe fatigue in MG [15,40], but we did not identify this association. No previous studies have found that cohabitation and job status are related to fatigue in patients with MG.

PHYSICAL ACTIVITY

In study I, 53% (n=386) of the patients reported low levels of PA measured by the SGPALS. In study II, 46% (n=32) of the patients had lower levels than the recommended 150 min/week of MVPA measured by accelerometry, and 57% (n=38) did not meet 10,000 steps/day. In the survey sample, 48% (n=343) did not meet the recommendations of min 150 min/week measured by the IPAQ. Compared to other patient cohorts, the findings were in line with the patient with myotonic dystrophy[113] but higher than seen for patients with mitochondrial diseases[114].

Compared to previous studies in MG, our findings indicate more active patients. In previous studies, only 22% and 30% of the patients met the recommendations for MVPA in bouts of ≥10 min, and only 22% met the 10,000 steps per day measured by accelerometer[38,39]. However, the study by O'Connor[38] also found that 78% achieved the minimum requirements of 64 MET min/day recommended by the American Heart Association. Time spent on sedentary behavior was almost similar across studies, but our study demonstrated more time spent on MVPA and more steps per day than previous findings[32,38,39]. This might be explained by different accelerometers and thereby different algorithms, manufacturer software, and cut-off points to determine time spent on PA intensities, as our study was the only one using the ActiGraph. However, further research is needed to clarify this.

Comparisons with large-scale studies from the Danish background population were troublesome. Depending on which study we compared to, our patients went from being less active to more active than the general Danish population, even for studies using the same measurements[73,115,116]. The complexity of measuring self-reported PA in larger settings is well-known[117].

In general, contrariety in findings stresses the complexity of measuring habitual physical activity. Study conclusions depend on the PA outcome measure of the studies (e.g., MET, time spent in different PA intensities, CPM measured by the accelerometer) and the comparable PA recommendations. This challenges the comparison of study findings across clinical or population-based studies. Moreover, the wording of the recommendations is essential. Patients meeting the ≥150 min per week might not meet the ≥30 min/day for at least five days (used in the study by O'Connor[38] and close to the Danish recommendations of 30 min per day[118,119]), as some patients accumulate their exercise in few days. Also, the WHO recommendations have changed from 2010[105] to 2020[22], where bouts of a minimum of 10 minutes are no longer required. Our

findings were almost similar across different measurements (SGPALS, IPAQ, and accelerometry), indicating high validity.

Remarkably, around half of the included patients did not follow the international recommendations of PA, which suggests that PA counseling should be an integrated part of the rehabilitation of patients with MG.

FATIGUE AND PHYSICAL ACTIVITY

We found strong associations between low levels of all fatigue domains (except for mental fatigue in the sub-sample) and high levels of PA after adjusting for several patients- and disease-related variables. These findings are new, as previous results from cross-sectional studies did not measure PA in relation to separated fatigue domains[40,120].

Due to our studies' cross-sectional design, causal relationships are not evaluated, and future interventional studies are needed to assess the direction of this association. Previous interventional exercise studies found minimal and unsustainable[41] or no[30] improvement of fatigue after an intervention. However, in one of these studies[41] (combining exercise with psychological interventions), the sample size was small (n=10), and exercise was mainly stretching and light activities. A combination of exercise and cognitive-behavioral therapy has previously been beneficial to manage chronic fatigue in patients with facioscapulohumeral muscular dystrophy[120].

The study by Rahbek et al.[30], observed a decrease in MG-related quality of life after 8-weeks of supervised aerobic training. Also, the study of home-based rowing exercise in 43 patients with gMG found no improvements in MG-related quality of life after intervention[121]. These findings are discouraging, and further elaboration regarding type and mode of exercise, intensity, and duration are needed.

PATIENT ACCEPTABLE SYMPTOM STATE

One-third (n=33) of the patients in study III reported dissatisfaction with their current symptom state. Dissatisfaction was associated with severe MG, severe fatigue, depression, short disease duration, and low MG-related QoL. Regarding MG severity, the odds for reporting dissatisfaction were higher when the MG symptoms were patient-reported (MG-ADL) than clinician-derived (QMG), emphasizing that the patient perspective is relevant in the rehabilitation. Despite different cultural settings and treatment regimens, previous studies have reported a proportion of PASS-negative patients[10,11] similar to our findings, which indicates that dissatisfaction is complex and might involve non-identified factors.

One study[11] estimated cut-off points for the MG-ADL, MGC, and MG-QoL15 in relation to PASS status. When applied to our cohort, these cut-off points overestimated the number of PASS-negative answers. However, this might be explained by differences in study designs, as previous studies[10,11] were retrospective and did not compare PASS and MG outcomes in the same cohort. Our cohort resembled previous cohorts in terms of age, sex, and disease duration.

Severe fatigue was associated with PASS-negative status. The odds for reporting dissatisfaction were similar for physical- and general fatigue, emphasizing what we also found in study I, that general fatigue negatively impacts the patient's well-being at the same level as the disease-induced physical fatigue.

One-fifth of the patients reported depression defined by the MDI. Even though this number seems high, previous studies have reported a similar number in patients with MG[15,86,107] and other diseases(rheumatoid arthritis, multiple sclerosis)[122,123]. It might therefore be related to the burden of living with a chronic disorder. For comparison, the prevalence of depression measured by the MDI in 14,787 Danes (≥20 years) was 2.3%[124]. We did not find any associations between comorbidity and PASS-status, which might relate to the findings in the sub-sample in study I where comorbidity and fatigue were not associated.

Treatment with steroids and adverse effects are likely to be related, as prednisolone is known to cause undesirable adverse effects when used long-term and/or at high doses. However, treatment with steroids was not associated with PASS-negative status in our findings. In the Canadian study[11], the proportion of dissatisfied patients were similar to our results, despite higher ratios of steroid treatment (55-58%) compared to our study (24%), which might indicate that the influence of

steroids on PASS status is low. However, further research is needed to determine the impact of medical treatment on dissatisfaction across different cultures and treatment regimens.

We found that MG duration was associated with PASS-negative status, and the PASS-negative patients had half the median disease duration than the PASS-positive patients (Table 7). An explanation might be that newly diagnosed patients are not yet in stable MG medical treatment and might be troubled by contracting a new, chronic disease.

WALKING-RELATED FATIGUABILITY

In study IV, we identified walking-related fatiguability in the 6MWT in patients with various neuromuscular diagnoses. Inspired by this study, we performed study V. We found that the 6MWT was useful to measure walking-related fatigability in patients with MG and that RAS improved gait speed and walked distances compared to standard 6MWT.

In study IV, we found that the absolute decrease in gait speed was similar for the patients and the healthy controls; however, the percentage drop was larger in the patients. Also, in study V, we found that disease severity did not influence walking distance. This might indicate that walking-related fatiguability is not only related to the ability to perform. It occurs in both patients and healthy controls and might also be related to motivation and strategic approaches to the walk test. Therefore, it could be relevant in future studies to include measurements of other fatigue domains (e.g., the MFI-20 domains general fatigue, reduced activity, and reduced motivation) to explore the influence of these domains on walking-related fatiguability.

Gait speed and walking distance improved at a RAS frequency of 110% of the patient's fastest gait speed. The 110% was a pragmatic balance between obtaining an effect of RAS and a realistic gait speed for the patients. We believe that this threshold was optimal, as a previous study[114] found reduced synchronization between steps and auditory stimuli when the frequency was further increased. Compared to standard 6MWT, RAS improved gait speed and walking distance without additional exertion. As we usually rely on, that increasing workload leads to an increase in HR and Borg score, these findings were surprising. Previous studies have given a possible explanation of this phenomenon: locomotor–respiratory coupling, where respiration synchronizes with locomotion,

e.g., during walking, resulting in decreased energy expenditure[125,126]. However, further studies are needed to unravel this.

Bohannon et al. found that the minimal clinically important difference in repeated 6MWTs was 14-30.5 meters in adults (multiple patient groups, no neuromuscular diseases)[127]. Compared, we can discuss the relevance of our findings (8.3 meters) in study V. However, in the review by Bohannon, the learning effect explained a substantial part of the improvements in the repeated tests. As patients performed the walk tests in study V in a randomized order, there was no learning effect. Also, compared to the findings in study IV, the walked distance in patients with MG was closer to the performance of healthy controls than in patients with a variety of neuromuscular diseases. Therefore, there might be upper limits for further improvements in the included MG patients. The patients in study V were slightly younger than those in our other studies (Table 3), and the findings might have differed if we had included older patients.

Findings from study V indicate that walking-related fatiguability might be susceptible to influences from external stimuli, e.g., music. These findings add new strategies to existing MG rehabilitation.

STUDY LIMITATIONS

One essential type of bias was selection bias. As the survey design did not allow us to verify the diagnoses of the included patients, we might have included non-MG individuals. However, all analyses were repeated in a sub-sample of patients on current MG medical treatment AND regularly followed by a neurologist. As the study findings of fatigue and PA were almost similar in the overall- and the sub-sample, the influence of selection bias and thereby disproportion of the results were likely low. Also, the findings of PA in study I were confirmed by accelerometer testing in patients with a verified MG diagnosis in study II, indicating a low influence of non-MG individuals.

As seen in Figure 2, the youngest and oldest patients with MG are underrepresented in the survey. Most aging patients did not subscribe e-Boks, and the youngest patients did not respond to the survey. However, compared to the other studies, where we consecutively recruited patients from the clinic (studies II, III, V), the mean age of the included patients with MG was almost similar across study designs (Table 3).

Information bias might be present in the survey, e.g., for medical treatment and comorbidity, as we could not verify this information by medical records due to the study design. In study III, where we obtained information about medical treatment from the medical records, 24% of the patients were treated with steroids, whereas this was 18% in the survey. Also, compared to study III, where the prevalence of depression measured by the MDI (which is a validated diagnostic tool for depression in the clinic) was 20%, it was 8% in the survey. Therefore, it might be likely that depression and steroid treatment were under-reported in the survey, leading to weakened associations of fatigue.

Social desirability bias describes a tendency to distort self-reports in a favorable direction and is well-known in self-reports of PA[128,129]. However, social desirability bias seems limited in web-based surveys[130]. Also, as our patient-reported findings of PA were similar to the objective measurements of accelerometry (study II), we expect minimal social-desirability bias.

Covariates were chosen a priori based on evidence from literature and experiences from the clinic. However, to avoid multiple testing errors and very comprehensive analyses, the number of covariates was limited. Therefore, the amount of residual confounding is unknown and might be present.

We excluded patients with severe medical conditions from study II-V. Therefore, these findings might not be representative of most disabled patients. In addition, strong and well-treated patients,

who already had positive experiences with PA, might be over-represented in the accelerometry measurements of PA. However, variables such as age, sex, BMI, MG severity, and MG-related quality of life were almost similar across studies, including the survey (Table 3). The 779 patients enrolled in the survey comprised a large proportion of the previously estimated Danish MG cohort of around 1000 patients [5]. As we expect a minimal influence of bias on our results, we assume that our findings are generalizable to the Danish cohort of patients with MG. Cultural differences in perception of fatigue and PA, and differences in treatment regimens of MG, may question the generalizability of the findings outside Denmark. However, none of our results conflicted with previous international findings, indicating comparable study samples.

IMPLICATIONS

In general, our findings indicate that the well-being and satisfaction of patients with MG depend on various factors and not MG symptoms and medical treatment exclusively. Therefore, a more patient-centered approach to MG treatment is relevant in the clinic.

In this thesis, we have elucidated that fatigue in MG is widespread and complex, and we have identified factors associated with fatigue. However, future interventional studies are needed to unveil causality and dose-response between fatigue and relevant lifestyle factors, such as PA habits. As many patients reported low levels of PA, barriers for PA need to be identified, e.g., in qualitative study designs with the patient in focus.

We hope that the findings of this thesis will inspire and motivate other researchers to explore these topics even further in a way that benefits patients with MG.

REFERENCES

1. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14:1023–36.

2. Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Ann Thorac Surg. 2000;70:327–34.

3. Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. J Autoimmun. 2007;29:1–9.

4. Pedersen EG, Hallas J, Hansen K, Jensen PEH, Gaist D. Late-onset myasthenia not on the increase: a nationwide register study in Denmark, 1996-2009. Eur J Neurol. 2013;20:309–14.

5. Hansen JS, Danielsen DH, Somnier FE, Frøslev T, Jakobsen J, Johnsen SP, et al. Mortality in myasthenia gravis: A nationwide population-based follow-up study in Denmark. Muscle Nerve. 2016;53:73–7.

6. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in Myasthenia Gravis. BMC Neurol. 2010;10:46.

7. Gilhus NE, Nacu A, Andersen JB, Owe JF. Myasthenia gravis and risks for comorbidity. Eur J Neurol. 2015;22:17–23.

8. Pedersen EG, Pottegård A, Hallas J, Friis S, Hansen K, Jensen PEH, et al. Risk of non-melanoma skin cancer in myasthenia patients treated with azathioprine. Eur J Neurol. 2014;21:454–8.

9. Thomsen JLS, Andersen H. Outcome Measures in Clinical Trials of Patients With Myasthenia Gravis. Front Neurol. 2020;11:596382.

10. Menon D, Barnett C, Bril V. Comparison of the single simple question and the patient acceptable symptom state in myasthenia gravis. European Journal of Neurology [Internet]. [cited 2020 Sep 23];n/a. Available from: http://onlinelibrary.wiley.com/doi/abs/10.1111/ene.14397

11. Mendoza M, Tran C, Bril V, Katzberg HD, Barnett C. Patient-acceptable symptom states in myasthenia gravis. Neurology. 2020;

12. Chaudhuri A, Behan PO. Fatigue in neurological disorders. Lancet. 2004;363:978–88.

13. Heine M, van de Port I, Rietberg MB, van Wegen EEH, Kwakkel G. Exercise therapy for fatigue in multiple sclerosis. Cochrane Database Syst Rev. 2015;CD009956.

14. Wu S, Kutlubaev MA, Chun H-YY, Cowey E, Pollock A, Macleod MR, et al. Interventions for post-stroke fatigue. Cochrane Database Syst Rev. 2015;CD007030.

15. Ruiter AM, Verschuuren JJGM, Tannemaat MR. Fatigue in patients with myasthenia gravis. A systematic review of the literature. Neuromuscul Disord. 2020;30:631–9.

16. Andersen H, Mantegazza R, Wang JJ, O'Brien F, Patra K, Howard JF, et al. Eculizumab improves fatigue in refractory generalized myasthenia gravis. Qual Life Res. 2019;28:2247–54.

17. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology. 2013;80:409–16.

18. Grohar-Murray ME, Becker A, Reilly S, Ricci M. Self-care actions to manage fatigue among myasthenia gravis patients. J Neurosci Nurs. 1998;30:191–9.

19. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res. 1995;39:315–25.

20. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports. 2015;25 Suppl 3:1–72.

21. Warburton DER, Bredin SSD. Health benefits of physical activity: a systematic review of current systematic reviews. Curr Opin Cardiol. 2017;32:541–56.

22. WHO guidelines on physical activity and sedentary behaviour [Internet]. [cited 2021 Jan 31]. Available from: https://www.who.int/publications-detail-redirect/9789240015128

23. Tudor-Locke C, Bassett DR. How many steps/day are enough? Preliminary pedometer indices for public health. Sports Med. 2004;34:1–8.

24. Hallam KT, Bilsborough S, de Courten M. 'Happy feet': evaluating the benefits of a 100-day 10,000 step challenge on mental health and wellbeing. BMC Psychiatry. 2018;18:19.

25. Leddy JJ, Chutkow JG. Myasthenia gravis in a collegiate football player. Med Sci Sports Exerc. 2000;32:1975–9.

26. Stout JR, Eckerson JM, May E, Coulter C, Bradley-Popovich GE. Effects of resistance exercise and creatine supplementation on myasthenia gravis: a case study. Med Sci Sports Exerc. 2001;33:869–72.

27. Lucia A, Maté-Muñoz JL, Pérez M, Foster C, Gutiérrez-Rivas E, Arenas J. Double trouble (McArdle's disease and myasthenia gravis): how can exercise help? Muscle Nerve. 2007;35:125–8.

28. Scheer BV, Valero-Burgos E, Costa R. Myasthenia gravis and endurance exercise. Am J Phys Med Rehabil. 2012;91:725–7.

29. Lohi EL, Lindberg C, Andersen O. Physical training effects in myasthenia gravis. Arch Phys Med Rehabil. 1993;74:1178–80.

30. Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U. Exercise in myasthenia gravis: A feasibility study of aerobic and resistance training. Muscle Nerve. 2017;56:700–9.

31. Westerberg E, Molin CJ, Lindblad I, Emtner M, Punga AR. Physical exercise in myasthenia gravis is safe and improves neuromuscular parameters and physical performance-based measures: A pilot study. Muscle Nerve. 2017;56:207–14.

32. Westerberg E, Molin CJ, Spörndly Nees S, Widenfalk J, Punga AR. The impact of physical exercise on neuromuscular function in Myasthenia gravis patients: A single-subject design study. Medicine (Baltimore). 2018;97:e11510.

33. Birnbaum S, Porcher R, Portero P, Clair B, Demeret S, Eymard B, et al. Home-based exercise in autoimmune myasthenia gravis: A randomized controlled trial. Neuromuscul Disord. 2021;31:726–35.

34. Wong SH, Nitz JC, Williams K, Brauer SG. Effects of balance strategy training in myasthenia gravis: A case study series. Muscle & Nerve. 2014;49:654–60.

35. Ambrogi V, Mineo TC. Benefits of Comprehensive Rehabilitation Therapy in Thymectomy for Myasthenia Gravis: A Propensity Score Matching Analysis. Am J Phys Med Rehabil. 2017;96:77–83.

36. Fregonezi GA de F, Resqueti VR, Güell R, Pradas J, Casan P. Effects of 8-week, interval-based inspiratory muscle training and breathing retraining in patients with generalized myasthenia gravis. Chest. 2005;128:1524–30.

37. Freitag S, Hallebach S, Baumann I, Kalischewski P, Rassler B. Effects of long-term respiratory muscle endurance training on respiratory and functional outcomes in patients with Myasthenia gravis. Respir Med. 2018;144:7–15.

38. O'Connor L, Westerberg E, Punga AR. Pattern of Habitual Physical Exercise in Myasthenia Gravis Patients. J Neuromuscul Dis. 2019;6:85–91.

39. Birnbaum S, Bachasson D, Sharshar T, Porcher R, Hogrel J-Y, Portero P. Free-Living Physical Activity and Sedentary Behaviour in Autoimmune Myasthenia Gravis: A Cross-Sectional Study. J Neuromuscul Dis. 2021;

40. Ruiter AM, Verschuuren JJGM, Tannemaat MR. Prevalence and associated factors of fatigue in autoimmune myasthenia gravis. Neuromuscul Disord. 2021;

41. Farrugia ME, Di Marco M, Kersel D, Carmichael C. A Physical and Psychological Approach to Managing Fatigue in Myasthenia Gravis: A Pilot Study. J Neuromuscul Dis. 2018;5:373–85.

42. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100:126–31.

43. Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, et al. Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. Int J Behav Nutr Phys Act. 2017;14:75.

44. Salci Y, Karanfil E, Balkan AF, Kütükçü EÇ, Ceren AN, Ayvat F, et al. Functional exercise capacity evaluated by timed walk tests in myasthenia gravis. Muscle Nerve. 2019;59:208–12.

45. Vinge L, Jakobsen J, Andersen H. Muscle weakness and functional disability in patients with myasthenia gravis. Muscle Nerve. 2019;59:218–23.

46. Cejvanovic S, Vissing J. Muscle strength in myasthenia gravis. Acta Neurol Scand. 2014;129:367–73.

47. Symonette CJ, Watson BV, Koopman WJ, Nicolle MW, Doherty TJ. Muscle strength and fatigue in patients with generalized myasthenia gravis. Muscle Nerve. 2010;41:362–9.

48. Van Geel F, Moumdjian L, Lamers I, Bielen H, Feys P. Measuring walking-related performance fatigability in clinical practice: a systematic review. Eur J Phys Rehabil Med. 2020;56:88–103.

49. Jordan B, Mehl T, Schweden TLK, Menge U, Zierz S. Assessment of physical fatigability and fatigue perception in myasthenia gravis. Muscle Nerve. 2017;55:657–63.

50. Moumdjian L, Buhmann J, Willems I, Feys P, Leman M. Entrainment and Synchronization to Auditory Stimuli During Walking in Healthy and Neurological Populations: A Methodological Systematic Review. Front Hum Neurosci. 2018;12:263.

51. Nascimento LR, de Oliveira CQ, Ada L, Michaelsen SM, Teixeira-Salmela LF. Walking training with cueing of cadence improves walking speed and stride length after stroke more than walking training alone: a systematic review. J Physiother. 2015;61:10–5.

52. Rocha PA, Porfírio GM, Ferraz HB, Trevisani VFM. Effects of external cues on gait parameters of Parkinson's disease patients: a systematic review. Clin Neurol Neurosurg. 2014;124:127–34.

53. Moumdjian L, Sarkamo T, Leone C, Leman M, Feys P. Effectiveness of music-based interventions on motricity or cognitive functioning in neurological populations: a systematic review. Eur J Phys Rehabil Med. 2017;53:466–82.

54. Moumdjian L, Moens B, Maes P-J, Van Geel F, Ilsbroukx S, Borgers S, et al. Continuous 12 min walking to music, metronomes and in silence: Auditory-motor coupling and its effects on perceived fatigue, motivation and gait in persons with multiple sclerosis. Mult Scler Relat Disord. 2019;35:92–9.

55. Erra C, Mileti I, Germanotta M, Petracca M, Imbimbo I, De Biase A, et al. Immediate effects of rhythmic auditory stimulation on gait kinematics in Parkinson's disease ON/OFF medication. Clin Neurophysiol. 2019;130:1789–97.

56. Knak KL, Andersen LK, Witting N, Vissing J. Reliability of the 2- and 6-minute walk tests in neuromuscular diseases. J Rehabil Med. 2017;49:362–6.

57. Knak KL, Andersen LK, Witting N, Vissing J. The 2- and 6-minute walk tests in neuromuscular diseases: Effect of heart rate correction on the learning effect. Int J Phys Med Rehab. 2017.

58. Smets EM, Garssen B, Schuster-Uitterhoeve AL, de Haes JC. Fatigue in cancer patients. Br J Cancer. 1993;68:220–4.

59. de Dreu MJ, Schouwenaars IT, Rutten GJM, Ramsey NF, Jansma JM. Fatigue in brain tumor patients, towards a neuronal biomarker. Neuroimage Clin. 2020;28:102406.

60. Christensen D, Johnsen SP, Watt T, Harder I, Kirkevold M, Andersen G. Dimensions of post-stroke fatigue: a two-year follow-up study. Cerebrovasc Dis. 2008;26:134–41.

61. Lexell J, Jonasson SB, Brogardh C. Psychometric Properties of Three Fatigue Rating Scales in Individuals With Late Effects of Polio. Ann Rehabil Med. 2018;42:702–12.

62. Visser MM, Goodin P, Parsons MW, Lillicrap T, Spratt NJ, Levi CR, et al. Modafinil treatment modulates functional connectivity in stroke survivors with severe fatigue. Sci Rep. 2019;9:9660.

63. Rytter HM, Westenbaek K, Henriksen H, Christiansen P, Humle F. Specialized interdisciplinary rehabilitation reduces persistent post-concussive symptoms: a randomized clinical trial. Brain Inj. 2019;33:266–81.

64. Mikula P, Nagyova I, Krokavcova M, Vitkova M, Rosenberger J, Szilasiova J, et al. The mediating effect of coping on the association between fatigue and quality of life in patients with multiple sclerosis. Psychol Health Med. 2015;20:653–61.

65. Binz C, Schreiber-Katz O, Kumpe M, Ranxha G, Siegler H, Wieselmann G, et al. An observational cohort study on impact, dimensions and outcome of perceived fatigue in adult 5q-spinal muscular atrophy patients receiving nusinersen treatment. J Neurol. 2021;268:950–62.

66. Grønvold M. Report of the translation of the Multidimensional Fatigue Inventory (MFI-20) from English to Danish. Copenhagen: Rigshospitalet; 1997 May.

67. Watt T, Groenvold M, Bjorner JB, Noerholm V, Rasmussen NA, Bech P. Fatigue in the Danish general population. Influence of sociodemographic factors and disease. J Epidemiol Community Health. 2000;54:827–33.

68. Saltin B, Grimby G. Physiological analysis of middle-aged and old former athletes. Comparison with still active athletes of the same ages. Circulation. 1968;38:1104–15.

69. IPAQ scoring protocol - International Physical Activity Questionnaire [Internet]. [cited 2020 Aug 25]. Available from: https://sites.google.com/site/theipaq/scoring-protocol

70. Hansen A-LS, Carstensen B, Helge JW, Johansen NB, Gram B, Christiansen JS, et al. Combined heart rate- and accelerometer-assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes: the ADDITION-PRO study. Diabetes Care. 2013;36:3062–9.

71. Rödjer L, H Jonsdottir I, Börjesson M. Physical activity on prescription (PAP): self-reported physical activity and quality of life in a Swedish primary care population, 2-year follow-up. Scand J Prim Health Care. 2016;34:443–52.

72. Hu G, Tuomilehto J, Silventoinen K, Barengo NC, Peltonen M, Jousilahti P. The effects of physical activity and body mass index on cardiovascular, cancer and all-cause mortality among 47 212 middle-aged Finnish men and women. Int J Obes (Lond). 2005;29:894–902.

73. Christensen A, Davidsen M, Ekholm O, Pedersen P, Juel K. Danskernes Sundhed - den nationale sundhedsprofil 2013 (National survey of the health of Danes - The Danish National Health Profile 2013). The Danish Health and Medicines Authority, Copenhagen;

74. Eriksen L, Grønbæk M, Helge JW, Tolstrup JS, Curtis T. The Danish Health Examination Survey 2007-2008 (DANHES 2007-2008). Scand J Public Health. 2011;39:203–11.

75. Bergström G, Berglund G, Blomberg A, Brandberg J, Engström G, Engvall J, et al. The Swedish CArdioPulmonary BioImage Study: objectives and design. Journal of Internal Medicine. 2015;278:645–59.

76. Schnohr P, Scharling H, Jensen JS. Changes in leisure-time physical activity and risk of death: an observational study of 7,000 men and women. Am J Epidemiol. 2003;158:639–44.

77. Reinholdsson M, Palstam A, Sunnerhagen KS. Prestroke physical activity could influence acute stroke severity (part of PAPSIGOT). Neurology. 2018;91:e1461–7.

78. Olsson OA, Persson HC, Alt Murphy M, Sunnerhagen KS. Early prediction of physical activity level 1 year after stroke: a longitudinal cohort study. BMJ Open. 2017;7:e016369.

79. Cook P, Sunnerhagen KS, Persson HC. Level of physical activity is positively correlated with perceived impact on life 12 months after stroke: A cross-sectional study. J Rehabil Med. 2020;52:jrm00056.

80. Persson CU, Hansson P-O. Early prediction of physical inactivity in 215 individuals 6 months after a mild stroke: The fall study of Gothenburg. J Rehabil Med. 2020;52:jrm00077.

81. Di Stefano V, Battaglia G, Giustino V, Gagliardo A, D'Aleo M, Giannini O, et al. Significant reduction of physical activity in patients with neuromuscular disease during COVID-19 pandemic: the long-term consequences of quarantine. J Neurol. 2021;268:20–6.

82. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. Neurology. 1999;52:1487–9.

83. Burns TM, Conaway MR, Cutter GR, Sanders DB, Muscle Study Group. Less is more, or almost as much: a 15-item quality-of-life instrument for myasthenia gravis. Muscle Nerve. 2008;38:957–63.

84. Burns TM, Sadjadi R, Utsugisawa K, Gwathmey KG, Joshi A, Jones S, et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15r. Muscle Nerve. 2016;54:1015–22.

85. Morin C. Insomnia: psychological assessment and management. New York: Guilford Press; 1993.

86. Hoffmann S, Ramm J, Grittner U, Kohler S, Siedler J, Meisel A. Fatigue in myasthenia gravis: risk factors and impact on quality of life. Brain Behav. 2016;6:e00538.

87. EQ-5D [Internet]. [cited 2020 Sep 28]. Available from: https://euroqol.org/

88. Wittrup-Jensen KU, Lauridsen J, Gudex C, Pedersen KM. Generation of a Danish TTO value set for EQ-5D health states. Scand J Public Health. 2009;37:459–66.

89. Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W. The sensitivity and specificity of the Major Depression Inventory, using the Present State Examination as the index of diagnostic validity. J Affect Disord. 2001;66:159–64.

90. Olsen LR, Jensen DV, Noerholm V, Martiny K, Bech P. The internal and external validity of the Major Depression Inventory in measuring severity of depressive states. Psychol Med. 2003;33:351–6.

91. Paul RH, Cohen RA, Goldstein JM, Gilchrist JM. Fatigue and its impact on patients with myasthenia gravis. Muscle Nerve. 2000;23:1402–6.

92. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–83.

93. Mouri H, Jo T, Matsui H, Fushimi K, Yasunaga H. Effect of Sugammadex on Postoperative Myasthenic Crisis in Myasthenia Gravis Patients: Propensity Score Analysis of a Japanese Nationwide Database. Anesth Analg. 2020;130:367–73.

94. Wijeysundera DN, Johnson SR. How Much Better Is Good Enough?: Patient-reported Outcomes, Minimal Clinically Important Differences, and Patient Acceptable Symptom States in Perioperative Research. Anesthesiology. 2016;125:7–10.

95. Salaffi F, Carotti M, Gutierrez M, Di Carlo M, De Angelis R. Patient Acceptable Symptom State in Self-Report Questionnaires and Composite Clinical Disease Index for Assessing Rheumatoid Arthritis Activity: Identification of Cut-Off Points for Routine Care. Biomed Res Int. 2015;2015:930756.

96. Arnold MB, Khanna D, Denton CP, van Laar JM, Frech TM, Anderson ME, et al. Patient acceptable symptom state in scleroderma: results from the tocilizumab compared with placebo trial in active diffuse cutaneous systemic sclerosis. Rheumatology (Oxford). 2018;57:152–7.

97. Tindall RS, Rollins JA, Phillips JT, Greenlee RG, Wells L, Belendiuk G. Preliminary results of a double-blind, randomized, placebo-controlled trial of cyclosporine in myasthenia gravis. N Engl J Med. 1987;316:719–24.

98. Burns TM, Conaway MR, Cutter GR, Sanders DB, Muscle Study Group. Construction of an efficient evaluative instrument for myasthenia gravis: the MG composite. Muscle Nerve. 2008;38:1553–62.

99. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111–7.

100. Van Allen MW. Aids to the Examination of the Peripheral Nervous System. Archives of Neurology. 1977;34:61–61.

101. Troiano RP. Large-scale applications of accelerometers: new frontiers and new questions. Med Sci Sports Exerc. 2007;39:1501.

102. Dyrstad SM, Hansen BH, Holme IM, Anderssen SA. Comparison of self-reported versus accelerometer-measured physical activity. Medicine and Science in Sports and Exercise. 2014;46:99–106.

103. Donaire-Gonzalez D, Gimeno-Santos E, Balcells E, Rodríguez DA, Farrero E, de Batlle J, et al. Physical activity in COPD patients: patterns and bouts. Eur Respir J. 2013;42:993–1002.

104. Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc. 1998;30:777–81.

105. WHO. Global recommendations on physical activity for health [Internet]. World Health Organization; 2010 [cited 2020 Aug 25]. Available from: https://apps.who.int/iris/handle/10665/44399

106. Boter H, Mänty M, Hansen AM, Hortobágyi T, Avlund K. Self-reported fatigue and physical function in late mid-life. J Rehabil Med. 2014;46:684–90.

107. Alekseeva TM, Gavrilov YV, Kreis OA, Valko PO, Weber KP, Valko Y. Fatigue in patients with myasthenia gravis. J Neurol. 2018;265:2312–21.

108. Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol. 2013;13:132.

109. Binz C, Schreiber-Katz O, Kumpe M, Ranxha G, Siegler H, Wieselmann G, et al. An observational cohort study on impact, dimensions and outcome of perceived fatigue in adult 5q-spinal muscular atrophy patients receiving nusinersen treatment. J Neurol [Internet]. 2021 [cited 2021 Apr 9];268:950–62. Available from: http://link.springer.com/10.1007/s00415-020-10227-5

110. Løppenthin K, Esbensen BA, Østergaard M, Jennum P, Tolver A, Aadahl M, et al. Physical activity and the association with fatigue and sleep in Danish patients with rheumatoid arthritis. Rheumatol Int. 2015;35:1655–64.

111. Elbers RG, van Wegen EE, Verhoef J, Kwakkel G. Impact of fatigue on health-related quality of life in patients with Parkinson's disease: a prospective study. Clin Rehabil. 2014;28:300–11.

112. Kittiwatanapaisan W, Gauthier DK, Williams AM, Oh SJ. Fatigue in Myasthenia Gravis patients. J Neurosci Nurs. 2003;35:87–93, 106.

113. Knak KL, Sheikh AM, Witting N, Vissing J. Physical activity in myotonic dystrophy type 1. J Neurol. 2020;

114. Apabhai S, Gorman GS, Sutton L, Elson JL, Plötz T, Turnbull DM, et al. Habitual physical activity in mitochondrial disease. PLoS ONE. 2011;6:e22294.

115. Danskernes Sundhed - Den Nationale Sundhedsprofil 2017 [Internet]. [cited 2021 Sep 3]. Available from: https://www.sst.dk/da/udgivelser/2018/danskernes-sundhed-den-nationale-sundhedsprofil-2017

116. Danskernes sundhed [Internet]. [cited 2020 Sep 21]. Available from: http://www.danskernessundhed.dk/

117. National monitorering af den officielle anbefaling om fysisk aktivitet. - Et metodestudie - PDF Gratis download [Internet]. [cited 2021 Apr 9]. Available from: https://docplayer.dk/7821925-National-monitorering-af-den-officielle-anbefaling-om-fysisk-aktivitet-et-metodestudie.html

118. Voksne under 65 år [Internet]. [cited 2021 Sep 3]. Available from: https://www.sst.dk/da/viden/fysisk-aktivitet/anbefalinger-om-fysisk-aktivitet/voksne-under-65-aar

119. +65-årige [Internet]. [cited 2021 Sep 3]. Available from: https://www.sst.dk/da/viden/fysisk-aktivitet/anbefalinger-om-fysisk-aktivitet/_65-aarige

120. Voet N, Bleijenberg G, Hendriks J, de Groot I, Padberg G, van Engelen B, et al. Both aerobic exercise and cognitive-behavioral therapy reduce chronic fatigue in FSHD: an RCT. Neurology. 2014;83:1914–22.

121. Home-based exercise in autoimmune myasthenia gravis: a randomized controlled trial - ScienceDirect [Internet]. [cited 2021 Jun 17]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0960896621001279

122. Matcham F, Rayner L, Steer S, Hotopf M. The prevalence of depression in rheumatoid arthritis: a systematic review and meta-analysis. Rheumatology (Oxford). 2013;52:2136–48.

123. Boeschoten RE, Braamse AMJ, Beekman ATF, Cuijpers P, van Oppen P, Dekker J, et al. Prevalence of depression and anxiety in Multiple Sclerosis: A systematic review and meta-analysis. J Neurol Sci. 2017;372:331–41.

124. Ellervik C, Kvetny J, Christensen KS, Vestergaard M, Bech P. Prevalence of depression, quality of life and antidepressant treatment in the Danish General Suburban Population Study. Nord J Psychiatry. 2014;68:507–12.

125. Hoffmann CP, Torregrosa G, Bardy BG. Sound stabilizes locomotor-respiratory coupling and reduces energy cost. PLoS One. 2012;7:e45206.

126. Hoffmann CP, Bardy BG. Dynamics of the locomotor-respiratory coupling at different frequencies. Exp Brain Res. 2015;233:1551–61.

127. Bohannon RW, Crouch R. Minimal clinically important difference for change in 6-minute walk test distance of adults with pathology: a systematic review. J Eval Clin Pract. 2017;23:377–81.

128. Adams SA, Matthews CE, Ebbeling CB, Moore CG, Cunningham JE, Fulton J, et al. The effect of social desirability and social approval on self-reports of physical activity. Am J Epidemiol. 2005;161:389–98.

129. Sallis JF, Saelens BE. Assessment of physical activity by self-report: status, limitations, and future directions. Res Q Exerc Sport. 2000;71:S1-14.

130. Crutzen R, Göritz AS. Does social desirability compromise self-reports of physical activity in web-based research? Int J Behav Nutr Phys Act. 2011;8:31.

Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 31 (2021) 716–725

www.elsevier.com/locate/nmd

Fatigue, physical activity and associated factors in 779 patients with myasthenia gravis

Linda Kahr Andersen[a,c,*], Mette Aadahl[b,c,d], John Vissing[a,c]

[a] Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital Rigshospitalet, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark

[b] Center for Clinical Research and Prevention, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark

[c] Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

[d] Department of Physical and Occupational Therapy, Copenhagen University Hospital, Bispebjerg and Frederiksberg, Copenhagen, Denmark

Received 18 January 2021; received in revised form 21 April 2021; accepted 19 May 2021

Abstract

The objective of the study was to examine the association between fatigue (measured by the Multidimensional Fatigue Inventory; MFI-20) and physical activity (measured by the Saltin-Grimby Physical Activity Level Scale; SGPALS) in a large cohort of patients (≥18 years) with myasthenia gravis (MG) including relevant disease - and lifestyle-related factors. A total of 1463 persons, registered at the Danish National Registry of Patients with a MG diagnosis, according to the International Classification of Diseases, received a web-based survey. A total of 779 patients (53% women, mean [SD] age 60.8 [15.5]) responded. The remaining persons were either non-responders ($n=390$) or could not confirm the MG diagnosis ($n=294$). The most prominent MFI-20 fatigue domains were general fatigue (median [inter-quartile ranges, IQR], 13 [10–16]) and physical fatigue (median [IQR], 13 [9–15]), and 386 (53%) patients reported low levels of physical activity. All fatigue domains were associated with physical activity ($p<.01$). Higher level of physical activity was associated with lower levels of fatigue. Important factors for the association were myasthenia gravis disease severity (measured by the Myasthenia Gravis Activities of Daily Living profile), body mass index, insomnia (measured by the Insomnia Severity Index) job-status, comorbidity, and cohabitation.

Keywords: Myasthenia gravis; Fatigue; Physical activity level; MG disease severity; Sleep; Web-based survey.

1. Introduction

Myasthenia gravis (MG) is a chronic, autoimmune disease with fluctuating strength of voluntary muscles and distinct muscular fatigue. However, many patients with MG in our clinic also complain about another type of fatigue; a severe, overwhelming and constant fatigue that does not disappear at rest and is perceived different from the well-known and well-described muscular fatigue.

No standard definition of fatigue exists, although many suggestions have been proposed. In one attempt of defining fatigue in neurological diseases, fatigue was categorized into peripheral and central fatigue [1]. Peripheral fatigue was defined as muscle fatiguability as found in patients with MG. Central fatigue was defined as a subjective sense of fatigue including both cognitive and mental fatigue [1]. When using this definition of central fatigue, previous studies in MG have reported a prevalence of patient-reported central fatigue between 42 and 82% [2]. Central fatigue has been related to MG severity, autonomic disturbances, depressive state, female sex, and sleep disturbances [3–7], and has a substantial impact on activities of daily living and quality of life [3,4]. Central fatigue seems to be improved by some novel therapies [8].

In MG research, a variety of patient-reported questionnaires, both generic and MG specific, are used to measure fatigue [2]. However, most of these questionnaires

* Corresponding author at: Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital Rigshospitalet, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark.
E-mail address: linda.kahr.andersen@regionh.dk (L.K. Andersen).

https://doi.org/10.1016/j.nmd.2021.05.007

only cover one or two fatigue domains, e.g., cognitive, mental and/or physical fatigue, summarize one total score across fatigue domains, include items not relevant for MG patients, or are to comprehensive to include in a survey. Using a multidimensional assessment tool for future research on fatigue in MG has been recommended in a recent systematic review of fatigue in MG [2]. The Multidimensional Fatigue inventory (MFI-20) [9] is such an assessment tool, which is easy to use and includes five fatigue domains; physical fatigue, general fatigue, reduced activity, reduced motivation and mental fatigue. This tool and these fatigue terms are used in the present study, where the domain, physical fatigue, is considered to correspond to the definition of peripheral fatigue, whereas the remaining domains represent different aspects of the central fatigue.

Recent trials [10,11], as well as older case-studies of patients with MG participating in endurance [12–14] or resistance training [15,16], suggest that exercise training is beneficial, especially by increasing muscle strength without apparent side effects. However, only one small study [17] has examined the level of habitual physical activity in patients with MG. Habitual physical activity refers to the physical activity (PA) that is integrated in peoples' everyday life in their natural environment, and thus covers the activity level of a whole day instead of dedicated exercise sessions. Habitual physical activity is a relevant and important outcome measure in research, as lifelong habitual PA at recommended intensities is important to obtain the well-known health benefits of being physically active. No previous studies have examined the influence of fatigue on habitual physical activity levels in MG, and a better understanding of the potential association between different types of fatigue and PA in these patients is needed.

The objective of the study was to describe fatigue and PA levels in a national cohort of patients with MG, and to examine the association between different fatigue domains and PA levels, including relevant disease - and lifestyle-related factors.

2. Materials and methods

All Danish citizens are assigned a unique 10-digit civil registration number at birth or upon immigration. This has been used since 1977 to store data on all hospital in- and outpatient discharges in the Danish National Registry of Patients (DNRP). Most Danes use e-Boks, a personal, digital mailbox, connected to the civil registration number, to communicate sensitive information e.g. with health authorities.

In this study, we obtained permission to extract contact information from the Danish National Registry of Patients on persons registered with a diagnostic code of MG. The persons were ≥18 years, residents of Denmark, subscribed to e-Boks, and were coded according to International Classification of Diseases eight edition (ICD-8: 733.09, 1971- 1993) or tenth edition (ICD-10; G.70.0, from 1994) in the period from 1977 to end of 2018.

Persons, who met these criteria, were invited to participate in a web-based survey, using the software REDCap (© 2018 Vanderbilt University). All responses to the survey were automatically stored in a secure database. The invitation was sent out three times; first, in June 2019 and to non-responders in July 2019 and November 2019.

The study was approved by the ethics committee of the Capital Region of Denmark (approval H-18,031,231), registered at the Danish Data Protection Agency (VD-2018–440, I-Suite nr.6694) and approved by the Danish Health Data Authority. Written informed consent was obtained from all participants.

2.1. Questionnaires

2.1.1. Fatigue

The MFI-20 [9] is a generic, self-reported questionnaire that measures fatigue severity. The development of the questionnaire was based on comprehensive patient interviews, literature studies and theoretical considerations [18]. In the original validation study, Cronbach's alpha was reported to be 0.84 [9]. The MFI-20 has been validated and/or used in several clinical and healthy populations since 1995, including patients with neurological diseases, e.g. multiple sclerosis, stroke, polio and spinal muscular atrophy [19–25]. The MFI-20 was in 2000, in a study of fatigue in the Danish background population, validated and translated into Danish [26,27]. MFI-20 contains 20 items and categorises fatigue into five domains: general fatigue (e.g., "I feel tired"), physical fatigue (e.g., "Physically, I feel I am in bad condition"), reduced activity (e.g., "I get little done"), reduced motivation (e.g., "I don't feel like doing anything") and mental fatigue (e.g., "It takes a lot effort to concentrate on things"). The response options consist of five check boxes ranging from "Yes, that is true" to "No, that is not true". The total score in each domain ranges from 4 to 20, with higher scores indicating higher levels of fatigue. A total score across fatigue domains is not recommended.

2.1.2. Physical activity level

The Saltin–Grimby Physical Activity Level Scale (SGPALS), which was developed in 1968 [28], has been modified in various ways [29,30]. As the SGPALS is an easy-to-use measurement tool for self-reported PA, it has been used in several large-scale population-based studies, also in Denmark [31–35]. SGPALS has been used in patients with stroke [36–39], but never in patients with MG. High reliability and validity of SGPALS has been demonstrated [40]. In the present study, we measured PA levels based on the original questionnaire, but with a slightly modified wording both in the following question and in the four response categories: "During the last year, how physically active have you been, including leisure time activities and

transportation? Please categorize yourself into one of four levels of physical activity":

I (Sedentary). Mainly sedentary or engaged in light physical activity less than 2 h per week (e.g., reading books, watching television, or going to the cinema).
II (Light to moderate). Light-to-moderate physical activity 2–4 h per week (e.g., walking, cycling for pleasure, gardening, housework, light exercise).
III (Regular moderate). Moderate physical activity more than 4 h per week, or more strenuous activities 2–4 h per week (e.g. brisk walking, fast bicycling, heavy gardening, or exercises that makes you short of breath).
IV (Regular vigorous). More strenuous physical activities more than 4 h per week, or regular vigorous exercise (e.g. competitive sport) several times a week.

2.1.3. MG severity

The Myasthenia Gravis Activities of Daily Living profile (MG-ADL) is an 8-item patient-reported questionnaire where a higher score indicates higher MG severity (total score range 0–24) [41]. The scale assesses common MG-symptoms and dysfunctions, including questions of ocular, bulbar, respiratory, and extremity functions. Patients were asked about symptoms endured in the past seven days. The MG-ADL is found valid as an outcome measure in research and clinical practice [42].

2.1.4. Quality of life

The MG-specific quality-of-life instrument (MG-QoL15) is a 15-item questionnaire assessing quality of life in patients with MG. Rating consists of a 5-point scale ranging from 0 to 4 indicating the patient's agreement with a given statement; summing up to a total score of 0–60 points (60=low quality of life). The MG-QoL15 was developed in 2008 [43], and has been found valid and reliable [8,44,45] in patients with MG. The MG-QoL15 has been revised (MG-QoL15r) [46] in 2016, but for comparison of results from older studies, the original MG-QoL15 is used in present study.

2.1.5. Insomnia

The Insomnia Severity Index (ISI) (Mapi Research Trust, Lyon, France) measures self-reported sleep quality and disturbances during the previous 2 weeks. The ISI comprises seven items assessing the perceived severity of insomnia, each item rated on a five-point scale (total score 0–28, most severe insomnia=28). A total score $\geq$ 8 indicate clinical insomnia. ISI was developed in 1993 [47], used in several clinical and healthy populations, including patients with MG [3]. The reliability and validity of ISI has been tested and found to be good [48].

2.1.6. Background information

Patients reported age, sex, height + weight (used to calculate body mass index, BMI), MG duration, current job status, frequency of follow-up at neurologist, thymectomy and family status (cohabitant with person(s) $\geq$ 18 years). In addition, there were questions (with yes/no answers) about comorbidities relevant for fatigue and physical activity, and questions about the most common medical treatment for MG and comorbidity.

2.2. Statistical analyses

Continuous variables were presented by means and standard deviation (SD). Non-parametric continuous variables were presented by medians and inter-quartile ranges (IQR). Normality was assessed visually by histograms and boxplots. Categoric variables were presented by numbers and percentages.

For a descriptive purpose, the SGPALS was categorized into two groups; low PA (levels I and II) and regular PA (levels III and IV). Differences in characteristics between low and regular levels of PA were investigated by unpaired *t*-test for continuous data, by Mann-Whitney test for non-normal continuous data, and by Fisher's exact test for categoric data, and presented in Table 2. We did not present such a descriptive table for fatigue, as no cut-off points for low/high fatigue exist for MFI-20. However, differences were reported in text, based on the sample median score in each fatigue domain.

To examine the association between fatigue and PA, a general linear regression model was applied, using MFI-20 scores in each fatigue domain as continuous outcome variables. Covariates, included in the statistical models, were selected a priori, and determined from known evidence, or experiences from the clinic. Age, BMI, MG duration, MG-ADL, MFI-20 and ISI scores were included as continuous variables. Sex, job-status, cohabitation, medication other than MG treatment, and comorbidity were included as categoric variables.

Collinearity among covariates were examined by correlation analyses included in the models. To prevent collinearity, MG-QoL15 was not included in the statistical models as collinearity was expected between MG-QoL15 and MG-ADL because many of the items in these scales are alike. The regression models were executed as complete-case analysis and checked by 1) goodness of fit test, 2) test for linearity of covariates by adding log transformed covariates into the model, 3) test of accumulated residuals by plots and p-values. Convergence criterion was satisfied in all analyses.

Some responders confirmed their MG diagnosis but were not regularly followed by a neurologist and/or pharmacologically treated for MG. Therefore, to avoid misleading conclusions due to including potential non-MG persons, all analyses were additionally executed on a sub-sample of patients ($n=486$). These patients were regularly followed by a neurologist and were on active MG treatment.

For analyses, a $p \leq 0.05$ (2-tailed testing) was considered significant. All statistical analyses were carried out using SAS enterprise guide 7.1.

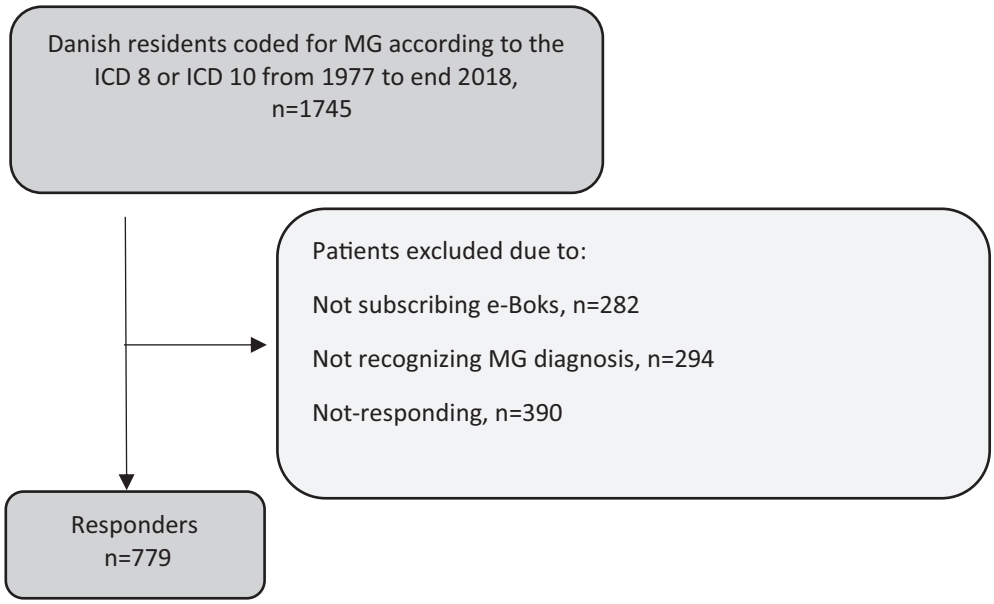


Fig. 1. Flowchart of included and excluded patients.

3. Results

3.1. Study population

A total of 1745 persons were registered at the DNPR with a diagnostic code of MG. However, 282 persons (mean age [SD], 78 [12.6] years, women 164 [58%]), did not subscribe to e-Boks, and 390 persons (mean age [SD], 55 [16.5] years, women 226 [58%]) never responded to the survey and were excluded. Another 294 persons phoned or emailed the study coordinator (LKA) because; 1) they were mistakenly registered at the DNPR with a MG diagnosis, but suffered from another neuromuscular disease, or 2) they haven't had any MG symptoms for years and were uncertain of the MG diagnosis. These 294 patients were excluded. This left 779 participants who completed the survey either completely ($n=662$) or partially ($n=117$). Of these 779 patients, 499 (64%) responded the first time the invitation was sent out (June 2019), 193 (25%) the second time (July 2019) and 87 (11%) the last time (November 2019).

The response rate was calculated according to recommended standards from the American Association for Public Opinion Research [49]. Response rate = (662+117/(662+117) +e (282+390)), where e is the estimated proportion of persons of unknown MG status that were expected to have a MG diagnosis. As the Danish MG population is estimated to be 1000 patients [50], and as 779 patients already responded, we expected that 221 (approximately one third) of the 672 with unknown eligibility ($e=0.33$) likely could have a diagnosis of MG. Therefore, the response rate of the survey was set at 78%. Enrolment pathway is illustrated in Fig. 1.

The demographic characteristics of the patient samples are presented in Table 1. The distribution of characteristics in the sub-sample ($n=486$) was very similar to the overall sample.

3.2. Fatigue

The MFI-20 domains general fatigue (median 13, IQR 10–16), physical fatigue (median 13, IQR 9–15) and reduced activity (median 12, IQR 9–15) were the most pronounced fatigue domains in this cohort (Table 1). Reduced motivation and mental fatigue were less pronounced with a sample median score of 9. This distribution was similar in the sub-sample ($n=486$), and in patients not regularly followed by a neurologist and not on MG treatment ($n=144$). The distribution pattern was similar in patients reporting comorbidity/no comorbidity, even though comorbid patients had higher scores in all fatigue domains vs. patients reporting no comorbidity ($p<.01$). Also, patients with another autoimmune disease besides MG and patients treated with steroids had higher fatigues scores than the remaining MG patients ($p<.01$).

Regarding general fatigue, patients with a median score ≥ 13 ($n=358$, 50%) compared to patients with a score <13 ($n=356$, 50%), were more often women (55 vs 45%, $p<.01$), more often retired due to illness (70 vs 30%, $p<.01$), more often reporting comorbidities (55 vs 45%, $p<.01$), more often lived alone (62 vs 39%, $p<.01$), had lower MG-related QoL (MG-QoL15, median 17 vs. 3, $p<.01$), had more problems sleeping (ISI, median 12 vs. 5, $p<.01$), were more often on medical treatment for diseases other than MG (54% vs 46%, $p=.01$), and reported higher level of disease severity (MG-ADL, median 4 vs. 1, $p<.01$). These patterns were similar for the remaining MFI-20 fatigue domains, also in the sub-sample.

3.3. Physical activity

The distribution of SGPALS levels (Table 1), resulted in 386 (53%) patients categorized in the group with low PA

Table 1
Characteristics of the study samples.

	Overall sample ($n=779$)	Sub-sample ($n=486$)
Age, years	60.8 (15.5)	62.5 (14.9)
Sex		
Female	413 (53%)	245 (50%)
Male	365 (47%)	241 (50%)
BMI, kg/m^2	27.2 (5.7)	27.6 (6.1)
MG duration, years	13.6 (12.4)	11.6 (11.0)
Job status		
Retired due to age	328 (42%)	229 (47%)
Working full- or part-time	300 (39%)	173 (36%)
Retired due to sickness	82 (11%)	46 (9%)
Out of work [a]	50 (6%)	28 (6%)
Student	17 (2%)	9 (2%)
Regularly follow-up by neurologist		
Yes	528 (68%)	486 (100%)
No	250 (32%)	0
Thymectomy		
Yes	235 (30%)	130 (27%)
No	542 (70%)	355 (73%)
MG Treatment [b]		
Pyridostigmine	448 (56%)	359 (74%)
Azathioprine	237 (30%)	224 (46%)
Glucocorticoids	92 (12%)	87 (18%)
Atropine	86 (11%)	74 (15%)
Mycophenolic acid	44 (6%)	37 (8%)
Methotrexate	34 (4%)	30 (6%)
Immunosuppressive drugs, other	74 (9%)	62 (13%)
Immunoglobulin	19 (2%)	17 (4%)
Plasmapheresis	8 (1%)	3 (1%)
No treatment	187 (24%)	0
Comorbidity [b]		
No comorbidity	234 (30%)	129 (27%)
Hypertension/high cholesterol	238 (31%)	162 (33%)
Pain in bone/joint	186 (24%)	119 (25%)
Conditions affecting PA level, other	123 (16%)	77 (16%)
Autoimmune disease, other	75 (10%)	40 (8%)
Diabetes, type 2	64 (8%)	48 (10%)
Chronic pain, other	61 (8%)	38 (8%)
Depression/anxiety	58 (8%)	38 (8%)
Chronic fatigue syndrome	44 (6%)	27 (6%)
Chronic obstructive lung disease	34 (4%)	22 (5%)
Severe cardiovascular disorder	26 (3%)	18 (4%)
SGPALS		
I: Sedentary	81 (11%)	52 (11%)
II: Light to moderate	305 (42%)	178 (39%)
III: Regular moderate	290 (40%)	201 (44%)
IV: Regular vigorous	55 (7%)	28 (6%)
MFI-20, total		
General fatigue	13 (10–16)	13 (10–15)
Physical fatigue	13 (9–15)	13 (10–15)
Reduced activity	12 (9–15)	12 (9–15)
Reduced motivation	9 (6–11)	9 (7–12)
Mental fatigue	9 (6–12)	10 (6–12)
MG-ADL, total	3 (1–5)	3 (1–5)
MG-QoL15, total	9 (2–19)	11 (4–21)
ISI, total	8 (4–13)	8 (4–13)

Legend Table 1: Age, BMI (Body mass index), MG duration: mean (standard deviation, SD). MFI-20 (Multidimensional Fatigue Inventory), MG-ADL (MG Activities of Daily Living profile), MG-QoL15 (MG-specific quality-of-life instrument), ISI (Insomnia Severity Index): median (Inter-quartile ranges, IQR). The SGPALS (Saltin-Grimby Physical Activity Level Scale) and the remaining variables are presented by numbers (n) and percentages (%).

[a] Out of work= unemployed, sick-leave, non-classified.

[b] Multiple answers so percentages do not add to 100%. As some responders completed the questionnaires partially there was missing data in the overall sample: age, sex, follow up at neurologist ($n=1$), Job-status, thymectomy ($n=2$), BMI ($n=6$), MG-duration ($n=7$), SGPALS ($n=48$), MFI-20 ($n=65$), MG-ADL ($n=72$), MG-QoL15 ($n=73$), ISI ($n=68$). Missing data in the sub-sample: Job-status ($n=1$), thymectomy ($n=1$), BMI ($n=4$), MG-duration ($n=2$), SGPALS ($n=27$), MFI-20 ($n=36$), MG-ADL ($n=39$), MG-QoL15 ($n=40$), ISI ($n=37$).

Table 2
Characteristics distributed between the physical activity levels; Low and Regular.

	Physical activity (PA) levels		
	Low level ($n=386$)	Regular level ($n=345$)	P-value
Age, years	62.3 (15.4)	58.5 (15.4)	<0.01
Sex			0.77
Female	201 (52%)	184 (53%)	
Male	185 (48%)	161 (47%)	
BMI, kg/m^2	27.9 (6.4)	26.4 (4.9)	<0.01
MG duration, years	14 (12.2)	14 (12.2)	0.97
Job status			<0.01
Retired due to age	182 (47%)	125 (36%)	
Working full- or part-time	129 (34%)	160 (46%)	
Retired due to sickness	44 (11%)	27 (8%)	
Out of work [a]	23 (6%)	24 (7%)	
Student	8 (2%)	9 (3%)	
Cohabitant [b]			0.86
No	83 (22%)	72 (21%)	
Yes	303 (78%)	273 (79%)	
Comorbidity [c]			<0.01
No comorbidity	121 (31%)	146 (42%)	
Comorbidity	265 (69%)	199 (58%)	
MFI-20, total			<0.01
Physical fatigue	14 (11–17)	11 (7–14)	
General fatigue	13 (11–16)	12 (8–15)	
Reduced activity	13 (11–16)	10 (8–13)	
Reduced motivation	10 (8–12)	8 (6–11)	
Mental fatigue	10 (7–12)	8 (6–12)	
MG-ADL, total	3 (1–6)	2 (1–4)	<0.01
MG-Qol15, total	12 (3–25)	7 (2–14)	<0.01
ISI, total	9 (5–14)	7 (3–12)	<0.01

Legend Table 2: Age, BMI (Body mass index), MG duration: mean (standard deviation, SD). MFI-20 (Multidimensional Fatigue Inventory), MG-ADL (MG Activities of Daily Living profile), MG-QoL15 (MG-specific quality-of-life instrument), ISI (Insomnia Severity Index): median (Inter-quartile ranges, IQR). The remaining variables are presented by numbers (n) and percentages (%).

[a] Out of work= unemployed, sick-leave, non-classified.

[b] with person(s)≥18 years.

[c] Comorbidity specified in Table 1. $N=731$ patients completed the SGPALS questionnaire. There was missing data on BMI ($n=1$), MG duration ($n=2$), MFI-20 ($n=17$), MG-ADL ($n=24$), MG-QoL15 ($n=25$), ISI ($n=20$).

(levels *I*+II) and 345 (47%) categorized in the group with regular PA (levels III+IV). An almost similar distribution was seen for the sub-sample (low PA: 50%, regular PA: 50%). Compared to patients in the group with regular PA (Table 2), patients in the group with low PA were slightly older (mean age 62 vs 59, $p<.01$), had a higher mean BMI (27.9 vs 26.4, $p<.01$), more often reported comorbidity (69 vs 58%, $p<.01$), more MG symptoms (MG-ADL, median 3 vs 2, $p<.01$), lower QoL (MG-QoL15, 12 vs 7, $p<.01$), more sleep problems (ISI, 9 vs 7, $p<.01$), and reported higher levels of fatigue in all domains ($p<.01$). These patterns were also present in the sub-sample.

3.4. Fatigue and physical activity

The association of fatigue and PA in the overall sample and the sub-sample are presented in Tables 3 and 4, respectively. There was a strong significant association between the MFI-20 fatigue domains and SGPALS, indicating that higher level of PA was associated with lower levels of fatigue, in both the overall sample and in the sub-sample, except for mental fatigue in the sub-sample. Statistically significant covariates influencing on this association were primarily MG severity (MG-ADL) and insomnia (ISI). Also, BMI, comorbidity (e.g., depression/anxiety), and job-status influenced on the association in the overall sample, but not in the sub-sample, while cohabitation was a statistically significant covariate in the sub-sample.

4. Discussion

In this survey of 779 patients with MG, self-reported high levels of fatigue and low levels of PA were frequent, and strongly associated. Important covariates in this association were MG disease severity, sleep quality, BMI, comorbidity, job-status, and cohabitation.

The level of general fatigue and physical fatigue were similar in this cohort, and these fatigue domains were the two most pronounced domains in the sample. This was also seen in the Danish background population [27]. Also, the MFI-20 domain for reduced activity was pronounced in this sample, whereas the median sample score of the domains reduced motivation and mental fatigue were lower. In one study of 4964 Danish adults in late mid-life (age 49–63) [51], the median sample score was 9 for general fatigue and physical fatigue, and 7 for the remaining domains, indicating an increased level of fatigue in patients with MG compared to a healthy population. An increased level of fatigue in MG compared to healthy controls has previously been reported [4,5]. However, patients reporting full remission (MG-ADL score=0) ($n=142$) had fatigue scores very similar to the non-MG background population [51]. The fatigue scores in the present cohort were slightly lower than found in patients with Parkinson disease [52], chronic fatigue syndrome [53], multiple sclerosis [24] and almost similar to fatigue scores in post-stroke patients [20] and patients with other autoimmune diseases [54]. One previous study has published results of MFI measurements in patients with neuromuscular diseases, finding a fatigue level in adult patients with spinal muscular atrophy lower (except for physical fatigue) than found in patients with MG [25]. Due to a large variation in measurement tools of fatigue across studies, comparison of results in previous MG studies are difficult. However, this study was the first to measure the level of fatigue in five different fatigue domains.

The grouping of SGPALS scores into low- and regular PA, as performed in other patient studies [54], gave us the opportunity to differentiate between patients meeting/not meeting the existing recommendations from WHO [55]. We assumed that patients who reported SGPALS scores III and IV met the WHO recommendations (minimum 150 min per week of moderate intensity), corresponding to 47% of the cohort. The 53% of the patients, not meeting the recommendations, corresponded to findings in patients

Table 3
Association of fatigue and physical activity level in the overall sample.

	General fatigue β (CI)	Physical fatigue β (CI)	Reduced activity β (CI)	Reduced motivation β (CI)	Mental fatigue β (CI)
	Univariate				
SGPALS	**−1.29 (−1.67–0.90)**	**−2.36 (−2.72–2.00)**	**−2.27 (−2.62–1.92)**	**−1.19 (−1.50—0.88)**	**−0.90 (−1.27—0.54)**
Age	−0.01 (−0.03–0.01)	0.01 (0.00–0.04)	**0.02 (0.00–0.04)**	**0.02 (0.00–0.04)**	−0.01 (−0.03–0.01)
Sex [a]	**0.96 (0.35–1.57)**	**0.63 (0.0–1.26)**	0.21 (−0.39–0.82)	−0.06 (−0.56–0.44)	0.70 (0.12–1.27)
BMI, kg/m^2	**0.06 (0.00–0.11)**	**0.15 (0.10–0.20)**	**0.09 (0.04–0.14)**	**0.06 (0.02–0.11)**	**0.00 (−0.05–0.05)**
Job-status [b]	**0.96 (0.34–1.58)**	**1.45 (0.82–2.08)**	**1.75 (1.15–2.36)**	**1.24 (0.74–1.74)**	**1.20 (0.61–1.78)**
Cohabitant [c]	**1.58 (0.83–2.33)**	**1.65 (0.89–2.41)**	**1.56 (0.82–2.30)**	**1.12 (0.52–1.73)**	**1.46 (0.75–2.16)**
MG duration	**−0.03 (−0.06–0.01)**	−0.01 (−0.04–0.01)	−0.02 (−0.04–0.01)	**−0.03 (−0.05—0.01)**	**−0.03 (−0.05—0.01)**
MG-ADL	**0.73 (0.63–0.83)**	**0.76 (0.66–0.86)**	**0.69 (0.60–0.79)**	**0.42 (0.33–0.50)**	**0.62 (0.53–0.72)**
Depression/anxiety [d]	**2.66 (1.49–3.82)**	**3.33 (2.16–4.51)**	**3.13 (1.99–4.28)**	**2.39 (1.44–3.33)**	**2.74 (1.65–3.84)**
Comorbidity [e]	**1.42 (0.80–2.04)**	**2.30 (1.68–2.92)**	**1.67 (1.06–2.28)**	**1.08 (0.57–1.59)**	**0.86 (0.26–1.45)**
Medication, others [f]	**1.00 (0.38–1.63)**	**2.12 (1.49–2.74)**	**1.70 (1.09–2.31)**	**0.95 (0.45–1.46)**	**0.71 (0.12–1.30)**
ISI	**0.38 (0.33–0.42)**	**0.31 (0.26–0.36)**	**0.27 (0.22–0.32)**	**0.18 (0.14–0.22)**	**0.33 (0.29–0.38)**
	Multivariable				
SGPALS	**−0.67 (−0.99–0.34)**	**−1.63 (−0.95–1.30)**	**−1.63 (−1.96–1.31)**	**−0.74 (−1.04—0.44)**	**−0.33 (−0.65—0.01)**
Age	−0.01 (−0.03–0.01)	−0.00 (−0.01–0.01)	0.01 (−0.00–0.02)	0.01 (−0.01–0.03)	−0.01 (−0.03–0.01)
Sex [a]	0.20 (−0.33–0.73)	0.30 (−0.04–0.60)	−0.28 (−0.60–0.03)	−0.20 (−0.70–0.29)	0.08 (−0.44–0.60)
BMI, kg/m^2	−0.01 (−0.05–0.04)	**0.06 (0.33–0.53)**	0.01 (−0.04–0.05)	0.02 (−0.03–0.06)	**−0.05 (−0.09—0.01)**
Job-status [b]	0.29 (−0.30–0.87)	−0.08 (−0.41–0.25)	**0.64 (0.06–1.22)**	0.50 (−0.05–1.04)	**0.73 (0.16–1.31)**
Cohabitant [c]	0.35 (−0.26–0.96)	0.14 (−0.25–0.54)	0.01 (−0.33–0.45)	0.50 (−0.07–1.07)	0.40 (−0.20–1.00)
MG duration	−0.02 (−0.04–0.00)	0.01 (−0.00–0.02)	−0.00 (−0.02–0.01)	−0.02 (−0–04–0.00)	−0.02 (−0.04–0.00)
MG-ADL	**0.43 (0.33–0.53)**	**0.43 (0.33–0.53)**	**0.43 (0.32–0.53)**	**0.22 (0.13–0.32)**	**0.37 (0.28–0.47)**
Depression/anxiety [d]	0.13 (−0.86–1.13)	0.35 (−0.29–0.98)	−0.16 (−0.78–0.47)	**0.96 (0.03–1.89)**	0.57 (−0.41–1.55)
Comorbidity [e]	0.48 (−0.14–1.09)	**0.81 (0.20–1.41)**	−0.39 (−0.77–0.00)	0.24 (−0.33–0.81)	−0.12 (−0.72–0.49)
Medication, others [f]	−0.13 (−0.75–0.50)	0.39 (−0.23–1.00)	0.37 (−0.04–0.78)	−0.13 (−0.71–0.45)	0.01 (−0.60–0.63)
ISI	**0.27 (0.23–0.32)**	**0.17 (0.13–0.22)**	**0.14 (0.10–0.19)**	**0.11 (0.06–0.15)**	**0.24 (0.20–0.29)**

Legend Table 3: Estimate (β), CI (confidence intervals), in bold: $p<.05$.
[a] female vs male.
[b] no work vs work.
[c] cohabitant (living alone vs living with person(s) ≥18 years).
[d] yes vs no.
[e] yes/no, diseases listed in Table 1.
[f] yes/no, medication other than MG medication e.g., benzodiazepines. Abbreviations: BMI (Body mass index), MG-ADL (MG Activities of Daily Living profile), ISI (Insomnia Severity Index), SGPALS (Saltin-Grimby Physical Activity Level Scale), MFI-20 (Multidimensional Fatigue Inventory). The analyses were made as complete-case analysis ($n=690$).

Table 4
Association of fatigue and physical activity level in the sub-sample.

	General fatigue β (CI)	Physical fatigue β (CI)	Reduced activity β (CI)	Reduced motivation β (CI)	Mental Fatigue β (CI)
	Multivariable				
SGPALS	**−0.65 (−1.07–0.22)**	**−1.68 (−2.09–1.28)**	**−1.58 (−2.00–1.16)**	**−0.92 (−1.32—0.52)**	−0.29 (−0.71–0.14)
Age	−0.01 (−0.04–0.02)	0.00 (−0.03–0.02)	−0.00 (−0.03–0.02)	−0.00 (−0.03–0.02)	−0.02 (−0.05–0.00)
Sex[a]	0.47 (−0.21–1.15)	0.18 (−0.47–0.83)	−0.41 (−1.08–0.26)	−0.28 (−0.92–0.37)	−0.20 (−0.88–0.48)
BMI, kg/m^2	−0.01 (−0.06–0.04)	**0.07 (0.02–0.12)**	0.00 (−0.05–0.05)	0.02 (−0.03–0.07)	−0.04 (−0.10–0.01)
Job-status[b]	0.14 (−0.63–0.91)	−0.11 (−0.85–0.62)	0.12 (−0.63–0.87)	0.23 (−0.49–0.96)	0.60 (−0.17–1.37)
Cohabitant[c]	0.49 (−0.26–1.25)	**0.85 (0.13–0.158)**	**0.78 (0.04–1.53)**	**0.82 (0.10–1.54)**	0.59 (−0.16–1.34)
MG duration	−0.02 (−0.05–0.01)	0.02 (−0.01–0.05)	0.00 (−0.02–0.02)	−0.02 (−0.05–0.00)	−0.02 (−0.05–0.00)
MG-ADL	**0.44 (0.31–0.57)**	**0.45 (0.32–0.57)**	**0.46 (0.33–0.59)**	**0.19 (0.07–0.32)**	**0.39 (0.26–0.52)**
Depression/anxiety[d]	0.51 (−0.73–1.75)	1.13 (−0.06–2.31)	1.09 (−0.13–2.30)	**1.57 (0.40–2.74)**	0.96 (−0.27–2.20)
Comorbidity[e],	−0.05 (−0.82–0.72)	0.61 (−0.12–1.35)	−0.26 (−1.01–0.50)	0.31 (−0.42–1.03)	−0.21 (−0.98–0.55)
Medication, others[f]	0.36 (−0.43–1.15)	0.09 (−0.67–0.84)	0.49 (−0.29–1.27)	−0.15 (−0.90–0.60)	0.35 (−0.44–1.14)
ISI	**0.24 (0.18–0.29)**	**0.17 (0.12–0.23)**	**0.16 (0.10–0.21)**	**0.12 (0.07–0.12)**	**0.25 (0.19–0.30)**

Legend Table 4: The sub-sample of patients were all on MG treatment and in regularly contact with a neurologist. Estimate (β), CI (confidence intervals), in bold: $p<.05$.
[a] female vs male.
[b] no work vs work.
[c] cohabitant (living alone vs living with person(s) ≥ 18 years).
[d] yes vs no.
[e] yes/no, diseases listed in Table 1.
[f] yes/no, medication other than MG medication e.g., benzodiazepines. Abbreviations: BMI (Body mass index), MG-ADL (MG Activities of Daily Living profile), ISI (Insomnia Severity Index), SGPALS (Saltin-Grimby Physical Activity Level Scale), MFI-20 (Multidimensional Fatigue Inventory). The analyses were made as complete-case analysis ($n=437$).

with myotonic dystrophy type 1 [56], whereas patients with MG seemed more physically active than patients with mitochondrial disease [57]. Comparing our findings of PA levels with findings in the Danish general population did not lead to any firm conclusions. The results from the large-scale Danish population studies were heterogeneous, and depending on which study we compared to, patients with MG were either more active or less active than the general Danish population [58–60]. The question used in these large-scale Danish population studies are found to be strongly dependent on the context of the examination and the mode of the administration of the questionnaire [61]. However, this hampers the possibility to compare the PA levels in MG with healthy counterparts, which is a limitation.

In general, it is noteworthy that assessment of PA level in both clinical and healthy cohorts depends on the chosen outcome measurement. This was the case in a study of PA patterns in 27 patients with MG [17], where 78% of the patients achieved the recommended minimum average of 64 MET min/day. However, when the study examined other outcome measures, e.g. steps per day, the patients' day was dominated by sedentary behaviour. Comparisons with other study results and recommendations are useful to obtain an overall interpretation of the activity level in the cohort, but larger studies including objective measurements is needed to fully examine the pattern of habitual PA in patients with MG.

Engaging in more PA was associated with less fatigue in both the overall sample and in the sub-sample, especially in the fatigue domains; physical fatigue and reduced activity. This finding could indicate that active patients experienced lower physical fatigue, or less fatigued patients engaged in more PA. More research is needed to determine the direction of the association, which cannot be identified by a cross-sectional study like the present.

Several covariates significantly influenced on the association between fatigue and PA. As reported previously [3–6,62,63], an increase in MG-severity was associated with an increased level of fatigue. This was seen in all fatigue domains, in both the overall and the sub-sample. Poor sleep quality (ISI score) strongly influenced on increasing fatigue, which have been reported previously [4,7]. In the present study, 55% of the patients reported insomnia (ISI score $\geq$8), which agrees with the finding of 59% in another MG cohort [7]. As insomnia is also associated with MG-related quality of life [7], the sleep problems in MG call for action, and must be taken into consideration when meeting these patients in the clinic. Also, higher BMI was associated with increased physical fatigue. This is interesting as a large proportion of patients (60%) of the cohort reported a BMI $\geq$ 25 (classified as overweight), which was also found in a previous study [62]. Reasons for obesity can be e.g., inactivity (caused by fatigue) or steroid use. However, the direction of the association cannot be assessed in a cross-sectional study, and prospective studies are needed to unravel this association.

Comorbidity was associated with increased physical fatigue in this cohort. These findings make sense as several diseases are known to induce fatigue in patients. Depression is previous found to be associated with fatigue perception in patients with MG [4,64]. However, in the present study, depression/anxiety only influenced on the fatigue domain reduced motivation, whereas depression was not associated with e.g., physical fatigue or mental fatigue. However, it is important to emphasize that the information on comorbidity (e.g. depression) might be prone to bias as no verification of comorbidity was possible in this study design. However, as the overall rate for depression among patients with MG has previously been found to be around 20%[3], the reported 8% in our study might be an underestimation, as well as the association with fatigue.

Female gender has previously been associated with fatigue in MG [4,6,65], which was not found in the present study. Living alone compared to living with another person $\geq$18 years was associated with an increase in fatigue in the sup-sample. However, this was only seen for the activity-oriented fatigue domains; physical fatigue, reduced activity and reduced motivation. For these domains, the largest reduction in fatigue was seen, when patients engaged in PA. An explanation to these findings could be that a cohabitant might motivate the patient for PA, or even participate in MG together with the patient, which may be motivational. The fact, that people living alone reported higher levels of fatigue was also seen in the Danish background population [27].

Even though potential confounders, included in the analyses, were selected a priori from the literature or from experience in our clinic, the influence of potential residual confounding cannot be ruled out. Interpretation of results should be done with caution knowing that the results indicate associations rather than causality. Self-reported outcomes are prone to bias, e.g. social desirability bias, where responders distort self-reports in a favourable direction. However, this bias has been demonstrated to be less pronounced [66], especially in an online setting where respondents' perceived privacy is higher [67].

The MFI-20 is generic, and a new instrument in MG research, even though it has been used and validated in neurological research since 1995. MFI-20 was chosen for its multidimensional approach to fatigue, which suited the objective of this study. The study confirmed our experiences from the clinic that general fatigue had a similar impact on the patient's everyday life as the MG-induced physical fatigue, which indicates that this instrument is useful to measure and separate these two different fatigue domains.

Due to the large sample of MG patients included, our study results are likely to represent the MG population of 1000 patients in Denmark [50]. The excluded persons (n=282) that did not subscribe to e-Boks had a higher mean age than the overall sample mean, indicating some selection bias towards the younger population, probably due to the web-based methods of this study. Opposite, the persons that did

not respond ($n = 390$) were younger compared to the overall sample. The total of excluded persons with unknown MG eligibility resembled the included patients regarding age and sex. However, we cannot rule out that these persons were markedly different from the included patients, regarding other characteristics. The results from the overall sample did not differ from the results in the sub-sample of patients in MG treatment, indicating no selection bias in the overall sample.

5. Conclusion

In this cross-sectional study, including 779 patients with MG, the levels of fatigue were increased compared to the general population. More than half of the cohort reported low levels of PA. Higher level of PA was associated with lower levels of fatigue, and important factors were MG severity, sleep quality, BMI, comorbidity, job-status and cohabitation. The findings suggest that PA may play an important role in managing fatigue in MG, however, the direction of the association needs further investigation. Also, important non-specific MG factors, e.g. weight and sleep quality, should be considered when planning rehabilitation programs for patients with MG.

Declaration of Competing Interest

Authors report no conflicts of interest.

References

[1] Chaudhuri A, Behan PO. Fatigue in neurological disorders. Lancet N Am Ed 2004;363:978–88.
[2] Ruiter AM, Verschuuren JJGM, Tannemaat MR. Fatigue in patients with myasthenia gravis. A systematic review of the literature. Neuromuscul Disord 2020;30:631–9.
[3] Hoffmann S, Ramm J, Grittner U, Kohler S, Siedler J, Meisel A. Fatigue in myasthenia gravis: risk factors and impact on quality of life. Brain Behav 2016;6:e00538.
[4] Alekseeva TM, Gavrilov YV, Kreis OA, Valko PO, Weber KP, Valko Y. Fatigue in patients with myasthenia gravis. J Neurol 2018;265:2312–21.
[5] Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol 2013;13:132.
[6] Tran C, Bril V, Katzberg HD, Barnett C. Fatigue is a relevant outcome in patients with myasthenia gravis. Muscle Nerve 2018;58:197–203.
[7] Martínez-Lapiscina EH, Martínez De Lapiscina EH, Erro ME, Erro Aguirre ME, Ayuso T, Ayuso Blanco T, et al. Myasthenia gravis: sleep quality, quality of life, and disease severity. Muscle Nerve 2012;46:174–80.
[8] Andersen H, Mantegazza R, Wang JJ, O'Brien F, Patra K, Howard JF, et al. Eculizumab improves fatigue in refractory generalized myasthenia gravis. Qual Life Res 2019;28:2247–54.
[9] Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995;39:315–25.
[10] Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U. Exercise in myasthenia gravis: a feasibility study of aerobic and resistance training. Muscle Nerve 2017;56:700–9.
[11] Westerberg E, Molin CJ, Lindblad I, Emtner M, Punga AR. Physical exercise in myasthenia gravis is safe and improves neuromuscular parameters and physical performance-based measures: a pilot study. Muscle Nerve 2017;56:207–14.
[12] Lucia A, Maté-Muñoz JL, Pérez M, Foster C, Gutiérrez-Rivas E, Arenas J. Double trouble (McArdle's disease and myasthenia gravis): how can exercise help? Muscle Nerve 2007;35:125–8.
[13] Scheer BV, Valero-Burgos E, Costa R. Myasthenia gravis and endurance exercise. Am J Phys Med Rehabil 2012;91:725–7.
[14] Leddy JJ, Chutkow JG. Myasthenia gravis in a collegiate football player. Med Sci Sports Exerc 2000;32:1975–9.
[15] Stout JR, Eckerson JM, May E, Coulter C, Bradley-Popovich GE. Effects of resistance exercise and creatine supplementation on myasthenia gravis: a case study. Med Sci Sports Exerc 2001;33:869–872.
[16] Lohi EL, Lindberg C, Andersen O. Physical training effects in myasthenia gravis. Arch Phys Med Rehabil 1993;74:1178–80.
[17] O'Connor L, Westerberg E, Punga AR. Pattern of Habitual Physical Exercise in Myasthenia Gravis Patients. J Neuromuscul Dis 2019;6:85–91.
[18] Smets EM, Garssen B, Schuster-Uitterhoeve AL, de Haes JC. Fatigue in cancer patients. Br J Cancer 1993;68:220–4.
[19] de Dreu MJ, Schouwenaars IT, Rutten GJM, Ramsey NF, Jansma JM. Fatigue in brain tumor patients, towards a neuronal biomarker. Neuroimage Clin 2020;28:102406.
[20] Christensen D, Johnsen SP, Watt T, Harder I, Kirkevold M, Andersen G. Dimensions of post-stroke fatigue: a two-year follow-up study. Cerebrovasc Dis 2008;26:134–41.
[21] Lexell J, Jonasson SB, Brogardh C. Psychometric properties of three fatigue rating scales in individuals with late effects of polio. Ann Rehabil Med 2018;42:702–12.
[22] Visser MM, Goodin P, Parsons MW, Lillicrap T, Spratt NJ, Levi CR, et al. Modafinil treatment modulates functional connectivity in stroke survivors with severe fatigue. Sci Rep 2019;9:9660.
[23] Rytter HM, Westenbaek K, Henriksen H, Christiansen P, Humle F. Specialized interdisciplinary rehabilitation reduces persistent post-concussive symptoms: a randomized clinical trial. Brain Inj 2019;33:266–81.
[24] Mikula P, Nagyova I, Krokavcova M, Vitkova M, Rosenberger J, Szilasiova J, et al. The mediating effect of coping on the association between fatigue and quality of life in patients with multiple sclerosis. Psychol Health Med 2015;20:653–61.
[25] Binz C, Schreiber-Katz O, Kumpe M, Ranxha G, Siegler H, Wieselmann G, et al. An observational cohort study on impact, dimensions and outcome of perceived fatigue in adult 5q-spinal muscular atrophy patients receiving nusinersen treatment. J Neurol 2021;268:950–62. [Internet][cited 2021 Apr 9]Available from: http://link.springer.com/10.1007/s00415-020-10227-5 .
[26] Grønvold M. Report of the Translation of the Multidimensional Fatigue Inventory (MFI-20) from English to Danish, Copenhagen: Rigshospitalet; 1997. May.
[27] Watt T, Groenvold M, Bjorner JB, Noerholm V, Rasmussen NA, Bech P. Fatigue in the Danish general population. Influence of sociodemographic factors and disease. J Epidemiol Commun Health 2000;54:827–33.
[28] Saltin B, Grimby G. Physiological analysis of middle-aged and old former athletes. Comparison with still active athletes of the same ages. Circulation 1968;38:1104–15.
[29] Hansen ALS, Carstensen B, Helge JW, Johansen NB, Gram B, Christiansen JS, et al. Combined heart rate- and accelerometer-assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes: the ADDITION-PRO study. Diabetes Care 2013;36:3062–9.
[30] Rödjer L, H Jonsdottir I, Börjesson M. Physical activity on prescription (PAP): self-reported physical activity and quality of life in a Swedish primary care population, 2-year follow-up. Scand J Prim Health Care 2016;34:443–52.
[31] Hu G, Tuomilehto J, Silventoinen K, Barengo NC, Peltonen M, Jousilahti P. The effects of physical activity and body mass index on cardiovascular, cancer and all-cause mortality among 47 212 middle-aged Finnish men and women. Int J Obes (Lond) 2005;29:894–902.

[32] Christensen A, Davidsen M, Ekholm O, Pedersen P, Juel K. Danskernes sundhed - den nationale sundhedsprofil 2013 (National survey of the health of Danes - The Danish national health profile. Copenhagen: The Danish Health and Medicines Authority; 2013.
[33] Eriksen L, Grønbæk M, Helge JW, Tolstrup JS, Curtis T. The Danish health examination survey 2007–2008 (DANHES 2007–2008). Scand J Public Health 2011;39:203–11.
[34] Bergström G, Berglund G, Blomberg A, Brandberg J, Engström G, Engvall J, et al. The Swedish CArdioPulmonary BioImage study: objectives and design. J Intern Med 2015;278:645–59.
[35] Schnohr P, Scharling H, Jensen JS. Changes in leisure-time physical activity and risk of death: an observational study of 7,000 men and women. Am J Epidemiol 2003;158:639–44.
[36] Reinholdsson M, Palstam A, Sunnerhagen KS. Prestroke physical activity could influence acute stroke severity (part of PAPSIGOT). Neurology 2018;91:e1461–7.
[37] Olsson OA, Persson HC, Alt Murphy M, Sunnerhagen KS. Early prediction of physical activity level 1 year after stroke: a longitudinal cohort study. BMJ Open 2017;7:e016369.
[38] Cook P, Sunnerhagen KS, Persson HC. Level of physical activity is positively correlated with perceived impact on life 12 months after stroke: a cross-sectional study. J Rehabil Med 2020;52:jrm00056.
[39] Persson CU, Hansson P-O. Early prediction of physical inactivity in 215 individuals 6 months after a mild stroke: the fall study of Gothenburg. J Rehabil Med 2020;52:jrm00077.
[40] Grimby G, Börjesson M, Jonsdottir IH, Schnohr P, Thelle DS, Saltin B. The 'Saltin-Grimby Physical Activity Level Scale' and its application to health research. Scand J Med Sci Sports 2015;25(Suppl 4) 119–25.
[41] Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. Neurology 1999;52:1487–9.
[42] Muppidi S, Wolfe GI, Conaway M, Burns TM. MG COMPOSITE AND MG-QOL15 STUDY GROUP. MG-ADL: still a relevant outcome measure. Muscle Nerve 2011;44:727–31.
[43] Burns TM, Conaway MR, Cutter GR, Sanders DBMuscle Study Group. Less is more, or almost as much: a 15-item quality-of-life instrument for myasthenia gravis. Muscle Nerve 2008;38:957–63.
[44] Muppidi S, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, et al. Long-term safety and efficacy of eculizumab in generalized myasthenia gravis. Muscle Nerve 2019;60:14–24.
[45] Raja SM, Howard JF, Juel VC, Massey JM, Chopra M, Guptill JT. Clinical outcome measures following plasma exchange for MG exacerbation. Ann Clin Transl Neurol 2019;6:2114–2119.
[46] Burns TM, Sadjadi R, Utsugisawa K, Gwathmey KG, Joshi A, Jones S, et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15r. Muscle Nerve 2016;54:1015–22.
[47] Morin C. Insomnia: psychological assessment and management. New York: Guilford Press; 1993.
[48] Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Med 2001;2:297–307.
[49] Standard Definitions - AAPOR [Internet]. [cited 2020 Apr 22]. Available from: https://www.aapor.org/Publications-Media/AAPOR-Journals/Standard-Definitions.aspx
[50] Hansen JS, Danielsen DH, Somnier FE, Frøslev T, Jakobsen J, Johnsen SP, et al. Mortality in myasthenia gravis: a nationwide population-based follow-up study in Denmark. Muscle Nerve 2016;53:73–7.
[51] Boter H, Mänty M, Hansen AM, Hortobágyi T, Avlund K. Self-reported fatigue and physical function in late mid-life. J Rehabil Med 2014;46:684–90.
[52] Elbers RG, van Wegen EE, Verhoef J, Kwakkel G. Impact of fatigue on health-related quality of life in patients with Parkinson's disease: a prospective study. Clin Rehabil 2014;28:300–11.
[53] Natelson BH, Lin J-MS, Lange G, Khan S, Stegner A, Unger ER. The effect of comorbid medical and psychiatric diagnoses on chronic fatigue syndrome. Ann Med 2019;51:371–8.
[54] Løppenthin K, Esbensen BA, Østergaard M, Jennum P, Tolver A, Aadahl M, et al. Physical activity and the association with fatigue and sleep in Danish patients with rheumatoid arthritis. Rheumatol Int 2015;35:1655–64.
[55] WHO. Global recommendations on physical activity for health [Internet]. World Health Organization; 2010 [cited 2020 Aug 25]. Available from: https://apps.who.int/iris/handle/10665/44399.
[56] Knak KL, Sheikh AM, Witting N, Vissing J. Physical activity in myotonic dystrophy type 1. J Neurol 2020.
[57] Apabhai S, Gorman GS, Sutton L, Elson JL, Plötz T, Turnbull DM, et al. Habitual physical activity in mitochondrial disease. PLoS One 2011;6:e22294.
[58] National survey of the health of Danes - The Danish National Health Profile 2013 (sedentary) [Internet]. [cited 2020 Jun 16]. Available from: http://proxy.danskernessundhed.dk/SASVisualAnalyticsViewer/VisualAnalyticsViewer_guest.jsp?reportName=Stillesiddende%20fritidsaktivitet&reportPath=/Produktion/Danskernes_Sundhed/
[59] Eriksen L, Grønbæk M, Helge JW, Tolstrup JS, Curtis T. The Danish health examination survey 2007–2008 (DANHES 2007-2008). Scand J Public Health 2011;39:203–11.
[60] National survey of the health of Danes - The Danish national health profile 2017 (WHO recommendations) [Internet]. [cited 2020 Oct 13]. Available from: http://proxy.danskernessundhed.dk/SASVisualAnalyticsViewer/VisualAnalyticsViewer_guest.jsp?reportName=Opfylder%20ikke%20WHOs%20anbefalinger%20for%20fysisk%20aktivitet&reportPath=/Produktion/Danskernes_Sundhed/
[61] National monitorering af den officielle anbefaling om fysisk aktivitet. - Et metodestudie - PDF Gratis download [Internet]. [cited 2021 Apr 9]. Available from: https://docplayer.dk/7821925-National-monitorering-af-den-officielle-anbefaling-om-fysisk-aktivitet-et-metodestudie.html
[62] Kittiwatanapaisan W, Gauthier DK, Williams AM, Oh SJ. Fatigue in myasthenia gravis patients. J Neurosci Nurs 2003;35:87–106.
[63] Westerberg E, Landtblom A-M, Punga AR. Lifestyle factors and disease-specific differences in subgroups of Swedish Myasthenia Gravis. Acta Neurol Scand 2018;138:557–65.
[64] Jordan B, Mehl T, Schweden TLK, Menge U, Zierz S. Assessment of physical fatigability and fatigue perception in myasthenia gravis. Muscle Nerve 2017;55:657–63.
[65] Grohar-Murray ME, Becker A, Reilly S, Ricci M. Self-care actions to manage fatigue among myasthenia gravis patients. J Neurosci Nurs 1998;30:191–9.
[66] Motl RW, McAuley E, DiStefano C. Is social desirability associated with self-reported physical activity? Prev Med 2005;40:735–9.
[67] Crutzen R, Göritz AS. Does social desirability compromise self-reports of physical activity in web-based research? Int J Behav Nutr Phys Act 2011;8:31.

Journal of Neuromuscular Diseases xx (2021) x–xx
DOI 10.3233/JND-210693
IOS Press

Research Report

Habitual Physical Activity in Patients with Myasthenia Gravis Assessed by Accelerometry and Questionnaire

Linda Kahr Andersen[a,b,*] and John Vissing[a,b]
[a]*Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark*
[b]*Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*

Pre-press 28 July 2021

Abstract.
Background: Physical activity (PA) in patients with myasthenia gravis (MG) is considered safe and beneficial, and an active lifestyle is required to obtain the health benefits of exercise. However, as the disease leads to physical impairments an insight into the overall PA habits in this patient population is relevant but lacking.
Objective: To measure habitual physical activity in a Danish cohort of patients with MG measured by accelerometer and questionnaire, and to determine relevant predictors for PA intensities.
Methods: Habitual physical activity was assessed by: 1) the accelerometer *ActiGraph* in a cohort of patients recruited from our neuromuscular clinic, 2) the *International Physical Activity Questionnaire* (IPAQ) in a web-based survey. PA levels were compared to international recommendations. Predictors for PA (age, sex, body mass index, disease severity and duration) were included in the regression analyses.
Results: Habitual physical activity was measured by accelerometer for 7 days in 69 patients and by questionnaire in 691 patients. Measured by the accelerometer, 46% of the patients did not meet the international recommendations for PA at moderate/vigorous intensity and 57% were below the recommendations for steps per day. Measured by the IPAQ, 48% did not meet the recommendations. Disease severity and age were predictors for PA intensities.
Conclusions: This study found that around half of the included patients did not meet the recommendations for PA. This is a concern, as it increases the risk of life-style related diseases. Disease severity and age may be taking into consideration when counseling the patients about PA.

Keywords: Myasthenia gravis, physical activity, exercise, cross-sectional survey, patient outcome assessment, body mass index, epidemiological study, linear regression, population at risk, health promotion

INTRODUCTION

Habitual physical activity refers to the physical activity (PA) that is integrated in peoples' everyday life in their natural environment, and thus covers the activity level of a whole day instead of dedicated exercise sessions. Habitual physical activity is a relevant and important outcome measure in research, as lifelong PA at recommended intensities is important to obtain the health benefits of being active.

Myasthenia gravis (MG) is a chronic, autoimmune disease with a well-known pathogenesis. Blocking of signal transduction and destruction of the synaptic

*Correspondence to: Linda Kahr Andersen, Copenhagen Neuromuscular Center, section 8077, Copenhagen University Hospital – Rigshospitalet, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark. Tel.: +45 35456217; E-mail: linda.kahr.andersen@regionh.dk.

ISSN 2214-3599/$35.00

components, result in fluctuating strength of voluntary muscles, and distinct muscular fatigue with repeated muscle contractions. The Danish prevalence of MG is estimated to 180 per million people [1] with an annual incidence of 9.2 per million [2]. Recent trials [3, 4] as well as older case-studies of patients with MG participating in endurance exercise [5–7], or resistance training [8, 9], suggest that exercise training is beneficial, especially by increasing muscle strength without apparent side effects.

Over the last decades, new technology, such as accelerometers, has been developed that facilitates objective assessment of habitual physical activity. Accelerometers are considered objective but with some technical user problems or limitations. An alternative to accelerometry is self-reported outcomes such as questionnaires, which are simple to use and cost-efficient, but can be flawed by information bias. Habitual physical activity has previously been assessed in neuromuscular diseases by a wide range of accelerometers and questionnaires [10].

In patients with MG, habitual physical activity has been measured in three recent cross-sectional studies by either accelerometry [11, 12] or questionnaire [13], finding that patients with MG were less active than non-MG persons. Two interventional exercise studies examined baseline PA patterns in 10 and 11 patients with both accelerometry and questionnaire, even though this was a minor part of the studies' analyses [4, 14].

The purpose of the present study was to measure habitual physical activity in patients with MG by both accelerometer (ActiGraph) and a standardized questionnaire (the International Physical Activity Questionnaires, IPAQ), and to identify important predictors for different PA intensities.

MATERIALS AND METHODS

Patients

Habitual physical activity was measured by questionnaire (IPAQ) in a web-based survey and by accelerometer in patients ($\geq$ 18 years) recruited from the neuromuscular clinic at Copenhagen University Hospital – Rigshospitalet.

The survey was conducted from June to November 2019 and has been described in a recent study [13]. Patients were recruited from the Danish National Registry of Patients, which is a registry of all Danish hospital in- and outpatient discharges since 1977. The inclusion criteria were; resident of Denmark and a diagnosis code for MG according to International Classification of Diseases in either the eight edition (ICD-8: 733.09, 1971–1993) or tenth edition (ICD-10; G.70.0, from 1994) in the period from 1977 to the end of 2018. Also, patients should subscribe to e-Boks, which is a personal, digital mailbox, connected to the patient's 10-digit civil registration number. Patients who met these criteria were invited to participate in the web-based survey, using the software REDCap (© 2018 Vanderbilt University). All responses to the survey were automatically stored in a secure database. As a part of the survey, patients completed the IPAQ, and the MG-ADL (MG Activities of Daily Living profile) as a measure for disease severity. Also, background information was registered; age, sex, height and weight (to calculate the body mass index, BMI), MG treatment and MG duration.

A group of patients were recruited from the neuromuscular clinic in the period November 2018 until February 2020. Patients arriving for their regular follow-up at the neurologist, were informed about the project, and, if meeting the inclusion criteria, invited to participate. The inclusion criteria were a MG diagnosis verified by a typical clinical history and symptom improvement with acetylcholinesterase inhibitors coupled with either positive acetylcholine receptor antibodies and/or significant decrement/increased jitter on electromyography. The included patients had a history of symptoms corresponding to categories II-IV on the Myasthenia Gravis Foundation of America clinical classification [15]. If patients met the inclusion criteria and agreed to participate, they were instructed to wear the accelerometer at home, 24 hours a day (unless when showering and swimming) for 7 full days. While in the clinic, patient completed the MG-ADL and the IPAQ, and background information was registered; age, sex, height and weight (to calculate the body mass index, BMI), MG treatment and duration.

Measurements

The ActiGraph wGT3X-BT accelerometer (ActiGraph, LLC, Pensacola, FL) was used to measure the patients' PA level. Acceleration intensity and duration of hip motion (e.g., steps and other movements such as rising from a chair) were monitored in three axes (up–down, left–right, forward–backward). The accelerometer (weight 19 g, dimension 4.6 cm $\times$3.3 cm $\times$1.5 cm) was worn in an elastic belt on the right side of the patient's waist. The ActiGraph monitors were set to activity counts in triaxial mode,

using a 10-second epoch. The number of counts increased with the frequency and intensity of movement. Patients with 7 days of at least 10 hours of recording during daytime were included in the analyses. Wear time was defined by subtracting non-wear time from 18 h because all data between 12:00 a.m. and 6:00 a.m. were registered and excluded as sleeping time as done in other studies on habitual PA using accelerometry [16, 17]. Non-wear time was defined as intervals of at least 60 consecutive minutes of zero counts [18]. Light, moderate, and vigorous intensities were extracted, and were defined as: light = 100–1951 counts per minute (CPM); moderate = 1952–5724 CPM; vigorous $\geq$ 5725 CPM in bouts of minimum 10 minutes [19]. CPM $\leq$ 99 were defined as sedentary time. CPM were converted to minutes per week (m/w) of light, moderate and vigorous physical activities. Sedentary time was reported in minutes per day (m/d). Steps per day were also reported.

IPAQ short form [20] was used to measure self-reported time spent on PA undertaken across a comprehensive set of domains; leisure time PA, domestic and gardening activities, work-related PA, and transport-related PA. IPAQ assessed three specific types of activity; walking, moderate-intensity activities and vigorous-intensity activities. Patients were asked to report number of days, hours and minutes spent on these activity intensities during the last 7 days. Also, the number of hours and minutes of sitting on one of these days were reported. From these data, the minutes per week (m/w) in each intensity were calculated. For sitting time, minutes per day (m/d) were calculated. The PA level of the patients was determined according to the following international recommendations: 1) $\geq$150 min at a moderate intensity or $\geq$75 min at vigorous intensity per week [21], 2) a minimum of 10,000 steps per day [22].

The MG-ADL is an 8-item patient-reported questionnaire where higher score indicates higher MG severity (total score range 0–24) [23]. The scale assesses common MG-symptoms and dysfunctions, including questions of ocular, bulbar, respiratory, and extremity functions. The MG-ADL is a validated outcome measure in research and clinical practice [24].

The study was approved by the ethics committee of the Capital Region of Denmark (approval H-18031231). Written informed consent was obtained from all patients, but according to the ethics committee of the Capital Region of Denmark, written informed consent was not required for the survey. Permission was obtained from the Danish Health Data Authority to extract patient contact information from the Danish National Registry of Patients.

Analyses

Age, BMI and disease duration were presented by means and standard deviations (SD). MG-ADL, IPAQ and ActiGraph results were presented by medians and inter-quartile ranges (IQR) due to non-normal distributions. Normality was assessed visually by histograms and boxplots. Sex and treatment were presented by numbers and percentages. Differences in variables with continuous data where investigated by either unpaired *t* test or Mann-Whitney test, depending on normality. Differences in categorical data were investigated by Fisher's exact test.

A commercially available software, ActiLife v6.11.6, was used for the analyses of raw accelerometer data. The 10-second epochs were for analyses collapsed into 60-second epochs, which was required by the ActiGraph software to set sleep time periods.

For analyses, minutes spent on moderate and vigorous activities were merged into one outcome; MVPA (moderate-vigorous physical activity), as many patients reported zero minutes spent on vigorous activities. The outcomes of the analyses were the activity levels measured by accelerometer (sedentary, light, MVPA, steps) and by IPAQ (sitting, walking, MVPA). Covariates were selected a priori and included in the general linear regression models as continuous variables (age, BMI, MG-ADL score, disease duration) or as categorical variable (sex). The number of covariates was limited to sample size divided by ten to avoid mass significance. For the same reason MG treatment was not included in the models. The following model assumptions were checked; independence of observations, linearity of covariates, homogeneous and normally distributed residuals. In case of violation, the analyses were conducted on log-10 transformed data.

For analyses, a $p \leq 0.05$ (2-tailed testing) was considered significant. All statistical analyses were carried out using SAS enterprise guide 7.1.

RESULTS

A total of 1745 persons were registered at the Danish National Registry of Patients with a diagnosis code of MG. N = 282 persons (164 women [58.2%]; mean [SD] age, 78.0 [12.6] years) did not subscribe to e-Boks and were excluded. N = 1463 persons received an information for the survey.

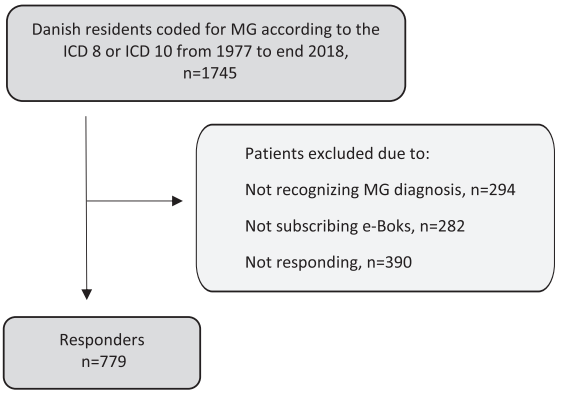


Fig. 1. Flowchart of in- and exclusion of the survey.

N = 294 persons (173 women [58.8%]; mean [SD] age, 62.2 [15.1] years) contacted the study coordinator (LKA) due to uncertainty of the MG diagnosis and were excluded, and 390 persons (226 women [57.9%]; mean [SD] age, 55.0 [16.5] years) never responded to the survey. Of the 779 responders (413 women [53.0%]; mean [SD] age, 60.8 [15.5] years), there were missing data on IPAQ ($n = 59$), MG-ADL ($n = 24$), BMI ($n = 1$) and MG duration ($n = 4$), leaving 691 patients for analyses. Flowchart of in- and exclusion can be seen in Fig. 1.

A total of 69 patients were included for accelerometer measurements from the clinic. Wear time was on average (mean ± SD) 16 ± 1.4 hours/day during the 7 days. There was missing data on MG-ADL ($n = 5$) and IPAQ ($n = 6$) due to lack of time for completing the questionnaires.

Table 1
Patient demographics

Variable	Clinic sample ($n = 69$)	Survey sample ($n = 691$)
Age, years	58.9 (17.3)	60.5 (15.5)
Body mass index, BMI	27.5 (6.5)	27.1 (5.9)
Sex, female	44 (64%)	365 (53%)
MG duration, years*	8.0 (8.9)	13.8 (12.6)
MG-ADL, total score	2 (0–6)	3 (1–5)
Pyridostigmine treatment#	50 (73%)	399 (58%)
Immunosuppressive treatment#	53 (77%)	351 (51%)

Age, BMI and MG duration are presented as means and standard deviations (SD). Sex and treatment are presented as number and percentages. MG-ADL scores are presented as medians and inter-quartile ranges (IQR). Abbreviations: MG-ADL = MG Activities of Daily Living profile. *Significant ($p < .001$) difference between the two samples. #Some patients were in a combination treatment with both pyridostigmine and immunosuppressive drugs. Immunosuppressive drugs: prednisolone, Azathioprine, Methotrexate, Mycophenolic acid, Tacrolimus, Rituximab.

Patient demographics are shown in Table 1. The only difference between the two samples was MG duration ($p < 0.001$). The mean BMI indicated overweight (BMI ≥ 25) in both samples.

Time spent in different activity intensities are shown in Table 2. The time spent on activities of moderate intensity was 157 minutes (m) per week (w) measured by accelerometer and 150 m/w measured by IPAQ. Time spent on vigorous activities was 0 m/w and 60 m/w measured by accelerometer and IPAQ, respectively. The proportion of patients not meeting the international recommendations of PA was high. Measured by accelerometer, patients that did not meet the recommendations of moderate intensity (≥ 150 m/w) ($n = 32$, 46%) were older (67 vs 52 years, $p < 0.01$) compared to patients, that met the recommendations. In the survey sample, patients that did not meet the recommendations of moderate intensity ($n = 343$, 48%) had a higher median score on MG-ADL (3 vs 2, $p < 0.01$) and were more often women (58 vs. 42%, $p = 0.01$) compared to patients, who met the recommendations.

More than half of the patients assessed by accelerometry ($n = 38$, 57%) walked less than the recommended 10,000 steps per day. There were no significant differences between the patients that walked more or less than 10,000 steps per day regarding age, sex, BMI, disease severity and duration.

Significant predictors for PA were MG-ADL score and age. High scores of MG disease severity increased time spent on sedentary activities (regression coefficient (β): 6.1, confidence interval (CI): 0.0; 12.1) and sitting time (β: 2.5, CI: 0.9; 4.0). Severe self-reported MG symptoms also decreased walking time (β: –3.6, CI: –6.5; –0.4). Higher age decreased time spent on MVPA (β: –4.0, CI: –6.3; –1.6) and steps per day (β: –90.8, CI: –168.0; –13.5) measured by accelerometer. Measured by IPAQ, increasing age decreased time spent in sitting (β: –0.5, CI: –0.8; 0.2) and increased time spent on MVPA (β: 0.7, CI: 0.1; 1.3) (Table 3).

DISCUSSION

This is the first study to assess habitual physical activity measured by both accelerometer and questionnaire in a large cohort of patients with MG.

The study demonstrates that around half of the included patients with MG met the international recommendations for PA, which was found for both accelerometry and questionnaire assess-

Table 2
Physical activity levels measured by accelerometer and questionnaire

Accelerometer		IPAQ		
	$N = 69$		$N = 63^*$	$N = 691$
Sedentary, m/d	654 (596–694)	Sitting, m/d	420 (300–600)	420 (300–600)
Light, m/w	2037 (1643–2371)	Walking, m/w	150 (80–420)	225 (90–525)
Moderate, m/w	157 (91–292)	Moderate, m/w	120 (0–240)	150 (15–360)
Vigorous, m/w	0 (0–3)	Vigorous, m/w	30 (0–180)	60 (0–210)
Steps per day	9299 (5562–13,255)			

Data are presented as medians and inter-quartile ranges (IQR). Abbreviations: m = minute, d = day, w = week, IPAQ = the International Physical Activity Questionnaires. *patients both wearing an accelerometer and completing the IPAQ (missing data: $n = 6$).

ments. These findings are in line with previous findings from our research group using another questionnaire (Saltin-Grimby Physical Activity Level Scale) in a large cohort of patients with MG [13].

Our study findings indicate a more active patient group, than previously reported [11, 12]. In those previous studies, only 22% and 30% of the patients met the recommendations for MVPA in bouts of $\geq$10 min, and only 22% met the 10,000 steps per day measured by accelerometer [11]. Time spent on sedentary behavior was almost similar across studies, but our study demonstrated more time spent on MVPA and more steps per day than previous findings [11, 12, 14]. The disparity might be explained by the use of different accelerometers (O'Connor, Birnbaum, Westerberg used DynaPort Movemonitor) and thereby different algorithms, manufacturer software and cut-off points to determine time spent on PA intensities. Also, the wording of the recommendations is important. Patients meeting the recommendations of $\geq$150 min per week, might not meet the recommendations of $\geq$30 min/day for at least 5 days, as some patients accumulate their exercise in few days. However, our findings are strengthened by the combination of accelerometry and patient-reported outcomes, and by the large sample size. As we solely analyzed data (time spent on PA intensities) that were comparable between the IPAQ and accelerometry, we did not include other PA metrics. However, it might be relevant for future research to compare findings of other PA parameters, e.g. energy expenditure.

In a study of patients with myotonic dystrophy type 1 ($n = 67$) [25], around 50% of the patients met the recommendations for moderate intensity as measured by ActiGraph, which is in line with our results. Compared to a study of patients with mitochondrial diseases ($n = 100$) and healthy controls ($n = 100$) [26], MG patients walked more steps daily than the mitochondrial patients (mean $\pm$ SD, 9,602 $\pm$ 5,016 steps/day vs. 6,883 $\pm$ 3,944 steps/day) and were closer to the results of healthy controls (9,924 $\pm$ 3,944 steps/day). In the Copenhagen City Heart Study [27], the PA levels of 1670 adults ($\geq$ 18 years) living in Copenhagen were measured by ActiGraph accelerometers for 24 hours/day, 7 consecutive days. This study found a median (IQR) of 579 (509–646) min/day spent on sedentary activities and a median (IQR) of 9288 (6932 –12003) steps/day, indicating only minor differences between patients with MG and the background population. However, in another Danish population study using IPAQ, only 29% didn't meet the WHO recommendations [28], indicating that patients in the present study were less active. This emphasizes that comparisons with other patient- or healthy cohorts must be performed cautiously, due to differences in diseases, sampling (age, sex) and methods used.

Even though it is positive that patients with MG seem more active than reported earlier, it is still concerning that around half of the patients did not meet the recommendations of PA. Inactivity in MG is both associated with physical – and general fatigue [13], and as inactivity increases the risk of life-style related diseases [21] and disuse atrophy in all individuals, it is a concern if disuse atrophy exacerbates the disease-related fatigue/weakness in patients with MG. However, further research is needed to determine this.

We found that severe MG (MG-ADL) was associated with increasing time spent in sitting and sedentary activities. This finding has not been detected in the smaller, previous studies [11, 12], and might be explained by the large number of patients included in our study. However, no associations were found between disease severity and time spent on physical activities in any of the studies. This could be explained by the cross-sectional design of these studies, but also in the interventional exercise stud-

Table 3
Associations of physical activity, disease severity and demographics

A: Accelerometer

	Sedentary, m/d				Light, m/w				MVPA, m/w			
	R^2 adjusted: 0.53				R^2 adjusted: 0.81				R^2 adjusted: 0.42			
	β	95%CI β	SE β	*P* value	β	95%CI β	SE β	*P* value	β	95%CI β	SE β	*P* value
Intercept	712.4	610.6; 814.3	50.9	<0.01	1904.9	1052.8; 2757.0	425.5	<0.01	506.4	276.6; 736.2	114.8	<0.01
Age[#]	–0.4	–1.5; 0.6	0.52	0.40	–6.9	–15.6; 1.9	4.4	0.12	**–4.0**	**–6.3; –1.6**	**1.2**	**<0.01**
Sex, female	17.6	–20.2; 55.3	18.9	0.36	–109.2	–425.0; 206.6	157.7	0.49	–9.1	–94.3; 76.1	42.5	0.83
BMI	–2.1	–4.8; 0.6	1.35	0.12	20.3	–2.4; 43.0	11.3	0.08	–1.7	–7.9; 4.4	3.1	0.57
MG-ADL[¶]	**6.1**	**0.0; 12.1**	**3.0**	**<0.05**	–9.2	–59.8; 41.3	25.2	0.72	–2.0	–15.6; 11.7	6.8	0.77
MG duration[#]	–1.6	–3.6; 0.3	1.0	0.10	7.1	–9.3; 23.4	8.2	0.39	–0.8	–5.3; 3.6	2.2	0.70

B. Accelerometer, steps per day

	Steps per day			
	R^2 adjusted: 0.75			
	β	95%CI β	SE β	*P* value
Intercept	15,757.8	82499.2; 23264.5	3749.2	<0.01
Age[#]	**–90.8**	**–168.0; –13.5**	**38.6**	**0.02**
Sex, female	–1133.3	–3915.8; –1649.3	1389.6	0.42
BMI	9.6	–190.2; 209.5	99.8	0.92
MG-ADL ¶	2.3	–443.0; 447.6	222.4	0.99
MG duration[#]	–46.2	–190. 2; 97.9	71.9	0.52

C: IPAQ

	Sitting time, m/d				Walking, m/w				MVPA, m/w			
	R^2 adjusted: 0.20				R^2 adjusted: 0.91				R^2 adjusted: 0.35			
	β	95%CI β	SE β	*P* value	β	95%CI β	SE β	*P* value	β	95%CI β	SE β	*P* value
Intercept	408.1	312.8; 532.5	14.5	<0.01	265.1	152.6; 460.3	32.4	<0.01	361.1	206.2; 632.4	33.0	<0.01
Age[#]	**–0.5**	**–0.8; 0.2**	**0.1**	**<0.01**	0.1	–0.4; 0.7	0.3	0.62	**0.7**	**0.1; 1.3**	**0.3**	**0.02**
Sex, female	–1.5	–9.9; –7.7	4.7	0.74	6.2	–11.3; 27.0	9.6	0.51	–11.2	–25.8; 6.2	9.6	0.19
BMI	0.3	–0.1; 1.4	0.4	0.06	0.2	–1.5; 1.8	0.8	0.85	–1.1	–2.6; 0.5	0.8	0.17
MG-ADL ¶	**2.5**	**0.9; 4.0**	**0.8**	**<0.01**	**–3.6**	**–6.5; –0.4**	**1.6**	**0.03**	–2.1	–5.2; 1.2	5.3	0.22
MG duration[#]	0.1	–0.2; 0.5	0.2	0.41	–0.3	–1.0; 0.4	0.4	0.46	–0.3	–1.0; 0.4	0.4	0.45

β = regression coefficient, SE β = standard error of the regression coefficient, CI β = confidence intervals of the regression coefficient. Significance in bold. Abbreviations: MVPA = moderate-vigorous physical activity, MG-ADL = MG Activities of Daily Living profile, m = minute, d = day, w = week, BMI = Body mass index ¶ There was missing data on MG-ADL ($n = 5$) due to lack of time for completing the questionnaire, [#]measured in years

ies, MG severity was constant [3, 4] or only slightly decreased [14] after the intervention period. These findings indicate, that even though exercise improves muscle parameters and functional capacity in patients with MG, the commonly used MG-measurements might not be sensitive enough to detect these changes. Whether isolated symptoms of MG, e.g. muscle weakness in the leg, play a specific role on PA levels, can't be ruled out in our study, as we used the total score of the MG-ADL. However, O'Connor[11] did not find any correlation between specified leg fatigue and PA levels.

Increasing age was associated with less time spent on MVPA and less steps per day measured by accelerometry. Contrary to these findings, increasing age was associated with less sitting time and increasing time on MVPA as measured by the IPAQ. These conflicting results could be due to bias in self-reported PA. The IPAQ has previously been found to underestimate sitting time and overestimate time on MVPA compared to accelerometry [29, 30], which might also be the case in the study (Table 2). This is also in line with the feedback from some patients, telling us that it was difficult to self-estimate time spent on sitting during a day. However, comparison of IPAQ and accelerometry should be performed with caution, as the cut-off points for intensity intervals assessed by accelerometry did not exactly correspond to the definitions of PA in the IPAQ. Also, as the IPAQ measured PA in certain PA domains (e.g. leisure time and transportation), accelerometry measured activities during the whole day. Social desirability bias might also explain the overestimation of vigorous activity measured by the IPAQ, compared to the accelerometer, but social desirability bias is found to be less pronounced in web-based surveys, where the online setting increases respondents' perceived privacy [31, 32]. Also, it was not relevant to compare walking time in IPAQ with light activity from the ActiGraph, as walking was specified as one certain activity, whereas light activity was every activity in-between sleep, sedentary, moderate and vigorous activities throughout the 24 hours.

There are several study limitations. Based on the available data from the survey, it was not possible to determine the proportion of patients with ocular/generalized MG. This is a limitation of the study, as it could be relevant to determine if patients with a history of solely ocular impairments and no experiences of MG-related muscular fatigue in the extremities have a different pattern of habitual physical activity.

The accelerometer was worn at the waist, which we thought was the most convenient for the patients. However, as measurements in this position required hip movements, some activities e.g. bicycling where the hip was held still, may have been underestimated. Furthermore, the accelerometer did not measure the added strain of carrying a load, or walking uphill or upstairs, and arm movements were not recorded. A solution for a future study might be to add heart rate measurements to the accelerometer measures to register PA without or with minimal hip movements.

It might be that only the best functioning and most active patients agreed to wear an accelerometer, leading to selection bias. However, as baseline demographics such as age, BMI, and MG-ADL score did not differ between the clinic and the survey sample, selection bias was not likely.

The study was strengthened by the large number of patients included and the combination of patient-reported and objective data of PA.

The survey sample was considered representative of the Danish MG cohort in terms of demographic characteristics, except for the most disabled MG patients [13, 33]. Persons not subscribing to e-Boks ($n = 282$) were older than the survey sample, which could have introduced some selection bias based on age. However, as the non-responders ($n = 390$) were younger, the age- and sex distribution were almost similar between in- and excluded persons. As the 69 patients, who were assessed by accelerometry, resembled the survey sample regarding demographics and MG severity, and as the study findings were almost similar between the smaller clinical and the larger survey sample, we think these 69 patients are representative of the larger survey cohort.

We found that accelerometry was useful to measure habitual physical activity in patients with MG. Accelerometry could be relevant in future longitudinal studies to identify associations between changes in the patient's disease and/or lifestyle and changes in the quantity or intensity of PA. Also, further research is needed to identify barriers to exercise for patients with MG, as this is not explained by MG severity. A previous, explorative study [34] identified, that barriers to exercise for patients with neuromuscular diseases were e.g. "lack of energy". As fatigue is a core symptom of MG, and as fatigue and PA are previously found to be strongly associated [13], it is relevant to examine if changes in fatigue result in changes in PA habits. Also, it is relevant to identify motivational factors for PA. Music during exercise might be motivational as music during walking is

found to improve walking distance in the 6MWT in patients with MG [35]. Further research is needed to elaborate this.

ACKNOWLEDGMENTS

LKA is grateful for a grant from the Research Foundation of Copenhagen University Hospital – Rigshospitalet.

CONFLICTS OF INTEREST

The authors have no conflict of interest to report.

REFERENCES

[1] Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. J Autoimmun. 2007;29:1-9.

[2] Pedersen EG, Hallas J, Hansen K, Jensen PEH, Gaist D. Late-onset myasthenia not on the increase: a nationwide register study in Denmark, 1996–2009. Eur J Neurol. 2013;20:309-14.

[3] Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U. Exercise in myasthenia gravis: A feasibility study of aerobic and resistance training. Muscle Nerve. 2017;56:700-9.

[4] Westerberg E, Molin CJ, Lindblad I, Emtner M, Punga AR. Physical exercise in myasthenia gravis is safe and improves neuromuscular parameters and physical performance-based measures: A pilot study. Muscle Nerve. 2017;56:207-14.

[5] Lucia A, Maté-Muñoz JL, Pérez M, Foster C, Gutiérrez-Rivas E, Arenas J. Double trouble (McArdle's disease and myasthenia gravis): how can exercise help? Muscle Nerve. 2007;35:125-8.

[6] Scheer BV, Valero-Burgos E, Costa R. Myasthenia gravis and endurance exercise. Am J Phys Med Rehabil. 2012;91:725-7.

[7] Leddy JJ, Chutkow JG. Myasthenia gravis in a collegiate football player. Med Sci Sports Exerc. 2000;32:1975-9.

[8] Stout JR, Eckerson JM, May E, Coulter C, Bradley-Popovich GE. Effects of resistance exercise and creatine supplementation on myasthenia gravis: a case study. Med Sci Sports Exerc. 2001;33:869-72.

[9] Lohi EL, Lindberg C, Andersen O. Physical training effects in myasthenia gravis. Arch Phys Med Rehabil. 1993;74:1178-80.

[10] Jimenez-Moreno AC, Newman J, Charman SJ, Catt M, Trenell MI, Gorman GS, et al. Measuring Habitual Physical Activity in Neuromuscular Disorders: A Systematic Review. J Neuromuscul Dis. 2017;4:25-52.

[11] O'Connor L, Westerberg E, Punga AR. Pattern of Habitual Physical Exercise in Myasthenia Gravis Patients. J Neuromuscul Dis. 2019;6:85-91.

[12] Birnbaum S, Bachasson D, Sharshar T, Porcher R, Hogrel J-Y, Portero P. Free-Living Physical Activity and Sedentary Behaviour in Autoimmune Myasthenia Gravis: A Cross-Sectional Study. J Neuromuscul Dis. 2021;

[13] Andersen LK, Aadahl M, Vissing J. Fatigue, physical activity and associated factors in 779 patients with Myasthenia Gravis. Neuromuscular Disorders [Internet]. 2021 [cited 2021 Jun 1]; Available from: https://www.sciencedirect.com/science/article/pii/S0960896621001322.

[14] Westerberg E, Molin CJ, Spörndly Nees S, Widenfalk J, Punga AR. The impact of physical exercise on neuromuscular function in Myasthenia gravis patients: A single-subject design study. Medicine (Baltimore). 2018;97:e11510.

[15] Jaretzki A, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Ann Thorac Surg. 2000;70:327-34.

[16] Dyrstad SM, Hansen BH, Holme IM, Anderssen SA. Comparison of self-reported versus accelerometer-measured physical activity. Medicine and Science in Sports and Exercise. 2014;46:99-106.

[17] Donaire-Gonzalez D, Gimeno-Santos E, Balcells E, Rodríguez DA, Farrero E, de Batlle J, et al. Physical activity in COPD patients: patterns and bouts. Eur Respir J. 2013;42:993-1002.

[18] Troiano RP. Large-scale applications of accelerometers: new frontiers and new questions. Med Sci Sports Exerc. 2007;39:1501.

[19] Freedson PS, Melanson E, Sirard J. Calibration of the Computer Science and Applications, Inc. accelerometer. Med Sci Sports Exerc. 1998;30:777-81.

[20] IPAQ scoring protocol – International Physical Activity Questionnaire [Internet]. [cited 2020 Aug 25]. Available from: https://sites.google.com/site/theipaq/scoring-protocol.

[21] WHO. Global recommendations on physical activity for health [Internet]. World Health Organization; 2010 [cited 2020 Aug 25]. Available from: https://apps.who.int/iris/handle/10665/44399.

[22] Tudor-Locke C, Bassett DR. How many steps/day are enough? Preliminary pedometer indices for public health. Sports Med. 2004;34:1-8.

[23] Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. Neurology. 1999;52:1487-9.

[24] Muppidi S, Wolfe GI, Conaway M, Burns TM. MG-ADL: Still a relevant outcome measure. Muscle & Nerve [Internet]. 2011 [cited 2019 Nov 14];44:727-31. Available from: http://onlinelibrary.wiley.com/doi/abs/10.1002/mus.22140

[25] Knak KL, Sheikh AM, Witting N, Vissing J. Physical activity in myotonic dystrophy type 1. J Neurol. 2020;

[26] Apabhai S, Gorman GS, Sutton L, Elson JL, Plötz T, Turnbull DM, et al. Habitual physical activity in mitochondrial disease. PLoS ONE. 2011;6:e22294.

[27] Johansson MS, Korshøj M, Schnohr P, Marott JL, Prescott EIB, Søgaard K, et al. Time spent cycling, walking, running, standing and sedentary: a cross-sectional analysis of accelerometer-data from 1670 adults in the Copenhagen City Heart Study: Physical behaviours among 1670 Copenhageners. BMC Public Health. 2019;19:1370.

[28] National survey of the health of Danes – The Danish National Health Profile 2017 (WHO recommendations) [Internet]. [cited 2020 Oct 13]. Available from: http://proxy.danskernessundhed.dk/SASVisualAnalyticsViewer/VisualAnalyticsViewer_guest.jsp?reportName=Opfylder%20ikke%20WHOs%20anbefalinger%20for%20fysisk%20aktivitet&reportPath=/Produktion/Danskernes_Sundhed/

[29] Ferrari G, Werneck AO, Silva DR, Kovalskys I, Gómez G, Rigotti A, et al. Agreement Between Self-Reported

and Device-Based Sedentary Time among Eight Countries: Findings from the ELANS. Prev Sci. 2021;

[30] Deliens T, Versele V, Jehin J, D'Hondt E, Verhavert Y, Clarys P, et al. Misreporting of Physical Activity and Sedentary Behavior in Parents-to-Be: A Validation Study across Sex. Int J Environ Res Public Health. 2021;18.

[31] Crutzen R, Göritz AS. Does social desirability compromise self-reports of physical activity in web-based research? Int J Behav Nutr Phys Act. 2011;8:31.

[32] Motl RW, McAuley E, DiStefano C. Is social desirability associated with self-reported physical activity? Prev Med. 2005;40:735-9.

[33] Hansen JS, Danielsen DH, Somnier FE, Frøslev T, Jakobsen J, Johnsen SP, et al. Mortality in myasthenia gravis: A nationwide population-based follow-up study in Denmark. Muscle Nerve. 2016;53:73-7.

[34] Phillips M, Flemming N, Tsintzas K. An exploratory study of physical activity and perceived barriers to exercise in ambulant people with neuromuscular disease compared with unaffected controls. Clin Rehabil. 2009;23:746-55.

[35] Andersen LK, Witting N, Vissing J. Effects of rhythmic auditory stimulation on walking during the 6-minute walk test in patients with generalised Myasthenia Gravis. European Journal of Physiotherapy [Internet]. 2021 [cited 2021 Feb 23];1-000. Available from: https://www.tandfonline.com/doi/full/10.1080/21679169.2021.1876760.

Journal of Neurology
https://doi.org/10.1007/s00415-021-10902-1

ORIGINAL COMMUNICATION

Causes of symptom dissatisfaction in patients with generalized myasthenia gravis

Linda Kahr Andersen[1,2] · **Anna Sofie Jakobsson**[1] · **Karoline Lolk Revsbech**[1,2] · **John Vissing**[1,2]

Received: 6 August 2021 / Revised: 8 November 2021 / Accepted: 10 November 2021

Abstract

Background Patient-centered assessments have attracted increasing attention in the last decade in clinics and research. The purpose of this study was to examine the association between patients' satisfaction with symptoms and several disease-specific and generic outcome measures in 100 patients with generalized myasthenia gravis (gMG).

Methods In this cross-sectional study, patients with gMG followed at the Copenhagen Neuromuscular Center from October 2019 to June 2020 participated in one test. The patients completed commonly used MG-specific outcome measures and generic questionnaires for depression (Major Depression Inventory), comorbidities (Charlson Comorbidity Index), fatigue (Multidimensional Fatigue Inventory), overall health state (EQ-5D-3L), and satisfaction with MG treatment. The analyses were anchored in the Patient Acceptable Symptom State (PASS).

Results $N = 190$ patients were screened for the study, and 100 patients were included. One-third of the patients reported dissatisfaction (negative PASS status) with the current symptom state. Increasing MG symptoms, fatigue, depression, low MG-related quality of life, and shorter disease duration were associated with negative PASS status. Age, sex, BMI, MG treatment, and comorbidity did not influence PASS status.

Conclusions This study shows that dissatisfaction with the current symptom level is high in patients with gMG and that dissatisfaction is associated with disease severity, disease length, depression, fatigue, and lower MG-related quality of life. The results emphasize the importance of a patient-centered approach to MG treatment to optimize patient satisfaction. The PASS question was useful in this study to investigate the causes of symptom dissatisfaction in gMG.

Keywords Myasthenia gravis · Patient acceptance of health care · Fatigue · Patient outcome assessment · Depression

Introduction

Myasthenia gravis (MG) is a chronic disease causing fluctuating weakness and fatigue in skeletal muscles, often due to antibodies against acetylcholine receptors in the neuromuscular junction [1]. The estimated number of patients in Denmark is around 1000 [2]. Approximately 380 patients with generalized myasthenia gravis (gMG) are followed at the Copenhagen Neuromuscular Center (CNMC), a specialized neuromuscular outpatient clinic at the Copenhagen University Hospital—Rigshospitalet.

Patient-centered assessments have attracted increasing attention in the last years in clinics and research. However, a challenge is to align patient satisfaction with various outcome measures. The threshold for satisfaction varies among patients, and a new approach to exploring this is the "patient acceptable symptom state" (PASS). PASS can be measured by a simple (yes/no) question, in which the patient answers whether they are satisfied with their current symptom state [3]. PASS has been used in two MG studies [4, 5], demonstrating that around 30% of the patients with MG were dissatisfied. However, previous studies were (1) retrospective, (2) did not compare PASS results with commonly used and clinician-reported MG measurements in the same group of patients, and (3) used limited generic outcome measures.

Linda Kahr Andersen and Anna Sofie Jakobsson authors contributed equally to this work.

✉ Linda Kahr Andersen
linda.kahr.andersen@regionh.dk

[1] Copenhagen Neuromuscular Center, Department of Neurology, Copenhagen University Hospital, Section 8077, Rigshospitalet, Inge Lehmanns Vej 8, 2100 Copenhagen, Denmark

[2] Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Published online: 21 November 2021

Therefore, the aim of this study was to determine factors related to PASS status, both MG-related factors, but also generic factors which might influence PASS status, such as fatigue, depression, and comorbidity.

Methods

Patients and procedures

In this cross-sectional study, patients were recruited from CNMC in the period October 2019–June 2020. Patients arriving consecutively for their regular follow-up were invited to participate. We decided a priori to include 100 patients, corresponding to more than a fourth of the total patients with gMG followed in our clinic.

Inclusion criteria for the study were: age ≥ 18 years, a verified diagnosis of gMG, and active medical treatment for MG. Verification of the diagnosis was; a typical clinical history and symptom improvement with acetylcholinesterase inhibitors coupled with either positive acetylcholine receptor, MUSK, or LR4P antibodies and/or significant decrement/increased jitter on electromyography. Exclusion criteria for the study were; competing, severe medical conditions that would interfere with the interpretation of the outcomes, current participation in clinical trials, pregnancy, ocular MG, and if patients were unable to understand Danish or English. For patients meeting the inclusion criteria and accepting to participate, a 1½-hour consultation was arranged. In this consultation, patients completed clinician-reported, MG-specific tests and several MG-specific and generic questionnaires. For patients on pyridostigmine treatment, the test was arranged 1½–2 h after the intake of the drug.

The tests were completed by one of three investigators (LKA, ASJ, or KLR). The three investigators trained together beforehand to conduct the assessments consistently.

The study was approved by the ethics committee of the Capital Region of Denmark. Informed consent was obtained from all included patients.

Outcome measures

The outcomes were explorative, and the analysis and interpretation of the outcomes were anchored in the patient acceptable symptom state (PASS) question.

The Patient Acceptable Symptom State (PASS) [3] was examined by one dichotomous yes/no question, which reads, "*Considering all the ways you are affected by Myasthenia gravis, if you had to stay in your current state for the next months, would you say that you are satisfied with your current disease state*"*?* The questions have previously been used in patients with MG [4, 5].

The Quantitative MG score (QMG) [6] is a 13-item, clinician-derived scale that measures muscle strength and endurance (score range 0–39, higher score indicates more severe MG status).

The MG Composite scale (MGC) [7] is a patient-reported and clinician-derived scale that measures the clinical status of the patients (range 0–56, higher score indicates more severe MG status).

The MG Activities of Daily Living profile (MG-ADL) [8] is an eight-question, clinician-directed but patient-reported questionnaire where higher scores indicate higher disease burden (total score range 0–24). The scale assesses common MG symptoms and dysfunctions. A total score below 3 is regarded as well-treated, and minimal symptom expression corresponds to a score of 0–1 [9].

The MG-specific quality of life (QoL) instrument (MG-QoL15) [10] is a 15-item patient-reported disease-specific QoL questionnaire, consisting of a five-point scale indicating the patient's agreement with a given statement about MG involvement. Total score ranges from 0 to 60, where higher scores indicate lower MG-related QoL.

The Multidimensional Fatigue Inventory (MFI-20) [11] is a patient-reported questionnaire that measures fatigue severity. MFI-20 categorizes fatigue into five domains: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. The total score in each domain ranges from 4 to 20, with higher scores indicating higher fatigue levels. MFI-20 has been used in several neurological diseases [12–14] and healthy populations but has never been used in patients with MG.

The Major Depression Inventory (MDI) [15, 16] is a patient-reported rating scale, where higher scores indicate more severe depression (range 0–50). The scale is used as a diagnostic tool for depression (score ≥ 20 means clinical depression). MDI has previously been used in patients with MG [17, 18].

The Charlson Comorbidity Index (CCI) [19] is an instrument for the long-term mortality prognosis in patients with comorbidities. The point-score ranges from 0 to 37 and is accumulated according to associated diseases and age ranges. A higher score predicts a shorter 10-year survival probability. CCI has previously been used in a study of MG [20].

The EQ-5D-3L [21] is a generic classification system for measuring health-related QoL, compromising the five dimensions; mobility, self-care, usual activities, pain/discomfort, and depression/anxiety. The index score is derived from three different questions in each dimension, giving an index ranging from 0 to 1; 0 (death) to 1 (perfect health) [22]. An EQ-VAS scale ranging from 0 to 100 (100 = the best health you can imagine) was also included, indicating the patient's self-rated overall health status. EQ-5D-3L has previously been used in patients with MG [4].

As we expected that the satisfaction with adverse effects of the MG medical treatment would influence on PASS status, and as no such easy-to-use instrument is developed for patients with MG, we simply asked the patient one question: "*How satisfied are you with the current adverse effects of your medical treatment for MG?* Satisfaction was scored on a VAS scale ranging from 1 to 10 (1 = not at all satisfied to 10 = very satisfied).

Patient- and disease-related background information was obtained: age, sex, weight, and height (used to calculate body mass index, BMI), occupational status, MG duration, MG treatment, and antibody- and thymectomy status.

Statistical analyses

Continuous variables were presented by means ± standard deviation (SD) when data were normally distributed and medians and interquartile ranges (IQR) when this was not the case. Normality was determined visually by histograms and boxplots. Categoric variables were presented by numbers (*n*) and percentages (%).

The difference in distribution was investigated by unpaired *t* test for continuous data, Mann–Whitney test for non-normal distributed continuous data, and Fisher's exact test for categorical data. Correlation between tests was estimated by the non-parametric Spearman's rank correlation coefficients.

To identify factors associated with PASS status, logistic regression models were applied, using PASS as a dichotomous outcome: positive (yes) /negative (no). In the adjusted analyses, the covariates were age, sex, disease length, MG disease severity (QMG), and depression (MDI), which were selected a priori and determined from available evidence or experiences from the clinic. The QMG was included as this was the only entirely objective measurement of disease symptoms, and depression score was included as depressive symptoms were expected to influence symptom satisfaction. More covariates could be relevant, but the numbers were limited to avoid mass significance in the analyses. The regression models were executed as complete-case analysis and checked for (1) overall Goodness-of-fit, (2) linearity of covariates by adding log-transformed covariates into the model, (3) interaction and (4) test of accumulated residuals by plots and p-values. The convergence criterion was satisfied in all analyses.

For analyses, a $p \leq 0.05$ (two-tailed testing) was considered significant. All statistical analyses were carried out using SAS enterprise guide 7.1.

Data availability statement

The individual de-identified participant data, the study protocol, and statistical analyses plan will be shared by request from any qualified investigator.

Results

To reach the desired number of 100 patients, we consecutively screened 190 patients for eligibility (by checking their medical records) when they arrived in the clinic for their regular follow-up with a neurologist. Of these, 34 did not meet the inclusion criteria (due to ocular MG ($n = 8$), currently not on medical treatment for MG ($n = 11$), or severe comorbidity that could influence the interpretation of test results, e.g., severe COPD or hemiparalysis ($n = 15$)). Of the remaining 156 invited to participate in the study, one patient was pregnant, one patient had language issues, and 54 patients refused for practical issues, such as lack of time or long transportation.

Of the included patients, 57 were women with a mean age at disease onset of 45.6 ± 19.8 years. Men were generally older at disease onset; 56.7 ± 15.3 years ($p < 0.001$). The median disease duration was 6 (IQR 3–13) years. In total, 66 patients were on pyridostigmine treatment, of whom 20 patients were treated solely with this drug. Twenty-four patients were in prednisolone treatment (mean dose 15.8 mg/daily) combined with other MG drugs. Table 1 shows some characteristics of the sample.

Thirty-three patients answered "no" to the PASS question, while 67 patients were satisfied. Compared to PASS-positive patients, the dissatisfied patients had shorter disease duration (median 3 vs. 8 years, $p = 0.001$) and were more often unemployed or on disability ($p = 0.033$). Also, the PASS-negative patients had higher scores on all outcome measures ($p < 0.05$), except for comorbidity ($p = 0.767$) compared to satisfied patients. The MG medical treatment differed between PASS groups ($p = 0.028$), whereas this was not the case for age, sex, BMI, and antibody- and thymectomy status (Table 2). There were no differences in prednisolone dose between the groups ($p = 0.120$).

PASS-negative status was associated ($p < 0.05$) with both clinician-measured outcome (QMG), patient-reported outcomes (MG-ADL, MG-QoL15, MFI-20, MDI), and disease duration. Increasing MG severity, MG-related quality of life scores, fatigue, and depression, as well as short disease duration, increased the odds for negative PASS answers. Age, sex, BMI, MG medical treatment (both overall and prednisolone), MGC scores, and comorbidity were not associated with PASS status in the adjusted analyses (Table 3).

The correlation between MG-ADL and QMG was low ($r = 0.50$, $p < 0.0001$) to modest ($r = 0.79$, $p < 0.0001$), depending on the inclusion/exclusion of outliers. The distribution of patients with the most prominent distinction between patient-reported and clinician-derived outcomes was equally between PASS-negative and -positive respondents ($p = 0.596$). Also, a prominent distinction was not associated with a PASS-negative answer ($p = 0.208$).

Table 1 Characteristics of the sample ($N = 100$)

Characteristic	Statistic
Age, years	60.2 ± 15.4
Sex, women	57
BMI, kg/m^2	28.1 ± 6.1
MG duration, years	6 (3–13)
Job-status, *n*	
Working full- or part-time	39
Unemployed/sick leave	10
Retired due to MG	5
Retired due to age	43
Student	3
Antibody status, *n*	
AChR positive	94
AChR negative	5
MUSK positive	1
Thymectomy, *n*	
Yes	41
MG Treatment ¶	
Pyridostigmine only	20
Pyridostigmine + Prednisolone	11
Pyridostigmine + Immunosuppressant	26
Immunosuppressant only	30
Immunosuppressant + prednisolone	4
Pyridostigmine + prednisolone + immunosuppressant	9
MG-ADL	3 (0–5)
QMG	9 (5–11)
MGC	5 (3–10)
MG-QoL15	8 (3–21)
MFI-20 general fatigue	12 (9–16)
MFI-20 physical fatigue	13 (10–16)
MFI-20 reduced activity	11 (6–14)
MFI-20 reduced motivation	8 (6–11)
MFI-20 mental fatigue	8 (5–12)
MDI	10 (5–18)
EQ-5D-3L	0.8 ± 0.2
EQ-5D-VAS	75 (65–80)
CCI	3 (2–5)

Values are presented as mean ± standard deviation (SD), median and interquartile ranges (IQR) or numbers. As the sample size is 100, the percentages are obvious and not shown

BMI body mass index, *CCI* charlson comorbidity index, *MG-ADL* MG Activities of Daily Living Profile, *MG-QoL15* MG-specific QoL instrument, *MDI* major depression inventory, *MGC* MG composite scale, *QMG* quantitative MG score, *MFI-20* multidimensional fatigue inventory. ¶ Immunosuppressant including Azathioprine, Methotrexate, Tacrolimus, Rituximab, and Mycophenolic acid

Twenty patients had an MDI score ≥ 20, indicating depression. The distribution of depressed patients differed between PASS groups ($p = 0.007$), with 38% ($n = 12$) of the PASS-negative patients having clinical depression, vs. 12% ($n = 8$) in the PASS-positive group. A high MDI score increased the odds for PASS-negative status (odds ratio, OR: 1.10, confidence interval, CI: 1.03–1.17, $p = 0.003$).

The median score of satisfaction with current adverse effects of MG medical treatment was 8 (IQR 5–9). $N = 41$ patients reported a score below the sample median of 8. Of the PASS-negative patients, 64% ($n = 21$) had low satisfaction (< 8) with adverse effects, whereas this was only 30% ($n = 20$) of the PASS-positive patients ($p = 0.002$).

Discussion

In this study, we used PASS to probe into the underlying cause of dissatisfaction by simultaneously assessing a wide range of MG-specific and -generic outcomes in a cohort of 100 patients with gMG. The results demonstrate that one-third of the included patients were dissatisfied with their current symptom state. Our findings show that dissatisfaction is associated with disease severity, short disease duration, depression, fatigue, and low MG-related quality of life.

High MG-ADL scores were associated with higher odds for dissatisfaction than the clinician-derived QMG, which underscores the relevance of subjective, patient-reported scores in assessing MG symptoms. No previous studies have compared PASS status with these MG measurements in the same group of patients.

The PASS-negative patients had higher self-reported physical- and general fatigue scores than the PASS-positive patients, and fatigue was strongly associated with PASS-negative status. Increasing general fatigue had almost similar odds for PASS-negative status as physical fatigue, emphasizing that in addition to physical fatigue as a core symptom in MG, general fatigue plays a vital role in the well-being and rehabilitation of patients with gMG.

One-fifth of the included patients had an MDI score indicating clinical depression, aligning with previous studies on depression in MG [23–26]. In non-MG studies that included patients with rheumatoid arthritis and multiple sclerosis, the prevalence of depression was found to be even higher (≥ 30%) [27, 28]. High MDI scores were a significant factor for PASS-negative status.

Comorbidity was not associated with PASS-negative status, which likely relates to the low occurrence of non-age-related comorbidities in the cohort. In comorbid patients, the most frequent diseases were diabetes type 2 and hypertension, with a prevalence corresponding to the general Danish population [29, 30]. As in the background population, there was a higher incidence of comorbidities with increasing age.

More PASS-negative patients reported low satisfaction with adverse treatment effects than PASS-positive patients. Prednisolone is known to cause undesirable adverse effects when used long-term and/or at high doses, which could

Table 2 Demographics and disease characteristics according to pass status

Characteristic	PASS negative ($n=33$)	PASS positive ($n=67$)	p value
Age, years	58.3 ± 14.0	61.1 ± 16.1	.409
Sex, women	18 (55%)	39 (58%)	.831
BMI, kg/m^2	28.9 ± 6.8	27.7 ± 5.7	.372
MG length, years	3 (1–6)	8 (4–15)	.001
Job-status, *n*			
Working full- or part-time	12 (37%)	26 (39%)	.033
Unemployed/sick leave	7 (21%)	3 (5%)	
Retired due to sickness	3 (9%)	2 (3%)	
Retired due to age	11 (33%)	32 (48%)	
Student	0	3 (5%)	
Antibody status, *n*			
AChR positive	29 (88%)	64 (97%)	.135
AChR negative	3 (9%)	2 (3%)	
MUSK positive	1 (3%)	0	
Thymectomy, *n*			
Yes	14	27	1.00
MG treatment ¶			
Pyridostigmine only	6	14	.028
Pyridostigmine + prednisolone	4	7	
Pyridostigmine + immunosuppressant	11	15	
Immunosuppressant only	3	1	
Immunosuppressant + prednisolone	4	26	
Pyridostigmine + prednisolone + immunosuppressant		4	
MG-ADL	5 (4–7)	1 (0–3)	< .0001
QMG	11 (9–13)	8 (5–10)	.0001
MGC	9 (4–13)	5 (2–8)	.001
MG-QoL15	25 (12–32)	6 (1–12)	< .0001
MFI-20 general fatigue	17 (12–18)	10 (7–14)	< .0001
MFI-20 physical fatigue	17 (14–18)	11 (9–14)	< .0001
MFI-20 reduced activity	13 (12–16)	9 (6–12)	< .0001
MFI-20 reduced motivation	9 (7–13)	7 (5–10)	.001
MFI-20 mental fatigue	11 (7–13)	8 (5–11)	.003
MDI	17 (12–25)	8 (3–14)	< .0001
EQ-5D-3L	0.71 (0.66–0.78)	0.83 (0.78–1.00)	< .0001
EQ-5D-VAS	60 (50–75)	80 (70–85)	< .0001
CCI	3 (1–5)	3 (2–5)	.767

Values are presented as mean ± standard deviation (SD), median and interquartile ranges (IQR), or as numbers and percentages (%)

BMI body mass index, *CCI* Charlson comorbidity index, *MG-ADL* MG Activities of Daily Living Profile, *MG-QoL15* MG-specific QoL instrument, *MDI* major depression inventory, *MGC* MG composite scale, *QMG* Quantitative MG score, *MFI-20* Multidimensional fatigue inventory. ¶ Immunosuppressant including azathioprine, methotrexate, tacrolimus, and mycophenolic acid

explain the dissatisfaction. An American register study [31] reported that 42% of patients received corticosteroids and 55–58% in a Canadian study [4]. These ratios are significantly higher than the 24% observed in our cohort, and we speculate if dissatisfaction with adverse effects is higher in other parts of the world. However, we found that MG treatment was not associated with PASS-negative status. Also, despite the frequent use of corticosteroids in the Canadian cohort [4], the proportion of PASS-negative patients was similar to our findings. However, the results might be biased by differences in the cohorts, as we exclusively enrolled patients with gMG.

Disease duration in the PASS-negative patients was more than halved compared to PASS-positive patients. A possible

Table 3 Associations of pass negative status

	PASS negative ($n=33$)	
	OR (CI)	p value
	Univariate analyses	
MG-ADL	1.83 (1.42–2.36)	<.0001
QMG	1.24 (1.10–1.40)	.0004
MG-QoL15	1.16 (1.10–1.23)	<.0001
MGC	1.16 (1.06–1.26)	.002
MDI	1.11 (1.06–1.17)	<.0001
Physical fatigue (MFI-20)	1.39 (1.20–1.60)	<.0001
General fatigue (MFI-20)	1.31 (1.16–1.48)	<.0001
MG duration, years	0.93 (0.87–0.99)	.020
Age, years	0.99 (0.96–1.02)	.392
Sex ¶	0.86 (0.37–2.00)	.728
BMI	1.03 (0.97–1.12)	.337
CCI	0.99 (0.81–1.19)	.876
Medical treatment, overall	0.95 (0.74–1.22)	.684
Medical treatment, prednisolone	2.62 (1.02–6.74)	.050
	Adjusted analyses	
MG-ADL	1.65 (1.22–2.23)	.001
Physical fatigue (MFI-20)	1.28 (1.09–1.51)	.003
General fatigue (MFI-20)	1.25 (1.06–1.46)	.007
QMG	1.23 (1.06–1.42)	.007
MG-QoL15	1.14 (1.06–1.23)	.0006
MDI	1.10 (1.03–1.17)	.003
MG duration, years	0.92 (0.86–0.99)	.016
MGC	1.01 (0.90–1.15)	.807
Age, years	0.99 (0.96–1.03)	.669
Sex ¶	0.79 (0.26–2.39)	.672
BMI	1.03 (0.95–1.13)	.444
CCI	0.89 (0.63–1.25)	.495
Medical treatment, overall	0.98 (0.72–1.34)	.901
Medical treatment, prednisolone	1.90 (0.39–9.40)	.430

Analyses were adjusted for age, sex, MG duration (years), MG severity (total score of QMG), and depression (total score of MDI). ¶ Women vs. men

BMI body mass index, *CCI* Charlson comorbidity index, *CI* confidence intervals, *MG-ADL* MG Activities of Daily Living profile, *MG-QoL15* MG- specific QoL instrument, *MDI* major depression inventory, *MGC* MG composite scale, *OR* odds ratio, *QMG* quantitative MG score, *MFI-20* multidimensional fatigue inventory

explanation might be that newly diagnosed patients still have not reached stabilization on optimal treatment (i.e., without unacceptable side effects) or are psychologically troubled by contracting a new, chronic disease that changes ADL and social functions.

In one retrospective validation cohort [4], PASS answers determined cut-off scores for MG-ADL, MGC, and MG-QoL15. Applying these cut-off scores to our population led to a proportion of 49–70% PASS-negative patients, which is different from the actual 33%. Although our cohort resembled previous cohorts [4] regarding age, sex, disease duration, and the number of PASS-negative patients, the cut-off points for MG measurements are not aligned. This might be explained by cultural differences or, more importantly, due to differences in study design, as our study is cross-sectional, while previous ones were retrospective studies.

We chose a wording of the PASS questions inspired by Mendoza et al. [4]. The Danish version of this PASS question has never been validated in Danish MG patients. However, as PASS is an approach to evaluating patient satisfaction individually, not a questionnaire, the wording differs between studies. Therefore, we believe that a validation of the Danish version is not needed.

The study design allowed us to combine PASS and MG measurements in one sample, which reduced the risk of selection bias. However, a total of 54 patients refused the study invitation for practical reasons. If lack of willingness relied on dissatisfaction with MG symptoms, this could lead to a selection of too many satisfied patients. However, none of the 54 patients expressed dissatisfaction as a reason for refusing, and we do not believe that these patients were more dissatisfied than the included patients. Still, it is not possible to estimate the impact of this potential selection bias.

We believe our findings represent the Danish MG population, as Danish MG patients are treated according to national guidelines and allocated at hospitals based on residence and not by disease severity or socioeconomic status. We used a combination of patient-reported and clinician-derived measurements, both MG-specific and generic, which allowed us to examine the PASS status from a broader perspective. The PASS question could be a valuable tool for assessing patient perspectives in follow-up and clinical trials based on this study. However, further evaluations of to what extent the PASS question applies to other MG populations are needed, primarily when treatment algorithms differ.

Conclusion

One-third of the included patients with gMG reported dissatisfaction with their current symptom state. These patients reported severe MG symptoms, low disease duration and MG-related quality of life, and high levels of fatigue and depression but resembled the remaining patients regarding demographics and disease status. The results suggest that a broader perspective on the disease, than strictly focusing on objective symptoms and treatment, is vital in the clinic to understand the underlying causes of dissatisfaction. For this, the PASS question could be a relevant and easy-to-use tool. A particular focus on newly diagnosed patients is crucial, as these patients seem more often dissatisfied.

Author contributions LKA and AJ have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Vissing. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: All authors. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Andersen. Obtained funding: Vissing. Administrative, technical, or material support: Andersen, Jakobsson. Supervision: Vissing.

Funding N/A.

Declarations

Conflicts of interest Vissing received an unrestricted grant from Argenx to cover salary and patient transport expenses. The company had no influence on study design or in the writing of the manuscript. Andersen, Jakobsson, and Revsbech report no disclosures.

Ethics approval The study was approved by the ethics committee of the Capital Region of Denmark.

Consent to participate Informed consent was obtained from all included patients.

Consent for publication N/A.

References

1. Gilhus NE (2016) Myasthenia Gravis. N Engl J Med 375(26):2570–2581
2. Hansen JS, Danielsen DH, Somnier FE, Frøslev T, Jakobsen J, Johnsen SP et al (2016) Mortality in myasthenia gravis: A nationwide population-based follow-up study in Denmark. Muscle Nerve 53(1):73–77
3. Wijeysundera DN, Johnson SR (2016) How much better is good enough?: Patient-reported outcomes, minimal clinically important differences, and patient acceptable symptom states in perioperative research. Anesthesiology 125(1):7–10
4. Mendoza M, Tran C, Bril V, Katzberg HD, Barnett C (2020) Patient-acceptable symptom states in myasthenia gravis. Neurology. https://doi.org/10.1212/WNL.0000000000010574
5. Menon D, Barnett C, Bril V (2020) Comparison of the single simple question and the patient acceptable symptom state in myasthenia gravis. Eur J Neurol 27(11):2286–2291
6. Tindall RS, Rollins JA, Phillips JT, Greenlee RG, Wells L, Belendiuk G (1987) Preliminary results of a double-blind, randomized, placebo-controlled trial of cyclosporine in myasthenia gravis. N Engl J Med 316(12):719–724
7. Burns TM, Conaway MR, Cutter GR, Sanders DB (2008) Muscle study. Group construction of an efficient evaluative instrument for myasthenia gravis: the MG composite. Muscle Nerve 38(6):1553–1562
8. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ (1999) Myasthenia gravis activities of daily living profile. Neurology 52(7):1487–1489
9. Vissing J, Jacob S, Fujita KP, O'Brien F, Howard JF (2020) REGAIN study group "Minimal symptom expression" in patients with acetylcholine receptor antibody-positive refractory generalized myasthenia gravis treated with eculizumab. J Neurol 267(7):1991
10. Burns TM, Conaway MR, Cutter GR, Sanders DB (2008) Muscle study. Group Less is more, or almost as much: a 15-item quality-of-life instrument for myasthenia gravis. Muscle Nerve 38(2):957–963
11. Smets EM, Garssen B, Bonke B, De Haes JC (1995) The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 39(3):315–325
12. Mikula P, Nagyova I, Krokavcova M, Vitkova M, Rosenberger J, Szilasiova J et al (2015) The mediating effect of coping on the association between fatigue and quality of life in patients with multiple sclerosis. Psychol Health Med 20(6):653–661
13. Visser MM, Goodin P, Parsons MW, Lillicrap T, Spratt NJ, Levi CR et al (2019) Modafinil treatment modulates functional connectivity in stroke survivors with severe fatigue. Sci Rep 9(1):9660
14. Lexell J, Jonasson SB, Brogardh C (2018) Psychometric properties of three fatigue rating scales in individuals with late effects of polio. Ann Rehabil Med 42(5):702–712
15. Bech P, Rasmussen NA, Olsen LR, Noerholm V, Abildgaard W (2001) The sensitivity and specificity of the major depression inventory, using the present state examination as the index of diagnostic validity. J Affect Disord 66(2–3):159–164
16. Olsen LR, Jensen DV, Noerholm V, Martiny K, Bech P (2003) The internal and external validity of the major depression inventory in measuring severity of depressive states. Psychol Med 33(2):351–356
17. Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U (2017) Exercise in myasthenia gravis: A feasibility study of aerobic and resistance training. Muscle Nerve 56(4):700–709
18. Paul RH, Cohen RA, Goldstein JM, Gilchrist JM (2000) Fatigue and its impact on patients with myasthenia gravis. Muscle Nerve 23(9):1402–1406
19. Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40(5):373–383
20. Mouri H, Jo T, Matsui H, Fushimi K, Yasunaga H (2020) Effect of sugammadex on postoperative myasthenic crisis in myasthenia gravis patients: propensity score analysis of a japanese nationwide database. Anesth Analg 130(2):367–373
21. EQ-5D [Internet]. [cited 2020 Sep 28]. Available from: https://euroqol.org/
22. Wittrup-Jensen KU, Lauridsen J, Gudex C, Pedersen KM (2009) Generation of a danish TTO value set for EQ-5D health states. Scand J Public Health 37(5):459–466
23. Gavrilov YV, Alekseeva TM, Kreis OA, Valko PO, Weber KP, Valko Y (2020) Depression in myasthenia gravis: a heterogeneous and intriguing entity. J Neurol 267(6):1802–1811
24. Alekseeva TM, Gavrilov YV, Kreis OA, Valko PO, Weber KP, Valko Y (2018) Fatigue in patients with myasthenia gravis. J Neurol 265(10):2312–2321
25. Hoffmann S, Ramm J, Grittner U, Kohler S, Siedler J, Meisel A (2016) Fatigue in myasthenia gravis: risk factors and impact on quality of life. Brain Behav 6(10):e00538
26. Kittiwatanapaisan W, Gauthier DK, Williams AM, Oh SJ (2003) Fatigue in myasthenia gravis patients. J Neurosci Nurs J Am Assoc Neurosci Nurs 35(2):87–93
27. Matcham F, Rayner L, Steer S, Hotopf M (2013) The prevalence of depression in rheumatoid arthritis: a systematic review and meta-analysis. Rheumatol Oxf Engl 52(12):2136–2148
28. Boeschoten RE, Braamse AMJ, Beekman ATF, Cuijpers P, van Oppen P, Dekker J et al (2017) Prevalence of depression and anxiety in multiple sclerosis: A systematic review and meta-analysis. J Neurol Sci 372:331–341
29. Carstensen B, Rønn PF, Jørgensen ME (2020) Prevalence, incidence and mortality of type 1 and type 2 diabetes in Denmark

1996–2016. BMJ Open Diabetes Res Care. https://doi.org/10.1136/bmjdrc-2019-001071
30. Ibsen H, Jørgensen T, Jensen GB, Jacobsen IA (2009) Hypertension–prevalence and treatment. Ugeskr Laeger 171(24):1998–2000
31. Cutter G, Xin H, Aban I, Burns TM, Allman PH, Farzaneh-Far R et al (2019) Cross-sectional analysis of the myasthenia gravis patient registry: disability and treatment. Muscle Nerve 60(6):707–715

Two- and 6-minute walk tests assess walking capability equally in neuromuscular diseases

Linda Kahr Andersen, PT, MSc*
Kirsten Lykke Knak, PT, MSc*
Nanna Witting, MD, PhD
John Vissing, MD, PhD

Correspondence to L.K. Andersen: linda.kahr.andersen@regionh.dk

ABSTRACT

Objective: This methodologic study investigates if the 2-minute walk test (2MWT) can be a valid alternative to the 6-minute walk test (6MWT) to describe walking capability in patients with neuromuscular diseases.

Methods: Patients (n = 115) with different neuromuscular diseases were invited to participate on 2 test days, each consisting of 1 2MWT and 1 6MWT separated by a minimum 30-minute period of rest. The order of the walk tests was randomly assigned via sealed envelopes. A group of 38 healthy controls completed 1 6MWT.

Results: The mean walking distance for the 2MWT was 142.8 meters and for the 6MWT 405.3 meters. The distance walked in the 2MWT was highly correlated to the distance walked in the 6MWT ($r = 0.99$, $p < 0.001$). There was a significant decrease in walking speed from the first to last minute in the 6MWT, both among patients and healthy controls, which was not evident in the 2MWT. Results were consistent across diagnoses and levels of disease severity.

Conclusion: The 2MWT is a potential alternative to the 6MWT to describe walking capability among patients with neuromuscular diseases during clinical trials. *Neurology*® 2016;86:442–445

GLOSSARY

2MWT = 2-minute walk test; **6MWT** = 6-minute walk test; **MRC** = Medical Research Council.

The 6-minute walk test (6MWT) is widely used to measure treatment efficacy and disease progression in patients with neuromuscular diseases. However, the 6MWT is time-consuming and often not tolerated by patients with severe lower limb muscle weakness. A considerable variation is found in repeated 6MWTs due to a learning effect,[1–3] and pretrial walk tests are recommended to improve reliability.

Three previous studies have found that the 2-minute walk test (2MWT) is a valid alternative to the 6MWT when assessing walking capability in patients with multiple sclerosis,[4] sporadic inclusion body myositis,[5] and stroke,[6] but it is unknown whether the 2MWT can be an alternative to the 6MWT in patients with a wide range of neuromuscular diagnoses.

The objective of the present study was to examine if the 2MWT and 6MWT assess walking capability equally well in different neuromuscular diseases with a wide range of disease severities.

METHODS

Patients were recruited from our Neuromuscular Center by consecutive sampling from March 2014 to January 2015. Patients had variable weakness of proximal and distal muscles according to their primary disease of muscle, motor neurons, or peripheral nerves. The inclusion criteria were age ≥18 years, ability to walk ≥60 meters in a 6MWT, and a genetic or biopsied proven neuromuscular diagnosis. The exclusion criteria were any other medical condition that could interfere with the interpretation of walking capability, e.g., heart failure. Patients who walked <60 meters in 6 minutes were excluded due to a potential variability of performance that may occur with a marginal ambulatory status.

Patients were invited to participate in 2 test days, separated by 1–2 weeks. The second day was used to test whether a learning effect potentially would alter any relationship found between the 2MWT and 6MWT on the first test day. Each test day consisted of 1 2MWT and 1 6MWT, performed randomly via sealed envelopes, and separated by 30 minutes of rest.

The walk tests were administered according to the American Thoracic Society Guidelines.[7] Habitual assistive devices were permitted, and if needed, were used on both days.

*These authors contributed equally to this work.

From the Copenhagen Neuromuscular Center, Department of Neurology, Rigshospitalet, University of Copenhagen, Denmark.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

On the first test day, bilateral muscle strength was assessed in the lower limb (flexion/extension in ankle, knee, and hip) by manual muscle testing (Medical Research Council [MRC] scale).

A total of 115 patients with the following diagnoses were included: myotonic dystrophy type 1, limb-girdle muscular dystrophy (types 1B, 2A, 2I, 2L), facioscapulohumeral dystrophy, Kennedy disease, Charcot-Marie-Tooth neuropathy (CMT1A and CMTX), mitochondrial myopathy, sporadic inclusion body myositis, Becker muscular dystrophy, and a mixed group of myopathies. Weak patients were a priori defined as patients with one or more muscle groups in the lower limb with a MRC score ≤4, and strong patients with universal strength above 4.

A total of 84 patients (73%) were classified as weak. Thirty-three patients used assistive devices (cane or wheeled walker). Ninety-three of the 115 patients participated in the second test day. Thirty-eight healthy controls were included to examine walking speed and fatigue during the 6MWTs. For characteristics of the study population, see the table.

Standard protocol approvals, registrations, and patient consents. The study was approved by the ethics committee of the capital region (approval H-4-2014-FSP), and informed consent was obtained from all involved participants.

Statistical analyses. Results are expressed as the mean ± SEM and range. Differences between the predicted and performed 6MWTs, and differences between the walking speed in the first and last minute of the walking tests, were assessed by a paired Student t test. A $p \leq 0.05$ (2-tailed testing) was considered significant. Pearson correlation coefficient was used to examine the relationship between the 2MWT and 6MWT. Subgroup analyses were performed to identify potential deviations from the main findings.

RESULTS

Correlation between walking tests. The distance walked in the 2MWT was highly correlated to that in the 6MWT ($r = 0.99$, $p < 0.001$) for the overall study population (figure 1) with similar results for the diagnosis subgroups (table). In the 93 patients who performed 2 test days, the correlation was $r = 0.98$, $p < 0.001$ on day 1, and $r = 0.99$, $p < 0.001$ on day 2. Thus, a learning effect does not influence the high correlation between 2MWT and 6MWT.

Walked distance. The mean walking distance on the first test day for the 2MWT was 142.8 meters (range 30–258 meters) and for the 6MWT 405.3 meters (range 65–750 meters). When comparing the mean walked distance in the performed 6MWT with the predicted 6MWT (the distance walked in the 2MWT

Table Population characteristics, correlation between 2MWT and 6MWT, and walking speed for subgroups

Characteristics of the study population	No.	Male/female	Age, y	BMI	Height, cm
Patients	115	75/40	52.6 (22-83)	25.2 (16.1-44.1)	173.9 (138-198)
Healthy	38	19/19	47.6 (25-76)	25.1 (19.6-33.5)	175.6 (155-192)
Weak patients	84	55/29	54.3 (22-83)	25.9 (16.1-44.1)	174.1 (138-196)
Strong patients	31	21/10	48.1 (24-79)	23.5 (16.8-38.3)	173.2 (154-198)

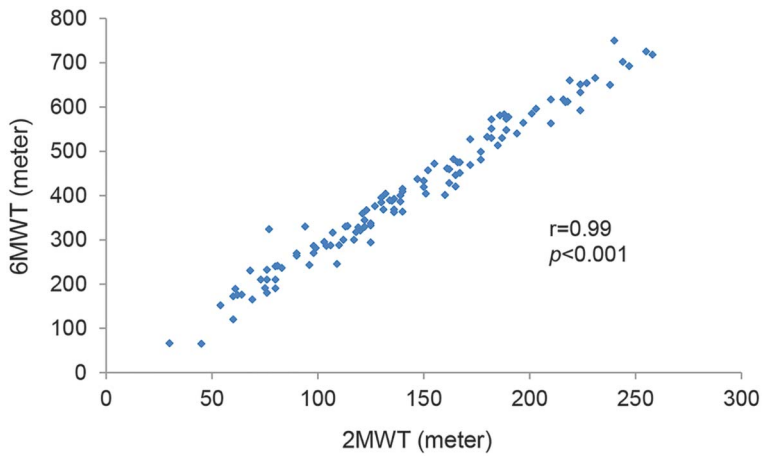
			Walking speed in 6MWT, m/s			
Correlation and walking speed for subgroups	No.	Correlation, r ($p < 0.001$)	First minute	Last minute	Decrease in speed, m/s	p Value
DM	20	0.99	1.39	1.34	0.05	0.01
LGMD	18	0.99	1.07	1.01	0.06	<0.001
FSHD	15	0.99	1.31	1.26	0.05	0.02
Kennedy disease	14	0.95	1.02	0.92	0.10	<0.001
CMT	14	0.96	1.2	1.18	0.04	0.17
Mitochondrial myopathy	10	0.99	1.18	1.11	0.07	0.03
IBM	10	0.98	0.73	0.73	0	0.53
BMD	8	0.99	1.43	1.36	0.07	0.04
Mixed[a]	6	0.99	1.18	1.13	0.05	0.12
Weak patients	84	0.98	1.06	1.01	0.05	<0.001
Strong patients	31	0.99	1.51	1.45	0.06	<0.001

Abbreviations: 2MWT = 2-minute walk test; 6MWT = 6-minute walk test; BMD = Becker muscular dystrophy; BMI = body mass index; CMT = Charcot-Marie-Tooth neuropathy (CMT1A and CMTX); DM = myotonic dystrophy type 1; FSHD = facioscapulohumeral dystrophy; IBM = sporadic inclusion body myositis; LGMD = limb-girdle muscular dystrophy (types 1B, 2A, 2I, 2L).

Data are from the first test day (n = 115). The top of the table shows age, BMI, and height of the participants. Values are mean (range). The lower left part of the table shows correlation between walking distance in the 2MWT and 6MWT for individual diagnostic subgroups. The lower right part of the table shows walking speed in the first and last minute of the 6MWT. Weak patients were defined as those with one or more muscle groups in the lower limb with a Medical Research Council score ≤4, and strong patients as those with universal strength above 4.

[a] Mixed group of myopathies (spinal muscular atrophy [n = 3], myotonia congenita [Thomsen disease] [n = 1], congenital myopathy [n = 1], polymyositis [n = 1]).

Figure 1 Correlation between distances walked in the 2- and 6-minute walk tests (2MWT and 6MWT)



Data from the first test day (n = 115).

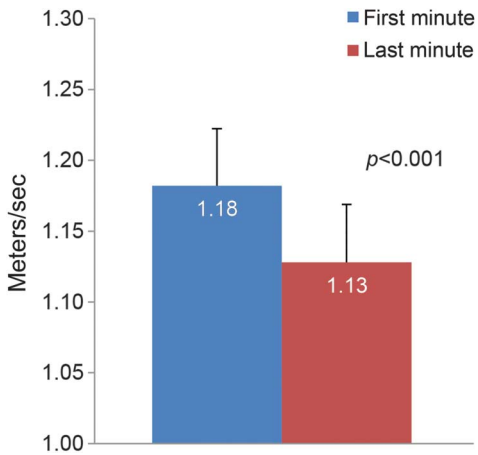
multiplied by 3 = 427.9 meters), the walked distance was shorter ($p < 0.001$) in the performed 6MWT.

The mean walked distance for patients classified as weak was 128.0 meters (range 30–247 meters) for the 2MWT and 362.1 meters (range 65–692 meters) for the 6MWT.

Walking speed. There was a small decrease in the walking speed (m/s) between the first and second minute in the 2MWT (1.21 vs 1.19 m/s in the 2nd minute, $p < 0.001$) on the first test day, which could not be detected ($p = 0.76$) on the second test day.

Walking speed in the 6MWT decreased by 4.6% from the first (1.18 m/s) to the last minute (1.13 m/s) ($p < 0.001$) on the first test day in patients with neuromuscular diseases (figure 2). Decrease in walking speed was seen across disease severities and in most of the disease subgroups (table).

Figure 2 Walking speed in the first and last minute of the 6-minute walk test



Data from the first test day (n = 115). The error bars show SEM.

A decrease of 1.4% in walking speed from the first (1.93 m/s) to last minute (1.90 m/s) ($p = 0.01$) was found in the healthy controls. The absolute fall in walking speed did not differ between patients and healthy controls ($p = 0.10$), but the percentage drop was higher in the patients ($p = 0.002$).

DISCUSSION

The main finding of the present study is that walking distance in 2MWT and 6MWT is highly correlated across multiple neuromuscular diseases of varying disease severities. This suggests that the 2MWT is a valid alternative to the widely used 6MWT to assess walking capability in patients with neuromuscular diseases who have a minimum walking distance of 60 meters in 6 minutes.

It has been shown that the 6MWT captures fatigue in patients with spinal muscular atrophy[8] and in patients with other diseases than neuromuscular diseases.[9] Fatigue among healthy adults in the 6MWT has also been found in one study.[2] We found a significant decrease in walking speed in the 6MWT in all participants, showing that the 6MWT is suitable to capture fatigue in patients with different neuromuscular diseases. We also found that the level of fatigue as indicated by the absolute drop in walking speed was similar among patients and healthy controls. In this perspective, a decrease in walking speed can also be interpreted as a matter of motivation, or strategic approach to the walk test common for all participants, indicating that the 6MWT does not necessarily provide additional clinically relevant information about fatigue as a part of the patient's neuromuscular disease.

Our results show that the 2MWT is too short to capture fatigue. The variable response in walking speed found between the first and second 2MWT emphasizes the importance of performing test trials to accustom test participants to experimental conditions.

We excluded patients with a walking distance <60 meters in 6 minutes. This can be a limitation of the study since our results may not apply to patients with severely impaired gait function.

The results of the present study are in line with a previous study showing that the 2MWT can be a valid alternative to the 6MWT for patients with the specific neuromuscular disease sporadic inclusion body myositis.[5] Our study expands these findings to a heterogeneous group of neuromuscular diseases with highly variable phenotypes.

AUTHOR CONTRIBUTIONS

L.K.A. and K.L.K. contributed to the conception and design of the study, made the statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. N.W. contributed to the conception and design of the study, provided critical input and advice during analysis and writing process, reviewed the manuscript, and approved the final

manuscript as submitted. J.V. conceptualized and designed the study, participated in the analysis and interpretation of results, reviewed the manuscript, and approved the final manuscript as submitted.

STUDY FUNDING

No targeted funding reported.

DISCLOSURE

L. Andersen, K. Knak, and N. Witting report no disclosures relevant to the manuscript. J. Vissing has received research and travel grants from Genzyme Corporation and serves on the global scientific advisory board for Pompe disease for Genzyme Corporation. Go to Neurology.org for full disclosures.

Received June 19, 2015. Accepted in final form October 7, 2015.

REFERENCES

1. Prahm KP, Witting N, Vissing J. Decreased variability of the 6-minute walk test by heart rate correction in patients with neuromuscular disease. PLoS One 2014;9:e114273.
2. Gibbons WJ, Fruchter N, Sloan S, Levy RD. Reference values for a multiple repetition 6-minute walk test in healthy adults older than 20 years. J Cardiopulm Rehabil 2001;21:87–93.
3. Casanova C, Celli BR, Barria P, et al. The 6-min walk distance in healthy subjects: reference standards from seven countries. Eur Respir J 2011;37:150–156.
4. Gijbels D, Eijnde BO, Feys P. Comparison of the 2- and 6-minute walk test in multiple sclerosis. Mult Scler 2011; 17:1269–1272.
5. Alfano LN, Lowes LP, Dvorchik I, et al. The 2-min walk test is sufficient for evaluating walking abilities in sporadic inclusion body myositis. Neuromuscul Disord 2014;24: 222–226.
6. Kosak M, Smith T. Comparison of the 2-, 6-, and 12-minute walk tests in patients with stroke. J Rehabil Res Dev 2004;41:103.
7. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–117.
8. Montes J, McDermott MP, Martens WB, et al. Six-Minute Walk Test demonstrates motor fatigue in spinal muscular atrophy. Neurology 2010;74:833–838.
9. Goldman MD, Marrie RA, Cohen JA. Evaluation of the six-minute walk in multiple sclerosis subjects and healthy controls. Mult Scler 2008;14:383–390.

European Journal of Physiotherapy

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iejp20

Effects of rhythmic auditory stimulation on walking during the 6-minute walk test in patients with generalised Myasthenia Gravis

Linda Kahr Andersen , Nanna Witting & John Vissing

To cite this article: Linda Kahr Andersen , Nanna Witting & John Vissing (2021): Effects of rhythmic auditory stimulation on walking during the 6-minute walk test in patients with generalised Myasthenia Gravis, European Journal of Physiotherapy, DOI: 10.1080/21679169.2021.1876760

To link to this article: https://doi.org/10.1080/21679169.2021.1876760

Published online: 27 Jan 2021.

EUROPEAN JOURNAL OF PHYSIOTHERAPY
https://doi.org/10.1080/21679169.2021.1876760

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Effects of rhythmic auditory stimulation on walking during the 6-minute walk test in patients with generalised Myasthenia Gravis

Linda Kahr Andersen, Nanna Witting and John Vissing

Department of Neurology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

Introduction: Rhythmic auditory stimulation (RAS) has been shown to improve gait parameters in several neurological diseases, both in walk-training interventions and in one-time walking tests, but the effect in myasthenia gravis (MG) is unknown.

Objective: The aim of this study was to examine if RAS improves walking distance and gait speed in patients with generalised myasthenia gravis (gMG) in the 6-minute walk test (6MWT).

Methods: Forty-eight patients with gMG walked two 6MWTs under different conditions: (1) walking with RAS with a frequency of 100% of the patient's fastest gait speed, (2) walking with RAS with a frequency of 110% of the patient's fastest gait speed, or (3) walking in silence.

Results: RAS with a frequency of 110% of the patient's fastest gait speed increased the walking distance by 8.3 metres in the 6MWT vs standard 6MWT ($p = 0.01$), without increasing average walking heart rate (HR) or Borg scores.

Conclusions: This study indicates that RAS may improve gait speed and walking distance in patients with gMG without additional exertion as judged by HR and Borg scores. Based on these results, RAS could be used as part of a physical rehabilitation program for patients with gMG.

ARTICLE HISTORY
Received 22 September 2020
Revised 1 January 2021
Accepted 8 January 2021

KEYWORDS
Rehabilitation; neurology; 6-min walk test; rhythmic auditory stimulation; myasthenia gravis

Introduction

Myasthenia gravis (MG) is a chronic, autoimmune disease with a well-known pathogenesis. Blocking of signal transduction and destruction of the synaptic components results in fluctuating strength of voluntary muscles, and distinct muscular fatigue with repeated contractions of skeletal muscles. MG affecting other muscles than ocular muscles is characterised as generalised myasthenia gravis (gMG).

As fatigue can be difficult to define, a proposal of a taxonomy of fatigue has been published I 2013 [1]. In this taxonomy, fatigue is divided into a performance-based fatigability and a fatigue perception. As the performance-based fatigability includes both cognitive and physical performances, the term 'muscle fatigability' is used in this study. This term defines the fatigability in skeletal muscles after repeated muscle contractions.

Muscle fatigability is known to occur in patients with MG after continued physical activity, e.g. when walking. Even for short distances of 400 m, and in timed walk tests, such as the 6-minute walk (6MWT), patients with MG have reduced gait speed and walk distance compared to healthy persons [2–4]. The 6MWT has been found useful and reliable to measure walking-related fatigability in patients with neuromuscular diseases, including patients with MG [3–8].

Auditory cues, also referred to as rhythmic auditory stimulation (RAS), is the concept of using e.g. music to synchronise movement and auditory stimuli [9]. The effect of auditory cueing relies on entrainment, which is defined by a temporal locking process in which one system's signal frequency affects the frequency of another system, meaning that e.g. external stimuli as rhythm from music is synchronised with human movement [10,11]. The neural basis of entrainment relies on the connections between the auditory and the motor systems [12].

RAS has been shown to improve gait parameters in repeated walk-training interventions in individuals affected by stroke, traumatic brain injury, Parkinson's disease, and multiple sclerosis [13–17]. However, improvements in spatio-temporal gait parameters, such as gait speed and stride length, are also found in one-time walking tests for patients with multiple sclerosis [18], Parkinson's disease [19] and chronic obstructive pulmonary diseases [20]. The effect of RAS has never been examined in patients with MG, but we hypothesise that RAS will influence on walk-related fatigability measured by gait speed and distance walked in these patients. If RAS is effective, this would add valuable knowledge to improve rehabilitation programs for patients with MG.

The objective of this study was to compare gait speed and distance walked in patients with gMG when performing

CONTACT Linda Kahr Andersen linda.kahr.andersen@regionh.dk Department of Neurology, Rigshospitalet, University of Copenhagen, Inge Lehmanns Vej 8, Copenhagen 2100, Denmark

a 6MWT with RAS (with a rhythm corresponding to either 100% or 110% of the patient's fastest gait speed) vs. a 6MWT without auditory stimulus.

Materials and methods

Participants

Patients with gMG, 18-80 years of age, were invited to participate in a 2-hour visit for testing. The patients were recruited from our neuromuscular clinic during their routine follow-up visit with their neurologist. The diagnosis of gMG was based on a typical clinical history and symptom improvement with acetylcholinesterase inhibitors coupled with either positive acetylcholine receptor antibodies and/or significant decrement/increased jitter on electromyography. Patients with a medical condition that could interfere with the interpretation of walking capability, e.g. heart failure or rheumatoid arthritis, were excluded. The included patients were treated by pyridostigmine, immunosuppressive drugs or a combination of both. For patients taking pyridostigmine, the tests were performed ½–2 h after intake of the drug.

Patients interested in participating were given verbal and written information about the study and those who accepted participation gave their written consent. This study was approved by the ethics committee of the Capital Region of Denmark (approval H-18031231).

Procedures

Patients completed a test battery conducted by one assessor. Two 6MWTs were completed in a random order by block randomisation, using sealed envelopes. RAS was applied during one of the 6MWTs, the other 6MWT was standard. Patients walked up and down a 30-metres walking course turning around cones at each end. The 6MWTs were administered according to the American Thoracic Society (ATS) Guidelines [21]. A timer started at the beginning of the test, keeping track of the 6 min. For every 30 metres, when the patient turned around a cone, the time was registered. As in previous studies [5,7], walking-related fatigability was determined by subtracting the gait speed in the first minute from the gait speed in the sixth minute.

MG severity was measured by the Myasthenia Gravis Composite Scale (MGC) [22]. The MGC consists of 10 items of objective and subjective character with a summary score from 0-50 (most severe = 50). A psychometric, multicentre study found that the MGC is a reliable and valid instrument to measure clinical status of patients with MG [23,24].

The order of tests was:

- Short 60-metre walk test to determine fastest gait speed
- MGC
- First 6MWT (with/without RAS)
- 30 min break
- Second 6MWT (with/without RAS)

In the initial 60-metre walk test, patients were asked to walk 60 metres "*as fast as possible, without running*", to determine the fastest gait speed. The distance of 60 metres was chosen for practical reasons, as it corresponded to one lap on the 30-m walk course. This length was expected not to exhaust patients while being long enough for the patient to gain confidence with the pace and rhythm in the walk test.

Average steps per minute were recorded, using an App, named BPM (version 3.04, developed by CHEEBOW), downloaded on an iPhone 7. For every single step the patient took in the 60-metre walk test, the tester tapped once on the phone, indicating a step. The recording started at the patient's first step and ended when the patient had completed one lap. The app calculated the average steps per minute during the 60 m, which was noted for each patient. The average steps per minute corresponded to the beat per minute in the music used in the 6MWT with RAS (with a frequency of 100%).

The auditory stimuli were vocal pop or rock music with a distinct rhythm. Playlists of music with different BPMs were made in advance. Prior to the 6MWTs with RAS, the rhythm of the music was indicated to the patient by clapping and stepping. The instruction, additional to the instructions of the ATS guidelines was: 'When you start to walk, I will play some music for you. Try to match your steps to the rhythm of the music. If you find it difficult to follow the rhythm, then focus on trying to walk as far as you can in the 6 min'.

The patient's heart rate (HR) was measured by a pulse watch (Suunto Quest, model SS018153000). The average HR was calculated by the pulse watch, based on all the HR's measured over the entire 6 min. Patients were asked beforehand to refrain from caffeine-containing beverages and smoking on the test day, to reduce the influence on HR. After each of the walk tests, patients were asked to determine 'how exhausting did you find the test?' on the Borg Scale of Perceived Exertion [25], where they could state their subjective experience of exhaustion from 6 ('very, very easy') to 20 (**"**very, very exhausting).

As no previous study has estimated the potential effect size of RAS or has examined the relationship between effect size and frequency of RAS in MG patients, we were unable to make a reasonable power calculation before the study start. Therefore, we started out with a RAS frequency of 100% of the patient's fastest gait speed. After 18 patients had completed the 6MWT, we did a post hoc sample size calculation based on the obtained effect size of RAS at this point (4.5 metres). These results suggested a sample size of 240 patients, which was not realistic to perform. To examine if a faster frequency of RAS would result in a larger effect size, the following 30 patients therefore walked to a frequency of 110% of the patient's fastest gait speed. A power calculation was performed after the first 12 patients had completed this protocol. The obtained effect size of RAS as this point was 9.7 m with a frequency of 110%, which would require a sample size of 30 patients. These two groups of patients were subsequently named FAST ($n = 18$) (100%) and FAST10 ($n = 30$) (110%). As it is common practice to calculate sample sizes before the study start, the choice of post hoc

sample size calculation was discussed with statistical experts. As no previous published effect sizes of RAS in patients with MG exist, the post hoc sample size calculation was recommended.

Statistical analyses

Age, BMI, walking distance, gait speed, and HR measurements were presented by means and standard deviation (SD). MGC and Borg scores were presented by medians and inter-quartile ranges (IQR) due to non-normal distributions. Normality was assessed visually by histograms and boxplots. Distribution of characteristics within or between the groups FAST and FAST10 was assessed by Fisher's exact test for sex differences, by Student's *t*-test for age and BMI, by paired t-test for walking distance, gait speed and HR average, and by Wilcoxon rank-sum test for MGC and differences in Borg scores.

To investigate the effect of RAS on walking distance and gait speed, a linear mixed model was conducted with age, sex, BMI, MGC and order of test as covariates. To account for the correlation in the repeated measurements as well as possible changes in variance, an unstructured covariance pattern was assumed. The following model assumptions were checked and in case of violation, the analyses were conducted on log10-transformed data: multivariate normal distribution, homogeneity of residuals, and convergence criteria.

Sample size calculations were based on alpha level = 0.05, power = 0.8, two-tailed test.

In general, a $p \leq 0.05$ (two-tailed testing) was considered significant. All statistical analyses are carried out using SAS enterprise guide 7.1.

Results

Demographics

A total of 48 patients (34 women) met the criteria for inclusion. The overall mean age of the sample was 55 (16.8) years, the BMI was 27 (6.6) and the median score (IQR) of MGC was 7.5 (3.5–13.5). Distribution of characteristics in the FAST and FAST10 groups can be seen in Table 1.

Gait parameters

In the FAST10 group (110% of fastest gait speed), walking distance in the RAS 6MWT was 8.3 (CI: 1.82–14.78, $p = 0.01$) metres longer than in the standard 6MWT. The order of tests ($p = 0.36$), the MG severity (MGC, $p = 0.07$) and sex ($p = 0.56$) did not influence on walked distance. However, increasing age and BMI decreased the walking distance (age; estimate: −2.10, CI: −3.29; −0.91, $p < 0.01$, BMI; estimate: −3.15, CI: −5.75; −0.55, $p = 0.02$).

In the FAST group (100% of fastest gait speed), walking distance in the 6MWT with RAS was 4.5 (CI: −7.91–16.91, $p = 0.45$) metres longer than in the standard 6MWT. However, this result was statistically insignificant.

Within each group there was a significant decrease in gait speed in both standard and RAS 6MWT, indicating walking-related fatigability. Gait speed in both the first and sixth minute of the RAS 6MWT was faster compared to standard 6MWT, indicating that RAS increased gait speed throughout the 6MWT.

We would have expected a nominal increase in average HR and Borg score due to a higher gait speed in the RAS tests, but surprisingly no increase was seen.

For the overall sample ($n = 48$), the mean (SD) walking distance in the standard 6MWT was 558 (67.8) metres, and 565 (72.9) metres in the RAS test. Gait parameters specified in the groups FAST and FAST10 are presented in Table 1.

Discussion

In this study, we found that RAS improved walking distance and gait speed in patients with gMG, when the RAS frequency was set 10% higher than the patients' fastest walking speed. Surprisingly, this did not result in increased average

Table 1. Patients characteristics and gait parameters.

	FAST ($n = 18$)	*p*-value	FAST10 ($n = 30$)	*p*-value
Sex	5 men (28%) 13 women (72%)		9 men (30%) 21 women (70%)	1.00
Age, mean (SD), years	57 (17.9)		54 (16.3)	0.59
BMI, mean (SD), kg/m^2	27 (5.6)		28 (7.2)	0.70
MGC, median (IQR), total score	6 (3-15)		9 (4-13)	0.69
Walking distance, mean (SD), metre				
Standard 6MWT	544 (77.2)	0.45	567 (61.2)	0.01
RAS 6MWT	548 (85.3)		575 (63.7)	
Gait speed standard 6MWT, mean (SD), m/s				
First minute	1.54 (0.22)	0.02	1.61 (0.16)	<0.01
Sixth minute	1.51 (0.21)		1.57 (0.17)	
Gait speed RAS 6MWT, mean (SD), m/s				
First minute	1.58 (0.25)	<0.01	1.62 (0.18)	<0.01
Sixth minute	1.52 (0.24)		1.59 (0.18)	
HR average, mean (SD), BPM				
Standard 6MWT	118 (18.7)	0.06	115 (16.9)	0.44
RAS 6MWT	109 (18.5)		113 (14.3)	
Borg, median (IQR), score				
Standard 6MWT	11.5 (10-13)	0.88	13.0 (11-14)	0.90
RAS 6MWT	11.0 (10-13)		13.0 (11-15)	

HR or Borg score. Increasing age and BMI, decreased walking distance, whereas sex, MG severity and the order of tests did not seem to have any influence on the results.

Our results are in line with previous studies finding that increase of RAS frequency leads to an increase in gait speed and walking distance in neurological conditions [20,21,27,28]. The mechanism behind RAS and the subsequent improvement of gait parameters is explained in several studies [11]. The mechanism is based on entrainment, where the auditory stimuli and the steps during walking align due to synchronisation. This synchronisation results in increasing gait speed with increasing RAS frequency.

The increase of walking distance in the RAS 6MWTs did not lead to increases in average HR or Borg score. This was surprising, as it is basic knowledge that increasing workload leads to an increase in HR, also in neuromuscular patients [26]. However, these findings may be explained by the locomotor–respiratory coupling (LRC), where the rhythm of a subject's respiration, synchronises to the rhythm of locomotion. LRC is seen in various species and in different kinds of movement, including human walking, and is found to significantly lower the energy expenditure. Also, RAS has been found useful to synchronise the locomotor- and the respiratory systems [27,28]. If we assume that this happened in this study, the stimuli of RAS may have facilitated the synchronisation of the patient's respiration and walking steps in the 6MWT. This will, according to previous findings, lead to a reduction in exergy expenditure, which can explain the lowering or stabilisation of HR and Borg in this study. Further studies are needed to confirm this, but the approach could be potentially useful and relevant in rehabilitation programs for patients with MG and severe fatigue.

The choice of RAS frequencies (100% and 110%) was mostly arbitrary due to lack of results from previous studies in MG patients. The chosen frequencies in this study were obtained in a pragmatic balance between obtaining an effect of RAS, and a realistic gait speed for the patients. It could be speculated, if a further increase of RAS frequency, e.g. 120%, could lead to a further increase of gait speed. This was seen in one study [29], where an increase of 20% of baseline walking speed resulted in the highest increase of gait velocity (but also a decrease of gait quality). However, in contrast to that study, the increase of 10% in this study, was based on a walk velocity already corresponding to the patient's fastest gait speed. Another study [30], found that increases above 8% of the preferred walking cadence resulted in an decrease in the ability to synchronise with the music. Based on these results, we presume that an additional increase above the 10% in patients with MG, would result in a decrease in gait quality and synchronisation to stimuli, but further studies are needed to test this.

Inspired by findings from previous studies, where instruction enhanced synchronisation [31,32], we instructed the patients to follow the rhythm in the music, when walking the 6MWT with RAS. However, some patients were concerned if they could succeed with it, often stating 'I am not very musical'. For these patients, the focus on following the rhythm, seemed to slow down their walking pace, and thereby lower the synchronisation between RAS and steps. However, it might be, that the RAS frequency was too high for these patients, and due to muscular fatigue, they had difficulties in following the rhythm. As discussed in another paper [31], synchronisation of RAS and steps, occurs more often when the RAS frequency matches to the walking cadence. As we did not measure synchronisation in this study, it is difficult to verify this, but this may have diminished the effect of RAS, and thereby weakened our results.

The only factors, despite RAS, that seemed to influence on walking distance and gait speed was age and BMI. An increase of age and BMI reduced the walking distance and the pace. These findings were expected, as this is already seen in the 6MWT for healthy adults [33]. It was more surprising that MG severity did not influence on the walking distance ($p = 0.07$). However, this result is in line with a previous study that found no correlation between MG severity and physical fatigability [3].

In a previous study of a heterogeneous group of neuromuscular patients ($n = 115$) [5], we found a mean walking distance of 405 metres and a mean gait speed in the first and sixth minute of the 6MWT of 1.18 and 1.13 m/s, respectively. For healthy controls ($n = 38$) the mean walking distance was 685 metres and the mean gait speed was 1.93 and 1.90 m/s in the first and sixth minute. Compared to these patients, the patients with gMG seemed mildly affected. However, due to huge differences in walking ability and underlying pathogenesis among different neuromuscular diagnoses, it is not possible to directly transfer our results to other neuromuscular patient's groups or healthy controls.

A considerable variation is found in repeated 6MWTs due to a learning effect [6,26,34,35] and pre-trial walk tests are recommended to improve reliability in both neuromuscular patients and healthy controls. However, to avoid exhausting the patients unnecessary, we chose a shorter pre-trial (60 m) to familiarise the patients to the test, and at the same time obtain step per minute in the patient's fastest gait speed. Also, as the 6MWT was performed in a randomised order, we did not expect learning effect to influence on our results. This was also confirmed in the analyses, where the order of the tests did not influence on walking distance ($p = 0.36$). This finding was in line with the results of another study of RAS and walk tests [20].

As no effect size of RAS in MG patients has been published, we did a post hoc sample size calculation, even though this is not standard method. The value of post hoc sample size calculations can be discussed, but after consultations with our statisticians, this was deemed the most correct method to use in this study. Future studies can benefit from our estimated effect size of RAS in MG patients.

A recent systematic review found a minimal clinically important difference (MCID) of 14-30.5 metres in repeated 6MWTs in adults across multiple patients groups (no neuromuscular diseases) [36]. As the increase in walking distance in our study was lower (8.3 metres), the relevance of our results can be questioned. However, the studies included in the systematic review, found a mean baseline walking distance (295-551 m) which was lower than the one we found

(≥544 m), indicating more severely affected patients. As the walking distances in our study were closer to healthy controls [5], we suggest that a ceiling effect limited the potential to increase walking distance in repeated 6MWTs in our study. Also, the increase of 8.3 metres in our study was present despite randomisation, whereas the study results presented in the systematic review, were influenced by the learning effect of repeated 6MWTs [36]. Furthermore, in a study of patients with COPD [20] patients walked 12 metres longer in the 6MWT with RAS, compared to walking without music or walking with music without rhythmic enhancement. This distance was closer to the results of this study, and also below the MCID in the systematic review [36] for 6MWTs without RAS.

This study is limited by missing measurements of synchronisation between RAS and steps, and missing measurements of LRC. Results of these measurements could have strengthened the conclusions of this study. The study was strengthened by the size of the cohort, which was large compared to other studies of RAS [11]. Also, that only patients with verified diagnosis of gMG were included, and that randomisation diminished the effect of learning in the 6MWT results. As this is the first study to examine the influence of RAS during walking in patients with gMG, we hope that the results and the estimated effect sizes of RAS can help and encourage other researcher to elaborate this topic into more details.

Conclusions

The results of this study indicate that gait speed and walking distance in the 6MWT in patients with gMG, can be increased by RAS without additional exertion as judged by HR and Borg scores. These observations are important theoretically and have potential implications for planning of physical rehabilitation program for patients with gMG.

Ethical approval

This study was approved by the ethics committee of the Capital Region of Denmark (protocol no. H-18031231), and oral and written informed consent was obtained from all involved participants.

Author contributions

Andersen has full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Andersen, Vissing; Acquisition, analysis, or interpretation of data: All authors; Drafting of the manuscript: All authors; Critical revision of the manuscript for important intellectual content: All authors; Statistical analysis: Andersen; Obtained funding: Andersen; Administrative, technical, or material support: Andersen; Supervision: Witting, Vissing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Linda Kahr Andersen http://orcid.org/0000-0002-4780-7739

References

[1] Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology. 2013; 80(4):409–416.

[2] Vinge L, Jakobsen J, Andersen H. Muscle weakness and functional disability in patients with myasthenia gravis. Muscle Nerve. 2019; 59(2):218–223.

[3] Jordan B, Mehl T, Schweden TLK, et al. Assessment of physical fatigability and fatigue perception in myasthenia gravis. Muscle Nerve. 2017;55(5):657–663.

[4] Salci Y, Karanfil E, Balkan AF, et al. Functional exercise capacity evaluated by timed walk tests in myasthenia gravis. Muscle Nerve. 2019;59(2):208–212.

[5] Andersen LK, Knak KL, Witting N, et al. Two- and 6-minute walk tests assess walking capability equally in neuromuscular diseases. Neurology. 2016;86(5):442–445.

[6] Knak KL, Andersen LK, Witting N, et al. Reliability of the 2- and 6-minute walk tests in neuromuscular diseases. J Rehabil Med. 2017;49(4):362–366.

[7] Van Geel F, Moumdjian L, Lamers I, et al. Measuring walking-related performance fatigability in clinical practice: a systematic review. Eur J Phys Rehabil Med. 2020;56(1):88–103.

[8] Montes J, Blumenschine M, Dunaway S, et al. Weakness and fatigue in diverse neuromuscular diseases. J Child Neurol. 2013; 28(10):1277–1283.

[9] Thaut MH. The discovery of human auditory-motor entrainment and its role in the development of neurologic music therapy. Prog Brain Res. 2015;217:253–266.

[10] Thaut MH, McIntosh GC, Hoemberg V. Neurobiological foundations of neurologic music therapy: rhythmic entrainment and the motor system. Front Psychol. 2014;5:1185

[11] Moumdjian L, Buhmann J, Willems I, et al. Entrainment and synchronization to auditory stimuli during walking in healthy and neurological populations: a methodological systematic review. Front Hum Neurosci. 2018;12:263.

[12] Bijsterbosch JD, Lee K-H, Hunter MD, Tsoi DT, et al. The role of the cerebellum in sub- and supraliminal error correction during sensorimotor synchronization: evidence from fMRI and TMS. J Cogn Neurosci. 2011;23(5):1100–1112.

[13] Hayden R, Clair AA, Johnson G, et al. The effect of rhythmic auditory stimulation (RAS) on physical therapy outcomes for patients in gait training following stroke: a feasibility study. Int J Neurosci. 2009;119(12):2183–2195.

[14] Nascimento LR, de Oliveira CQ, Ada L, et al. Walking training with cueing of cadence improves walking speed and stride length after stroke more than walking training alone: a systematic review. J Physiother. 2015;61(1):10–15.

[15] Rocha PA, Porfírio GM, Ferraz HB, et al. Effects of external cues on gait parameters of Parkinson's disease patients: a systematic review. Clin Neurol Neurosurg. 2014;124:127–134.

[16] Hurt C, Rice R, McIntosh G, et al. Rhythmic auditory stimulation in gait training for patients with traumatic brain injury. J Music Ther. 1998;35(4):228–241.

[17] Moumdjian L, Sarkamo T, Leone C, et al. Effectiveness of music-based interventions on motricity or cognitive functioning in neurological populations: a systematic review. Eur J Phys Rehabil Med. 2017;53(3):466–482.

[18] Moumdjian L, Moens B, Maes P-J, et al. Continuous 12 min walking to music, metronomes and in silence: Auditory-motor coupling and its effects on perceived fatigue, motivation and gait in persons with multiple sclerosis . Mult Scler Relat Disord. 2019;35: 92–99.

[19] Erra C, Mileti I, Germanotta M, et al. Immediate effects of rhythmic auditory stimulation on gait kinematics in Parkinson's disease ON/OFF medication. Clin Neurophysiol. 2019;130(10):1789–1797.
[20] Hernandez A, Bronas UG, Steffen AD, et al. Rhythmic auditory stimulation increases 6-Minute walk distance in individuals with COPD: A repeated measures study. Heart Lung. 2020;49(3): 324–328.
[21] ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111–117.
[22] Burns TM, Conaway MR, Cutter GR, et al.; Muscle Study Group. Construction of an efficient evaluative instrument for myasthenia gravis: the MG composite. Muscle Nerve. 2008;38(6):1553–1562.
[23] Burns TM. The MG composite: an outcome measure for myasthenia gravis for use in clinical trials and everyday practice. Ann N Y Acad Sci. 2012;1274:99–106.
[24] Burns TM, Conaway M, Sanders DB, MG Composite and MG-QOL15 Study Group. The MG Composite: a valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74(18): 1434–1440.
[25] Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982;14(5):377–381.
[26] Prahm KP, Witting N, Vissing J. Decreased variability of the 6-minute walk test by heart rate correction in patients with neuromuscular disease. PLoS One. 2014;9(12):e114273.
[27] Hoffmann CP, Torregrosa G, Bardy BG. Sound stabilizes locomotor-respiratory coupling and reduces energy cost. PLoS One. 2012;7(9):e45206.
[28] Hoffmann CP, Bardy BG. Dynamics of the locomotor-respiratory coupling at different frequencies. Exp Brain Res. 2015;233(5): 1551–1561.
[29] Cha Y, Kim Y, Chung Y. Immediate effects of rhythmic auditory stimulation with tempo changes on gait in stroke patients. J Phys Ther Sci. 2014;26(4):479–482.
[30] Moumdjian L, Moens B, Maes P-J, et al. Walking to music and metronome at various tempi in persons with multiple sclerosis: a basis for rehabilitation. Neurorehabil Neural Repair. 2019;33(6): 464–475.
[31] Buhmann J, Desmet F, Moens B, et al. Spontaneous velocity effect of musical expression on self-paced walking. PLoS One. 2016;11(5):e0154414.
[32] Mendonça C, Oliveira M, Fontes L, et al. The effect of instruction to synchronize over step frequency while walking with auditory cues on a treadmill. Hum Mov Sci. 2014;33:33–42.
[33] Mänttäri A, Suni J, Sievänen H, et al. Six-minute walk test: a tool for predicting maximal aerobic power (VO2 max) in healthy adults. Clin Physiol Funct Imaging. 2018;38(6):1038–1045.
[34] Gibbons WJ, Fruchter N, Sloan S, et al. Reference values for a multiple repetition 6-minute walk test in healthy adults older than 20 years. J Cardiopulm Rehabil. 2001;21:87–93.
[35] Casanova C, Celli BR, Barria P, on behalf of the Six Minute Walk Distance Project (ALAT), et al. The 6-min walk distance in healthy subjects: reference standards from seven countries. Eur Respir J. 2011;37(1):150–156.
[36] Bohannon RW, Crouch R. Minimal clinically important difference for change in 6-minute walk test distance of adults with pathology: a systematic review. J Eval Clin Pract. 2017;23(2):377–381.