Physical Fitness in Long-Term Juvenile Dermatomyositis

Thesis for the degree of philosophiae doctor (Ph.D.)

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Contents

Abbreviations ............................................................................................................................... 8
List of Papers .................................................................................................................................. 12

1 Introduction .................................................................................................................................. 13

1.1 Juvenile Dermatomyositis ........................................................................................................ 14

1.1.1 Epidemiology ....................................................................................................................... 14

1.1.2 Etiology and Pathophysiology ........................................................................................... 14

1.1.3 Diagnostic and Classification Criteria ................................................................................ 15

1.1.4 Clinical Manifestation .......................................................................................................... 16

1.1.5 Treatment ............................................................................................................................ 18

1.1.6 Disease Course and Long-Term Outcomes ....................................................................... 19

1.2 Physical Fitness ..................................................................................................................... 22

1.2.1 Exercise Physiology ............................................................................................................ 22

1.2.2 Cardiorespiratory Fitness .................................................................................................. 24

1.2.3 Muscle Structure, Fiber Types, and Myokines ................................................................. 24

1.3 Physical Fitness and Physical Function in Juvenile Dermatomyositis .................................... 25

1.3.1 Cardiorespiratory Fitness in JDM ....................................................................................... 25

1.3.2 Muscle Strength and Muscular Endurance in JDM .......................................................... 26

1.3.3 Balance/Basic Mobility Skills ............................................................................................ 26

1.3.4 Physical Activity in JDM .................................................................................................... 26

1.4 Establishment of our Cohort ................................................................................................... 27

2 Aims of the Study .................................................................................................................... 28

2.1 Main Aim ............................................................................................................................... 28

2.2 Specific Aims .......................................................................................................................... 28

3 Materials and Methodologic Considerations ........................................................................ 29

3.1 Study Design .......................................................................................................................... 29

3.2 Patients and Controls ............................................................................................................ 30
3.2.1 Patients.......................................................................................................................... 30
3.2.2 Controls .......................................................................................................................... 33

3.3 Data Collection .................................................................................................................. 33
3.3.1 Time and Location ......................................................................................................... 33
3.3.2 Stratification According to Disease Activity ................................................................. 33
3.3.3 Examinations Common to Visit 1 and 2 ....................................................................... 33
3.3.4 Examinations Exclusive to Visit 1 .................................................................................. 34
3.3.5 Examinations Exclusive to Visit 2 .................................................................................. 40

3.4 Statistical Considerations .................................................................................................. 49
3.5 Ethical Considerations ....................................................................................................... 51

4 Summary of Results ............................................................................................................. 53
4.1 PAPER I (Visit 1) ............................................................................................................. 53
4.2 PAPER II (Visit 2) ............................................................................................................. 53
4.3 PAPER III (Visit 2) ............................................................................................................. 54

5 Discussion .......................................................................................................................... 55
5.1 Representativeness ........................................................................................................... 55
5.2 Primary Outcomes in the Total Cohort Compared to Literature ..................................... 56
5.3 Differences between Patients with Active and Inactive Disease .................................... 57
5.4 Secondary Outcomes ....................................................................................................... 59
5.5 Active Disease or Disease Damage? ............................................................................... 61

6 Main Conclusions ................................................................................................................. 63

7 Clinical Implications and Future Perspectives .................................................................... 64

8 References ........................................................................................................................... 65

Appendix: PAPER I-III
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>ADL</td>
<td>Activities of Daily Living</td>
</tr>
<tr>
<td>ALAT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>Anti-HMGCR</td>
<td>Anti-3 hydrox-3 methyl-Glutaryl-Coenzyme A Reductase</td>
</tr>
<tr>
<td>Anti-MDA-5</td>
<td>Anti-Melanoma Differentiation Associated Protein 5</td>
</tr>
<tr>
<td>Anti-SRP</td>
<td>Anti-Signal Recognition Particle</td>
</tr>
<tr>
<td>ASAT</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CARRA</td>
<td>Children’s Arthritis and Rheumatology Research Alliance</td>
</tr>
<tr>
<td>cHAQ</td>
<td>Childhood Health Assessment Questionnaire</td>
</tr>
<tr>
<td>CHQ</td>
<td>Child Health Questionnaire</td>
</tr>
<tr>
<td>CK</td>
<td>Creatine Kinase</td>
</tr>
<tr>
<td>CMAS</td>
<td>Childhood Myositis Assessment Scale</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>CPET</td>
<td>Cardiopulmonary Exercise Testing</td>
</tr>
<tr>
<td>CPM</td>
<td>Counts Per Minute</td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory Fitness</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional Area (of the anterior thigh compartment)</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DAG</td>
<td>Direct Acyclic Graph</td>
</tr>
<tr>
<td>DAS</td>
<td>Disease Activity Score</td>
</tr>
</tbody>
</table>
DLCO  Diffusing Capacity of the Lungs for Carbon Monoxide

DLCOc  DLCO corrected for hemoglobin

DM  Dermatomyositis

DMARDS  Disease Modifying Anti-Rheumatic Drugs

E  Early Diastolic Transmitral Flow

é  Early Diastolic Tissue Velocity

ECG  Electrocardiogram

EDTA  Ethylenediaminetetra-acetic acid

EULAR  European League Against Rheumatism

FEV1  Forced Expiratory Volume during the first second

FI 2  Functional Index 2

FVC  Forced Vital Capacity

HAQ  Health Assessment Questionnaire

HRCT  High Resolution Computed Tomography

HRQOL  Health Related Quality Of Life

IL-6  Interleukin 6

ILD  Interstitial Lung Disease

INF  Interferon

IMACS  International Myositis Assessment & Clinical Study Groups

IP-10  Interferon Inducible Protein 10

IVIG  Intravenous Immunoglobulin

J  Joules

JDM  Juvenile Dermatomyositis
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>JIIM</td>
<td>Juvenile Idiopathic Inflammatory Myopathy</td>
</tr>
<tr>
<td>JPM</td>
<td>Juvenile Polymyositis</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MAA</td>
<td>Myositis Associated Autoantibody</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte Chemoattractant Protein</td>
</tr>
<tr>
<td>MCS</td>
<td>Mental Component Summary of the SF-36</td>
</tr>
<tr>
<td>MDAAT</td>
<td>Myositis Disease Activity Assessment Tool</td>
</tr>
<tr>
<td>MDI</td>
<td>Myositis Damage Index</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>MMT</td>
<td>Manual Muscle Test</td>
</tr>
<tr>
<td>MMT-8</td>
<td>The Unilateral Manual Muscle Test of 8 muscle groups</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSA</td>
<td>Myositis Spesific Autoantibody</td>
</tr>
<tr>
<td>MVPa</td>
<td>Moderate to Vigorous Physical Activity</td>
</tr>
<tr>
<td>MVV</td>
<td>Maximal Voluntary Ventilation</td>
</tr>
<tr>
<td>MYOACT</td>
<td>Myositis Disease Activity Assessment Visual Analogue Scale</td>
</tr>
<tr>
<td>MYODAM-VAS</td>
<td>Myositis Disease Damage by Visual Analogue Scale</td>
</tr>
<tr>
<td>Nm</td>
<td>Newton Meter</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>O₂pulse</td>
<td>Oxygen Pulse</td>
</tr>
<tr>
<td>PCS</td>
<td>Physical Component Summary of the SF-36</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary Function Test</td>
</tr>
<tr>
<td>PhyGloVas</td>
<td>Physician Global Visual Analogue Scale</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PRINTO</td>
<td>Pediatric Rheumatology International Trials Organization</td>
</tr>
<tr>
<td>PROMS</td>
<td>Patient Reported Outcome Measures</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>SHARE</td>
<td>Single Hub and Access point for pediatric Rheumatology in Europe</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short Form-36</td>
</tr>
<tr>
<td>6MWT</td>
<td>Six-Min Walk Test</td>
</tr>
<tr>
<td>TLC</td>
<td>Total Lung Capacity</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TUG</td>
<td>Timed Up and Go</td>
</tr>
<tr>
<td>VA</td>
<td>Alveolar Volume</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral Fat</td>
</tr>
<tr>
<td>VC</td>
<td>Vital Capacity</td>
</tr>
<tr>
<td>VE</td>
<td>Ventilation</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$</td>
<td>Maximal Oxygen Uptake/maximal aerobic exercise capacity/cardiorespiratory fitness</td>
</tr>
<tr>
<td>VT</td>
<td>Ventilatory Threshold</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
List of Papers

PAPER I:

Submaximal Exercise Capacity in Juvenile Dermatomyositis after Longterm Disease: The Contribution of Muscle, Lung, and Heart Involvement


PAPER II:

Cardiorespiratory Fitness in Long Term Juvenile Dermatomyositis; a Controlled, Cross-sectional Study of Active and Inactive Disease

Kristin Schjander Berntsen, Elisabeth Edvardsen, Bjørge Herman Hansen, Berit Flatø, Ivar Sjaastad, Helga Sanner.


PAPER III:

Functional and Structural Adaptations of Skeletal Muscle in long-term Juvenile Dermatomyositis; a controlled cross-sectional study

Kristin Schjander Berntsen, Truls Raastad, Henriette Marstein, Eva Kirkhus, Else Merckoll, Kristoffer Toldnes Cumming, Berit Flatø, Ivar Sjaastad, Helga Sanner.

Submitted.
1 Introduction

Physical disability in the initial phase of juvenile dermatomyositis (JDM) may be profound, with reduced muscle strength and endurance, fatigue, and sometimes cardiac and pulmonary involvement. With treatment, however, there is an astonishing improvement in the physical function of many patients, and the patient that once, according to the patient’s medical records, could hardly move, walks in to a clinical follow-up examination with no obvious physical impairment.

Despite the clinical improvement with immune modulating treatment, long-term morbidity in JDM is not insignificant. Long-term outcome studies have shown, among others, persistent disease activity and disease damage, reduced aerobic exercise capacity, and reduced quality of life compared to healthy controls.

This thesis involved two examinations of a Norwegian, open JDM cohort, labelled visit 1 and visit 2. Visit 1 took place between 2005 and 2008, and visit 2 between 2013 and 2016. During visit 1, although not recorded systematically, many patients complained that the only way the disease still bothered them, was that they were unable to reach the physical fitness goals they desired. Based on already published studies of reduced exercise capacity in long-term JDM, we assumed that this would also apply to our cohort. However, we wanted to further investigate whether there were differences between long-term outcome in patients with active and inactive disease, and try to get closer in the search of why these patients did not reach their expected physical potential.
1.1 Juvenile Dermatomyositis

Juvenile dermatomyositis (JDM) is the most common (80-85%) of the juvenile idiopathic inflammatory myopathies (JIIMs), a group of rheumatic connective tissue diseases affecting primarily muscle tissue in children (1, 2). The other JIIMs include overlap myositis (6-12%), juvenile polymyositis (JPM) (4-8%), amyopathic or hypomyopathic DM (1%), and inclusion body myositis (sporadic) (2).

JDM differs from the other JIIMs in the additional involvement of inflammation of the skin. It resembles adult dermatomyositis, however, with several dissimilarities such as different associations to myositis specific autoantibodies, less pulmonary affection, more calcinosis, and little or no association to cancer (3). It was first described in the early 20th century (4). Until the steroid era started around 1960 almost 1/3 of all patients died (5). Today, however, few die of the disease, and there has been a shift in research from a focus on mortality to a focus on long term morbidity.

1.1.1 Epidemiology

There are no valid prevalence studies on JDM. In a newly published meta-analysis of 6 epidemiologic studies from different parts of the world, the overall annual JDM incidence was 2.7/million children (6). The majority of patients (approximately 65-70%) are white (in contrast to approximately half of the JPM population) (1, 7, 8), and in the western world there is a female predominance with a female: male ratio between 1.7:1 and 5.1:1 (9), differing somewhat according to ethnicity (1, 9).

Median age at JDM onset ranges from 5-8y (8, 10); 25% are diagnosed before the age of 4y (11). The overall mortality of JDM today is around 2-3% (1, 12).

1.1.2 Etiology and Pathophysiology

The etiology of JDM is to a large extent unknown. The disease is commonly believed to be autoimmune, triggered by some external, environmental factors such as infectious agents like viruses in genetically susceptible individuals (13). This theory is supported by an overrepresentation of intercurrent infectious disease prior to symptom onset (11).

Like the etiology, much of the pathophysiology of JDM is also still unknown, and knowledge depends mostly on studies from the adult IIM population, or studies of juvenile patients around the time of diagnosis:

JDM is characterized by a vasculopathy, which in the active phase of the disease is believed to represent a systemic, perivascular inflammation that affects primarily skin and muscle tissue, but also internal organs such as the heart and lungs (14). The inflammation resembles autoimmune disease
with the presence of autoantibodies divided into myositis specific autoantibodies (MSAs) and myositis associated autoantibodies (MAAs), antigen-driven clonal B and T cell expansion, as well as genetic associations with immune related genes of the human leukocyte (HLA) region of the major histocompatibility complex (MHC) (13, 15). The genetic role in the pathophysiology of JDM embodies both an overexpression of certain risk factor genes as well as an underexpression of protective genes (16, 17). Also the innate immune system is involved through complex processes that involve e.g. complement activation (18) and cytokines/myokines (cytokines produced by muscle tissue) such as interferon (INF), tumor necrosis factor (TNF), interferon (INF)-inducible protein 10 (IP-10), interleukin 6 (IL-6), and monocyte chemoattractant proteins (MCP-1 and 2) (19-22).

There is an increasing evidence that also non-immune mechanisms such as altered energy metabolism (including a defect oxidative phosphorylation) play a role in the pathophysiology of JDM (19, 23, 24).

1.1.3 Diagnostic and Classification Criteria

Until 2017 the diagnostic criteria of dermatomyositis/polymyositis proposed by Bohan and Peter in 1975 were also used as classification criteria (in the absence of validated classification criteria) for clinical JDM studies (25):

- Characteristic cutaneous changes (e.g. heliotrope and Gottron’s sign/papules)
- Symmetric, often progressive proximal muscle weakness
- Elevated muscle enzymes (creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), and/or aldolase)
- Electromyographic changes characteristic of myopathy and denervation
- Muscle biopsy documenting histological evidence of perifascicular atrophy, perivascular inflammatory infiltrates and necrosis of muscle fibers.

A probable or definite DM required the skin criterion plus 2 or 3 of the muscle criteria respectively. However, these criteria were old and had their limitations: they had limited capability of excluding other forms of myopathies (26); new roles of autoantibodies had evolved; and in children the use of electromyography and muscle biopsies were often omitted in classic JDM cases; thus new classification criteria for juvenile and adult IIM were developed by the European League Against Rheumatism (EULAR) /American College of Rheumatology (ACR) and published in 2017 (26).

The new set of criteria is more extensive than the old, divided into two steps. The first step involves classification of IIM according to age, muscle weakness, skin manifestations, other clinical
manifestations, laboratory measurements and muscle biopsy features. The second step divides the IIM into subgroups, in which JDM is one (26).

1.1.4 Clinical Manifestation

The clinical picture of JDM may be heterogeneous, reflecting the systemic nature of the disease. The five most common clinical manifestations found in large JDM studies at time of diagnosis are:

Table 1:

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<tbody>
<tr>
<td></td>
<td>n=105</td>
<td>n=120</td>
<td>n=490</td>
<td>n=354</td>
</tr>
<tr>
<td>Proximal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weakness (95%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gottron’s rash (91%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heliotrope rash (83%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nailfold capillary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>change (80%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malar/facial rash (42%)</td>
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</table>

The classic symptoms of active JDM involve progressive muscle weakness (skeletal muscle weakness will be discussed separately) and skin manifestations of Gottron’s papules, Gottron’s sign and heliotrope rash (29). This triad of rashes is almost pathognomonic for JDM, and is enough to classify a definite JDM according to the new EULAR/ACR classification criteria (described previously) (26). Other skin manifestations include malar erythema and diffuse facial erythema, linear extensor erythema, shawl sign, periungual capillary changes, alopecia, and photosensitivity (30).

Muscle weakness may cause dysphonia or dysphagia, the latter posing a potential risk for aspiration (8). Internal organs such as the heart and lungs (discussed separately) as well as the gastrointestinal tract may be involved, the latter most often involving gastrointestinal vasculopathy that can result in lethal bleeding (27). Arthralgia and arthritis may cause pain and mobility problems. Systemic symptoms such as fatigue, fever, malaise, and headache may be pronounced, and hormonal changes (additionally to what treatment with steroids may cause, discussed under “Treatment”, section 1.1.5)
may influence growth and menarche. Lipid metabolism may be altered causing lipodystrophy, and calcinosis may develop in skin, connective tissue, or muscle tissue (29).

1.1.4.1 Muscle Involvement

Muscle involvement in JDM usually presents as painless muscle weakness and reduced muscular endurance although patients may experience myalgia (8). Muscle involvement is usually symmetric, proximal and axial (31). Distal muscles can, however, also be affected (32).

Muscle enzymes such as CK, LD, aldolase, ALAT and ASAT may leak into the blood stream (33). Electromyography (EMG) signaling may be pathologic (25), and edema (reflecting muscle infiltration) may be visible on magnetic resonance imaging (MRI) (34). Certain myositis phenotypes are also associated with myositis specific autoantibodies (MSAs), such as profound muscle weakness, muscle cramps, and calcinosis associated with anti-NXP2 (35, 36), and necrotizing myopathy with anti-signal recognition particle antibodies (anti-SRP) or anti-3-hydrox-3-methylglutaryl-coenzyme A reductase (anti-HMGCR). Muscle biopsies may reveal endomysial, perimysial, or perivascular infiltration of mononuclear cells, capillary dropout, and perifascicular fiber atrophy (37, 38).

1.1.4.2 Lung Involvement

In JDM, several factors may contribute to reduced function of the lungs. The most commonly detected lung pathology during active JDM is interstitial lung disease (ILD), with ground glass opacity and reticular patterns (39). ILD is associated with the antibody against melanoma differentiation-associated protein 5 (anti-MDA5) (40), and is most common in Asian JDM patients (41). Rapid, progressive ILD often results in respiratory failure and death (42), while chronic ILD is often subclinical and only visible on thoracic high resolution computed tomography (HRCT) scans (43). Muscles involved in respiration may also be affected by inflammation or muscle damage: In a whole-body magnetic resonance imaging (MRI) examination of 41 patients, 59% had detectable muscle inflammation in anterior neck muscles, and 56% had abdominal muscle inflammation (both these muscle groups representing accessory respiratory muscles) (32). Calcinosis may also develop in the chest wall, in the cutis, subcutis, fascia, or muscles, potentially reducing the expansion of the thorax (suggested earlier by our study group) (43).

1.1.4.3 Cardiac Involvement

Clinically overt cardiac manifestations in JDM are rare, but case reports of arrhythmias, cardiac vasculitis, and congestive heart failure in the initial phase of the disease do exist (44-46). Subclinical cardiac dysfunction early in the disease course is also rare: In a study on clinical features in 105
patients with JDM, none had changes on an electrocardiogram (ECG) suggestive of cardiac involvement (27). Another study found subclinical, abnormal ECG and echocardiography in 9% and 50% of patients respectively at time of diagnosis, and cardiac valve disease was the most common echocardiography-detected pathology (47). None of the patients developed clinically important cardiac disease over a 10y follow-up period. A recently published study assessing 14.5 million JDM hospitalizations in the United States, however, found an increase in cardiovascular comorbidities like hypertension, obesity, uncomplicated diabetes, and lipid abnormalities in patients versus controls. The same study also found higher odds of cardiovascular and cerebrovascular disorders compared to controls (48).

1.1.5 Treatment

Due to the focus of this thesis, treatment of JDM will only be discussed briefly. However, the management of JDM has evolved from hardly any available treatment 60 years ago, through the revolutionary introduction of steroids (unfortunately with harmful side effects) (49) to the addition of intravenous immunoglobulins (50), and other steroid sparing immune modulators (disease modifying anti-rheumatic drugs, DMARDS) (51). This change over time may have impact on long-term outcome studies as patients may have received different treatment strategies according to disease duration.

Even during the time scope of this work, there has been new advances in the management of the disease. Two randomized, controlled trials have been published (52, 53), and two new recommendations have been proposed: the Single Hub and Access point for pediatric Rheumatology in Europe (SHARE) recommendations (evidence based) (54), and the Children’s Arthritis and Rheumatology Research Alliance (CARRA) recommendations (consensus based) (51, 55-57).

Treatment strategies are divided into initial treatment, maintenance therapy after the initial disease phase, and the management of flare or treatment resistant disease. Early and aggressive treatment of newly diagnosed JDM has been found beneficial when it comes to quickly restoring physical function, retaining normal growth and puberty, and preventing disease damage (58). Still, despite the focus on early treatment, these goals are not always reached.

Recommendations differ slightly in approach according to symptoms, however, the backbone of JDM treatment is similar:

- High doses of Prednisone/Methylprednisolone (54, 59)
- Methotrexate (53)
Other commonly used drugs involve cyclosporine, intravenous immunoglobulin (IVIG), and hydroxychloroquine, depending on the presentation of clinical manifestations (51, 53, 55).

After the initial treatment, Prednisone is slowly tapered due to adverse side effects, such as hormonal and metabolic changes and increased cardiovascular risk (60). Methotrexate is often used as continuous immune modulating treatment, and can be slowly tapered after a year of clinical remission. In case of flare or treatment resistant disease, other immune modulating medication, including biologic agents may be attempted (54).

1.1.6 Disease Course and Long-Term Outcomes

The disease course of JDM is usually divided into three categories; monophasic (40-60%), polyphasic, or chronic (50-60% for the latter two combined) (61-63).

In JDM, disease activity refers to ongoing inflammation, while disease damage refers to persistent changes in anatomy, physiology, pathology, or function caused by inflammation, treatment, or other disease related events (64). Disease damage has been shown to increase with increased disease duration (65). It is usually regarded as more irreversible than disease activity, and it may be present despite disease remission. Inflammatory parameters are often absent, and muscle enzymes may be normal or low.

Disease course, disease activity, and disease damage are factors that complicate research on long-term outcomes and pathophysiology in JDM: Due to the different disease courses, at any time point studying long-term outcome some patients will have active inflammation and some patients will be in remission, clinical symptoms and signs after long-term disease may be mild, and it may be difficult to distinguish whether features such as muscle weakness or skin rashes represent disease activity or disease damage.

The World Health Organization (WHO) has defined the term outcome as “The effect the process has had on the people targeted by it” (66). Outcomes may include “changes in [patients’] self-perceived health status”, and “factors which are known to affect their health, well-being and quality of life” (66). Other than mortality, outcomes assessed in JDM have generally been related to disease activity and damage according to clinical scoring tools (67-69), patient reported outcome measures (PROMS) (70, 71), specific organ function (72, 73), and cardiorespiratory fitness (CRF) (74) (see section 1.3.1. Cardiorespiratory Fitness in JDM). There is no clear-cut definition of long-term in JDM research, but studies have commonly included patients with a disease duration of at least two years (after the initial inflammatory process) (65, 67, 68). However, due to the long disease duration of our patients, only the largest, most recent outcome studies of the longest disease duration will be presented here.
1.1.6.1 Long-Term Disease Activity

JDM studies have shown that disease activity is frequent, but relatively mild after long-term disease. One study found that approximately 60% had a total disease activity score (DAS) \(^1\) >0 after mean 7.7y disease duration (67), while two other studies found a total DAS score of mean 1.9 (2.9) and 4.7 (2.9) after mean 13.9y and median 16.8y disease duration respectively (68, 75). According to myositis disease activity assessment visual analogue scale (MYOACT) scores, the most frequent manifestations of long-term active disease were in the skin, muscle, and constitutional domains (and the skeletal domain ranked between skin and muscle in one study) (67, 75).

1.1.6.2 Long-Term Disease Damage

Disease damage has also been found to be frequent after long-term disease (Table 2).

Table 2. The most frequently presented JDM disease damage (according to domains of the myositis damage index (MDI)) after long-term disease.

<table>
<thead>
<tr>
<th></th>
<th>Ravelli 2010 (67)</th>
<th>Mathiesen 2012 (68)</th>
<th>Sanner 2009 (69)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=462</td>
<td>n=53</td>
<td>n=59</td>
</tr>
<tr>
<td>Follow-up: mean 7.7y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous scarring or atrophy (44%)</td>
<td>(40%)</td>
<td>(63%)</td>
<td></td>
</tr>
<tr>
<td>Muscle atrophy (24%)</td>
<td>Muscle dysfunction (34%)</td>
<td>Muscle weakness (47%)</td>
<td></td>
</tr>
<tr>
<td>Calcinosis (24%)</td>
<td>Muscle weakness (23%)</td>
<td>Joint contractures (45%)</td>
<td></td>
</tr>
<tr>
<td>Joint contractures (18%)</td>
<td>Calcinosis (21%)</td>
<td>Muscle dysfunction (40%)</td>
<td></td>
</tr>
<tr>
<td>Muscle dysfunction (16%)</td>
<td>Joint contractures (21%)</td>
<td>Calcinosis (37%)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent percentage frequency. The study by Sanner 2009 represents previous work on our Norwegian cohort.

Atrophic scarring and atrophy were the most commonly detected findings of disease damage.
Muscle symptoms, contractures and calcinosis then follow in all three studies, varying slightly according to frequency.

\(^1\) This chapter on long-term outcome refers to several scoring tools that are more thoroughly described under "Data collection", ch. 3.3.
1.1.6.3 Patient Reported Long-term Outcome

Regarding PROMs after long-term disease, one study found that the majority of patients had no impairment in physical function, defined by a childhood health assessment questionnaire (cHAQ) of 0, and only 6.5% had severe physical dysfunction. There was no difference in physical or psychological health between patients and controls in this study (tested by the child health questionnaire (CHQ)) (67). A study of our Norwegian cohort found that JDM patients after long-term follow-up had similar global quality of life (general satisfaction) compared to healthy controls, while health related quality of life, especially in the physical domain, was reduced (71).

1.1.6.4 Organ Specific Long-term Outcomes

More detailed investigation of organ specific long-term outcome has been performed primarily for muscle, heart and lungs.

Muscle. Muscle strength and endurance tested clinically by the unilateral manual muscle test (MMT-8) and the childhood myositis assessment scale (CMAS) was reduced compared to controls in a case-control, long-term outcome study of our Norwegian cohort (76). MRI detected muscle damage (such as muscle atrophy and fatty infiltration) was found in approximately 50% of patients. The serum cytokine eotaxin and the serum myokines MCP-1 and IP-10 were elevated in patients compared to controls (77). MCP-1 was also elevated in patients with active disease compared to respective controls, and correlated with disease damage in the whole patient group. Another study found that MMT and CMAS were normal in approximately 50% of patients, and that severe impairment in MMT or CMAS was rare (67).

Lung. Two studies describe lung function in JDM after long-term disease. In the first study of mean 14.3y disease duration, no patients reported pulmonary symptoms, 82% had normal pulmonary function compared to reference values adjusted for age, gender, and height, and 8% had restrictive interstitial lung disease (ILD) (72). A restrictive pulmonary function test was associated with increased disease damage (MDI, MYODAM-VAS, and physician global assessment) as well as increased activity (DAS). There were no differences in autoantibodies between patients with and without pulmonary changes, and none were anti-Jo-1 positive.

In our Norwegian cohort studied after 16.8y disease duration, 3% of patients reported dry cough and 12% dyspnea on exertion (43). A low total lung capacity (TLC) was found in 25% and a low diffusing capacity for carbon monoxide (DLCO) in almost 50% of patients, and the mean values of both as well as the forced vital capacity (FVC), forced expiratory volume during the first sec (FEV1), and the vital capacity (VC) were reduced compared to controls. However, there was no difference in DLCO
between patients and controls when corrected for lung volume. Together, lung function tests indicated reduced lung volumes in patients compared to controls. Thoracic HRCT scans revealed pathology in 21/57 (37%) patients; chest wall calcinosis in 8/57 (14%), airway disease in 9/57 (15%), and changes compatible with ILD in 8/57 (14%). Also examined in our patient group, HRCT-detected airway disease, reduced lung volumes and reduced DLCO were also associated with reduced nailfold capillary density (78).

Heart. Regarding cardiac manifestations after long-term disease, a study found increased cardiovascular risk in JDM patients after a median disease duration of 29 years compared to controls (79). Our research group has also found subclinical systolic as well as diastolic dysfunction tested at rest after median 16.8y disease duration compared to controls from the general population (73, 80). Twenty-two percent had elevated early diastolic transmitral flow/early diastolic tissue velocity (E/E’) indicative of diastolic dysfunction. Systolic function measured as long axis strain was also impaired in patients versus controls, but no differences were found between active and inactive disease. Cardiac dysfunction was associated with disease duration and damage (MDI) (80), elevated levels of eotaxin and MCP-1 (81), cholesterol levels in the upper, normal range (81), and reduced heart rate variability (82), but not with pathologic nailfold capillaroscopy (78). Seven (12%) patients had pericarditis during the disease course.

1.2 Physical Fitness

Health related physical fitness has been defined by The American College of Sports Medicine as a combination of cardiorespiratory fitness (CRF), muscle strength, muscle endurance, flexibility, and body composition (83). Balance, reaction time, and coordination, among others, are also involved. It is influenced by exercise habits, and is a contributing factor to physical function, a much less defined term describing the functional ability to perform activities of daily living (83, 84).

For the purpose of this thesis, we have assessed the following areas associated with physical fitness:

- Submaximal (PAPER I) and maximal (Cardiorespiratory fitness, PAPER II) exercise testing
- Muscle strength and muscular endurance (PAPER III)
- Submaximal exercise testing (PAPER I)
- Balance/ basic mobility (PAPER I)

1.2.1 Exercise Physiology

Exercise physiology is complex and will only be described in general terms for the purpose of this thesis. Exercise can be defined as aerobic or anaerobic (85).
Aerobic exercise involves the utilization of oxygen from the air for the energy production on a cellular level in order to induce muscular performance, in other words a coupling of external and cellular respiration (Figure 1) (85).

Figure 1. Aerobic exercise mechanisms

Oxygen is inhaled from the air, travels through the lungs and the cardiovascular system to the muscle cells (85). The muscle cells utilize the oxygen for oxidative phosphorylation in mitochondria to produce adenosine tri-phosphate (ATP), the energy-supplier for muscle contraction. The waist product, carbon dioxide (CO2), travels in the opposite direction and is exhaled into the air. Lung function requires the contribution of muscle strength, airway flow, and gas exchange across the border between the alveoli and the blood stream (86). During calm breathing the diaphragms is responsible for expanding the lungs during inspiration with a little help from intercostal muscles. Calm expiration during rest is passive. During exercise, accessory muscles such as the sternocleidomastoid and the scalene muscles contribute to increase the volume of the thoracic cavity to meet increased ventilatory demands (86). Forced expiration requires the use of abdominal muscles. Cardiac function is essential to transport oxygen from the lungs to the muscle tissue during exercise, and in healthy individuals the cardiac output is the limiting factor to maximal exercise capacity (85).
Anaerobic exercise, refers to exercise in which oxygen demand exceeds oxygen supply. During anaerobic exercise, substrates other than oxygen, such as glucose stored in the muscle tissue, are used to produce ATP in a less efficient manner (85). The waste product, lactate, accumulates in the muscle tissue causing lactic acidosis that will rapidly terminate the exercise.

1.2.2 Cardiorespiratory Fitness

Cardiorespiratory fitness (CRF, often used interchangeably with, among others, maximal aerobic exercise capacity or maximal oxygen uptake) refers to the maximal amount of oxygen an individual can consume during heavy exercise, and is expressed as VO_{2\text{max}} (85). It can be tested directly at maximal exercise, or be estimated from exercise at a submaximal level (maximal and submaximal tests are described under “submaximal exercise tests, section 3.3.4.1”). In healthy individuals several factors have been found to determine a person’s VO_{2\text{max}}, such as age, sex, exercise status and genetics, and there are large variations between individuals (87, 88). Genetics may also influence how well exercise may increase an individual’s VO_{2\text{max}} (89).

1.2.3 Muscle Structure, Fiber Types, and Myokines

Only muscle physiology relevant for the papers included in this thesis will be mentioned. Skeletal muscle consists of bundles of muscle fibers (Figure 2). Interspersed between the fibers and bundles are blood vessels, supplying the muscle cells with substances like oxygen needed to produce energy for the muscle cells to contract (86).

Figure 2. Structure of a Skeletal Muscle
There are two major types of skeletal muscle fibers (with several subtypes); slow-twitch, oxidative, type I fibers and fast-twitch type II fibers. The fiber types differ in terms of enzyme and calcium activity (important to contraction), and type I fibers are known to have a slower reaction to develop peak tension as well as a high resistance to fatigue compared to type II fibers (85). While type I fibers are associated with muscle endurance, type II fibers are associated with strength (91). In healthy individuals, the size of type II fibers are relatively larger than type I fibers, and in the vastus lateralis muscle (often exposed to muscle biopsies in JDM), the fiber type distribution is approximately 50% for each type (85). However, there are large individual variations dependent on genetics, age, sex, exercise, and innervation (85, 92).

Muscle tissue secretes myokines, small signaling proteins (cytokines) (93) in response to both health (such as exercise) and disease (such as inflammation) (94). They may exhibit an autocrine, paracrine, or endocrine function (93). They are important in regulating the innate and adaptive immune systems, and affect B- and T-cell growth, differentiation, recruitment, and activation (95).

1.3 Physical Fitness and Physical Function in Juvenile Dermatomyositis.

In JDM, physical function has traditionally been measured clinically by tools such as the childhood myositis assessment scale (CMAS) and the childhood health assessment questionnaire (cHAQ), found to be important contributors to health related quality of life (HRQOL) (96). However, CMAS and cHAQ may not detect minor changes (97), especially important in long-term outcome evaluation. Thus, there has been a growing use of more sensitive and objective measures of physical function in JDM, such as cardiorespiratory fitness and objectively tested muscle strength and muscular endurance in the evaluation of JDM (98).

1.3.1 Cardiorespiratory Fitness in JDM

Studies on cardiorespiratory fitness in JDM have consistently revealed lower VO$_{2\text{max}}$ in patients compared to controls, both early (99, 100) as well as after remission of the disease (74). Low values have generally been attributed to muscle weakness combined with deconditioning (74, 99). (74).

Even after remission, cardiorespiratory fitness was found to be decreased in the majority of patients, and was lower compared to healthy controls both in male, female, children and adults (74). A decreased maximal oxygen uptake was associated with longer disease duration of active disease (active disease defined by non-validated criteria). An aerobic training study of a part of the same cohort found a significant increase in cardiorespiratory fitness after a 12-week training program with no signs of corresponding muscle damage (101).
Prior to our study, detailed reports of cardiorespiratory response during cardiopulmonary exercise testing (CPET, the test used to measure cardiorespiratory fitness) were lacking, and most studies performed CPET using a bicycle (74, 99, 102) (discussed further under “cardiopulmonary exercise testing”, section 3.3.5.1).

1.3.2 Muscle Strength and Muscular Endurance in JDM

Clinical characteristics of muscle strength and muscular endurance in JDM were already described in detail under “clinical manifestations”, section 1.1.4, and “organ specific long-term outcome”, section 1.1.6.4.

To our knowledge, fiber composition has never been studied in JDM before, however, studies on adult patients with chronic polymyositis or dermatomyositis have shown a smaller proportion of type I fibers compared to healthy controls, while little difference has been found in fiber cross-sectional area of either type I or II (103). Twelve weeks of exercise increased the proportion of type I fibers by 10%, while the cross-sectional area of type II fibers increased by 25%.

1.3.3 Balance/Basic Mobility Skills

Although not part of the five major components of physical fitness (CRF, muscle strength, muscle endurance, flexibility, and body composition), an important contributor to physical fitness is balance and basic mobility skills (83). In JDM patients, these factors may be affected by e.g. muscle weakness, muscle and joint contractures, and calcinosis. The most common way to evaluate these functions in JDM is through the cHAQ/HAQ, the same tool that accesses physical function, with separate sections for, among others, reach, walking, and arising (104). A study of JDM patients after 1.5y disease duration found that the most impaired categories were dressing, arising, hygiene, reach, and activities (96). A physical function test of basic mobility skills and balance recently recommended by a JDM expert panel to be studied in JDM, is the Timed Up and Go (TUG) test (later described under “submaximal exercise tests, section 3.3.4.1” (105). To our knowledge, the Timed Up and Go test has never been systematically assessed in JDM yet.

1.3.4 Physical Activity in JDM

Up until recently, a commonly believed misconception was that exercise could be harmful to JDM patients and increase inflammation. Patients were therefore advised not to engage in physical activities. However, after the acknowledgement of exercise benefits in IIMs, and later also JDM, emerged around the millennium (106, 107), patients have been encouraged to exercise. Recently, the first published randomized controlled trial (RCT) on physical activity in JDM also acknowledged the benefits of exercise in this patient group (108). There are slightly different approaches to how to
organize exercise, and structured exercise programs are commonly used (109). In Norway, patients are usually encouraged to be involved in activities together with peers, not isolated through structured exercise programs reserved to JDM patients. The reason for this has been to increase compliance.

In JDM a Brazilian study of patients examined after mean 7.6±3.2y disease duration found that only 5% achieved the WHO recommendations of physical activity (110). Moderate to vigorous activity (MVPA) correlated negatively with disease duration and positively with VO2max and the current use of corticosteroids. A Danish study of patients examined after 14.1±9.9y found no significant difference in counts per minutes (CPM) between patients and historical comparator cases, and no correlations between CPM and VO2max (74).

1.4 Establishment of our Cohort

In 2005 MD PhD Helga Sanner started the work of establishing a Norwegian, nationwide JDM cohort (visit 1). Examination of the cohort after median 16.8y disease duration revealed that 90% of patients had cumulative organ damage, most commonly detected in muscle, skin and skeletal tissue (69), while 50% of patients classified as having active disease according to the original Pediatric Rheumatology International Trials Organization (PRINTO) criteria of inactive disease (75). Rather surprisingly, in addition to light muscle impairment, examinations also revealed subclinical pulmonary (43) and cardiac (73, 80) dysfunction already described in section 1.1.6.4.

Based on previous knowledge on reduced cardiorespiratory fitness in JDM, these findings led us to hypothesize that the subclinical nature of the cardiac and pulmonary dysfunction could become more pronounced during physical exertion and play a role in physical fitness after long term disease.
2 Aims of the Study

2.1 Main Aim

To explore components of physical fitness (cardiorespiratory fitness, muscle strength, and muscular endurance) in JDM after long-term disease duration, between patients and controls as well as between patients with active and inactive disease.

2.2 Specific Aims

After long-term disease duration:

- To compare submaximal exercise tests (the 6-min walk test (6MWT) and the TUG test) between JDM patients and controls, as well as between patients with active and inactive disease (PAPER I).
- To explore the contribution of muscle, lung and heart dysfunction to submaximal exercise test results in JDM patients (PAPER I).
- To compare cardiorespiratory fitness (maximal exercise testing) between JDM patients and matched controls, and between patients with active and inactive disease (PAPER II).
- To explore exercise limiting factors to CRF in JDM patients (PAPER II).
- To compare objective muscle strength and endurance between JDM patients and matched controls, and between patients with active and inactive disease (PAPER III).
- To explore potential contributing factors to reduced muscle strength and endurance in JDM patients through clinical, serologic, radiologic, and histologic (muscle fiber) assessment (PAPER III).
3 Materials and Methodologic Considerations

3.1 Study Design

This work is an observational, cross-sectional, case control study based on two separate examinations (visit 1 and visit 2) of an open, Norwegian, JDM cohort (Figure 3) (111). There was also a retrospective element in the design, used for scoring of diagnostic and classification criteria based on information from the time of diagnosis.

Figure 3:

Study design of the overall study.

Although it would be natural according to the presented design, to also look prospectively at data between visit 1 and 2, this was not possible to complete during the time scope of this work, and was saved for later, planned publications.
3.2 Patients and Controls

As each visit represented a separate cross-sectional controlled study, each visit also had a separate inclusion process. However, inclusions in visit 2 were based on inclusions in visit 1. The inclusion process is schematically shown in Figure 4.

3.2.1 Patients

Visit 1: The original JDM cohort established by Dr. Sanner was primarily identified through diagnostic searches (manual and electronic) at Oslo University Hospital. Also, all other major rheumatology or pediatric departments in Norway were encouraged to refer JDM patients that they had followed in the period 1995-2005. The inclusion criteria were 1) JDM diagnosed between 1970 and 2006, 2) a definite or probable diagnosis of dermatomyositis according to Bohan and Peter criteria (25), 3) onset of symptoms <18 years of age, 4) minimum 24 months between symptom onset and the study examination, and 5) age ≥6 years at examination.

There were no exclusion criteria. Details of the inclusion process have previously been described by Sanner (65). Sixty-seven JDM patients were identified; 4 were deceased. Inclusion criteria were met by 63 patients who were invited to participate.

Sixty (95%) accepted participation. Despite no formal exclusion criteria for patients, one 6-year-old was excluded due to difficulty in understanding instructions in most procedures, giving a cohort of 59/63 (94%) patients in PAPER I.

Visit 2: All 63 patients invited to visit 1 were re-invited to visit 2, except for one patient who, although fulfilling inclusion criteria, was retrospectively assumed to have been wrongly diagnosed with JDM, and one who had serious comorbidity. In addition, eight patients from a prospective JDM cohort at Oslo university hospital diagnosed after, or not reaching the age criteria for visit 1, were invited. As were three adult JDM-patients who were identified after visit 1. General inclusion criteria were the same as for visit 1 except for age at examination that was now raised to ≥10y due to complex examination procedures. As our work for this thesis began in 2013, inclusion of JDM patients were still based on the Peter and Bohan criteria. However, in order to compare our data to new research, PAPER II and III also included additional retrospective scoring of the new IIM classification criteria that were published in 2017 (26), using the web IIM classification calculator published by the Unit of Biostatistics, Karolinska Institutet, Stockholm, Sweden (112).

The exclusion criteria were different for each article:

PAPER II: The incapability to complete a CPET to voluntary exhaustion.
PAPER III: Neither the completion of objective muscle strength tests nor a muscle biopsy.

After being selected for invitation, patients were invited by mail, twice if not responding to the first. All positive repliers were contacted by phone to assess inclusion/exclusion criteria and organize examination.

Patients with a positive reply to participate were as follows:

- Re-invited patients: 48/61 (72%)
- Prospective cohort: 8/8 (100%)
- Other patients identified: 1/3 (33%)

Altogether this gave a positive response rate of 57/72 (79%). Four patients then withdrew before examination due to practical or personal reasons. After examinations and publishing of PAPER I, one patient was excluded from all further statistical analyses (from both visit 1 and 2, in PAPER II and PAPER III) due to a change of diagnosis. The final cohort thus consisted of 52/72 (72%) patients meeting inclusion criteria. Four patients wanted to only participate in clinical examinations at OUS, hence they were excluded from both PAPER II and PAPER III involving examinations performed at the NIH.

Further cohort selection was performed for each paper:

PAPER II:

Of 48 patients performing CPET, 3 were excluded due to incomplete CPET procedures: One fulfilled terminations criteria (section 5.5.3.1) and two stopped early due to lack of motivation. Hence 45/52 (87%) of the total cohort from visit 2 were included in final statistical analyses.

PAPER III:

Due to technical challenges with the isokinetic muscle testing apparatus, only 43 patients completed muscle strength and muscular endurance testing, however, as one patient without these tests volunteered for a new biopsy, there were 44/52 (85%) of the total cohort from visit 2 included in PAPER III. Biopsies were sampled from 17/44 (39%) patients (all above 18y of age).
Four patients from PAPER II were excluded in PAPER III, while three additional patients were added to the cohort. No= did not want to participate or did not reply to invitation.
### 3.2.2 Controls

**Visit 1:** Controls were summoned from Oslo and its surroundings through the Norwegian National Registry that provided a list of 10 controls per patient. The inclusion criterion was age and sex matched 1:1 with an included patient. The exclusion criteria were 1) mobility problems, 2) inflammatory rheumatic disease, 3) other autoimmune diseases treated with immunosuppressive agents, and 4) heart or lung disease (except for mild asthma).

Invitation was sent to 243 controls, and 69 accepted invitation, giving a response rate of 28%. Of accepting controls, ten were excluded, five due to exclusion criteria and five in excess of needed controls. Detailed information about the inclusion of controls has been described by Sanner (65).

**Visit 2:** Controls from visit 1 (n=59) were re-invited to visit 2. In addition, new controls from Oslo and its surrounding county were drawn randomly from the Norwegian National Registry, providing a list of 20 possible controls per patient. The inclusion criterion was the same as for visit 1, while the exclusion criteria were slightly modified. Inclusion criteria for visit 2 (with modifications highlighted in italic) were 1) mobility problems, 2) inflammatory rheumatic disease, 3) other active, autoimmune diseases, 4) *Treatment with immunosuppressive agents*, and 5) *serious* lung or heart disease.

The overall response rate for controls in visit 2 was 24% (75/350 contacted controls). Among controls from visit 1, the response rate was 36% (21/59). A total of 51 controls were included in the study, differing in number for each paper mentioned earlier according to the number of included patients. As patients and matched controls in visit 1 did not reply as a pair to participation in visit 2, new pairs were formed in visit 2 based on the closest age matching possible according to gender.

### 3.3 Data Collection

#### 3.3.1 Time and Location

**Visit 1:** Data collection took place between September 2005 and December 2009 at the Oslo University Hospital (Rikshospitalet and Ullevål).

**Visit 2:** Data collection took place between October 2013 and January 2016 at Oslo University Hospital, Rikshospitalet, and the Norwegian School of Sport Sciences.

#### 3.3.2 Stratification According to Disease Activity

Throughout this work we chose to divide our cohort into active and inactive disease according to the original PRINTO criteria for clinically inactive JDM (113). The reason for this was based on previous work by Sanner et.al. showing lower disease activity and disease damage, as well as higher physical
function and health related quality of life in our JDM patients with inactive compared to active disease after median 16.8y disease duration (75). These findings made us hypothesize that there might also be differences in physical fitness between the patient groups.

The criteria were published in 2012 and were the first validated criteria to define cutoffs between active and inactive disease. Any combination of a) Physician global visual analogue scale (PhyGloVAS)\(\leq 0.2\), b) MMT-8 \(\geq 78\), c) CMAS\(\geq 48\), and c) CK\(\leq 150\) define inactive disease.

However, there are challenges associated with the criteria. Firstly, both the CMAS (more on CMAS under “Clinical evaluation of muscle strength and endurance”, section 3.3.3.4) and the total sum of the PRINTO criteria are only validated in children with JDM (113, 114). But with no similar criteria of disease activity for adults with JDM, nor for adults with DM, and with previous findings of differences between active and inactive disease according to the criteria also in a population consisting primarily of adults with JDM, we chose to use the criteria for this study. Secondly, the criteria have been criticized for underestimating skin involvement, and new, revised criteria have been proposed (115). Despite this, due to the focus in this thesis on physical testing involving primarily muscle involvement rather than skin involvement, we chose to use the originally established criteria instead of the revised criteria.

### 3.3.3 Examinations Common to Visit 1 and 2

All examinations of visit 1 and 2 are schematically portrayed in Figure 5.
Figure 5. Examinations of visit 1 and visit 2.

Visit 1

Clinical examination (HS)
Heart/lung auscultation

Blood sampling
General blood samples

Scoring of core set measures:
DAS
MDAAT
MDI
Physician global activity

Self-evaluation:
SF-36
cHAQ/HAQ

Endurance tests:
6-MWT
TUG Test

Heart evaluation:
ECG
BP
Echocardiography

Lung evaluations:
Spirometry
Body plethysmography
DLCO
HRCT

Muscle evaluation:
MMT-8 (HS)
CMAS (Physiotherapist)
Muscle MRI

PAPER I

Visit 2

Clinical examination (KSB)
Heart/lung auscultation

Blood sampling
General blood samples

Scoring of core set measures:
DAS
MDI
Physician global activity
Physician global damage

Self-evaluation:
SF-36
cHAQ/HAQ

Physiological activity assessment:
Accelerometers

Cardiorespiratory fitness:
CPET

Muscle evaluation:
Objective muscle tests
Serum muscle enzymes
Serum Myokines
MMT-8 (KSB)
CMAS (physiotherapist)
Muscle MRI
Muscle biopsies

PAPER II:

PAPER III:

HS=Helga Sanner; KSB=Kristin Schjander Berntsen. Examination highlighted in red cursive were performed in both patients and controls. DAS=disease activity score; MDAAT=myositis disease activity assessment tool;
MDI=myositis damage index; SF-36=short form-36; cHAQ/HAQ= (childhood) health assessment questionnaire; 6-MWT=6 minute walking test; TUG= timed up and go; ECG=electrocardiogram; BP=blood pressure; DLCO=diffusing capacity of carbon monoxide; HRCT=high resolution computer tomography; MMT-8=unilateral manual muscle test of eight muscle groups; CMAS=childhood myositis assessment scale, MRI=magnetic resonance imaging; CPET=cardiopulmonary exercise testing.

### 3.3.3.1 Clinical Examination and Core Set Measures

Clinical examinations of patients were performed by separate physician in visit 1 and 2 (Sanner and Berntsen). Examinations included auscultation of heart and lungs, skin inspection, joint examination and isometric muscle strength testing (see under “manual muscle test”, section 3.3.3.4.).

A selection of JDM Core set measures of outcome and evaluation of response to treatment defined by the pediatric rheumatology international trials organization (PRINTO) (97, 116) and the International Myositis Assessment & Clinical Study Groups (IMACS) (117) (Table 3) were scored based on anamnestic information, clinical examination, and radiographic procedures (described later).

Table 3. PRINTO and IMAC core set measures for outcome and response to therapy.

<table>
<thead>
<tr>
<th></th>
<th>PRINTO (116)</th>
<th>IMACS (117)</th>
</tr>
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<tbody>
<tr>
<td><strong>Disease activity</strong></td>
<td>DAS</td>
<td>MDAAT</td>
</tr>
<tr>
<td></td>
<td>Physician global assessment of patient’s overall disease activity</td>
<td>Physician Global Disease Activity Assessment (DAS)</td>
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<td></td>
<td>MDAAT</td>
<td>MMT</td>
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<td></td>
<td>MMT</td>
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<tr>
<td><strong>Disease damage</strong> (97)</td>
<td>MDI</td>
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<td></td>
<td>Physician global damage</td>
<td>Parent Global Damage</td>
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<td></td>
<td>(MMT)</td>
<td>MMT</td>
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<tr>
<td><strong>Muscle evaluation/ physical function</strong></td>
<td>CMAS</td>
<td>CMAS</td>
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<td></td>
<td>cHAQ</td>
<td>cHAQ/HAQ</td>
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<tr>
<td></td>
<td>Parent’s global assessment of patient’s overall well-being</td>
<td>Parent/Patient Global Disease Activity Assessment</td>
</tr>
<tr>
<td><strong>Health related quality of life assessment</strong></td>
<td>Child Health Questionnaire</td>
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</table>
3.3.3.2 **Disease Activity Measures:**

**Disease Activity Score:** The DAS is the preferred assessment tool by PRINTO for global disease activity in pediatric JDM patients (116). It has also been recommended for adult and juvenile dermatomyositis studies by IMACS, although not validated in adults and not part of the core set measures for disease activity (118). In PAPER II and III we chose to DAS as the preferred measure of disease activity due to the complexity of MDAAT (described later). The tool has a potential score of 0-20 (in which 0=no activity); 0-11 regarding disease activity in muscle (divided into the domains “functional status” and “weakness”) and 0-9 regarding disease activity in the skin. The tool can be assessed as a whole, or separately for muscle and skin evaluation (97). We used DAS total for all papers included in this thesis, while in PAPER III we also assessed the DAS muscle.

**Myositis Disease Activity Assessment Tool:** The MDAAT is mentioned here as it is included as a disease activity measure by PRINTO and IMACS, although we chose to only use it in PAPER I. The tool has been validated in both juvenile and adult DM (119). It measures extramuscular as well as muscular disease activity in 7 organ systems and is divided into two scores, the Myositis Intention to Treat Activity Index (MITAX) and the Myositis Disease Activity Assessment Visual Analogue Scales (MYOACT)(97). The MITAX scores each item on a VAS scale ranging from 0-10, while the items in the MYOACT scores each item as follows: 0=not present, 1=improving, 2=stable, 3=worse, or 4=new.

**Physician Global Disease Activity VAS:** The Physician Global Disease Activity consists of a 10cm VAS scale or a Likert scale rating (0-4) (both with 0=no disease activity) (120). We used the VAS scale in all three papers included in this thesis. It is scored based on the physician’s overall evaluation of disease activity based on clinical appearance, medical history, examinations, laboratory testing, and the use of therapy (120).

3.3.3.3 **Disease Damage Measures:**

**Myositis Damage Index:** The MDI consists of an MDI extent of damage scale as well as an MDI severity of damage scale. The extent of damage scale contains 11 domains including e.g. muscle damage and internal organ damage and has a total score (damage index) of 35 in children, 37 in adolescents, and 38 in adults(64). The severity of damage scale consists of 10cm VAS scales for each of the 11 domains. There is also an extended version with 16 optional items not included in this thesis. The tool has shown good internal consistency and interrater reliability for the extent score as...
well as the severity score in most of the organ system assessed separately (121). The total MDI score has been validated thoroughly both in juvenile and adult DM (122), however, separate domains have not been systematically validated, although the muscle domain has been shown to correlate moderately with cHAQ, MMT-8, and MRI-scores, and inversely with serum creatinine (69, 76). For PAPER I and II we used the extent of damage scale (damage index) of the total MDI and for PAPER III we also included the extent and severity of damage scales for the muscle domain assessed separately.

**Physician Global Damage VAS:** Similar to physician global activity VAS it consists of a 10cm VAS scale based on an overall assessment of damage (as well as a Likert scale not used for this thesis) (64). We presented the physician global damage VAS in PAPER II and III.

### 3.3.3.4 Clinical Evaluation of Muscle Strength and Endurance:

#### Manual Muscle Test:

The MMT is defined as a core set measure of disease activity by both PRINTO and IMACS (116, 117), and it was also included in the 2016 IMACS candidate core sets of fitness and strength tests (98). We chose to use a unilateral version consisting of eight muscle domains (MMT-8), involving neck flexion, shoulder abduction, elbow flexion, wrist extension, hip abduction, hip extension, knee extension, and ankle dorsiflexion, which has shown good validity in both juvenile and adult DM (123). Isometric testing is performed against the examiner’s counter force. Each domain is scored from 0-10, giving a possible total sum of maximum 80 (normal force). The interrater reliability has been shown to be poor in distal and upper extremity proximal muscle groups, and it has been proposed only to use summary scores for research purposes (97, 124). Nevertheless, for this thesis we used the total score of MMT-8 in PAPER I-III, but also chose to separately evaluate the knee extension domain in order to compare it to objective muscle force of the quadriceps muscle in PAPER III.

#### Childhood Myositis Assessment Scale:

The CMAS is included in both the activity and damage core set measures by both PRINTO and IMACS (116, 117), however, in IMACS’ core set for disease damage it is classified as a functional score rather than a muscle strength score (117). It is composed of 14 different muscle endurance activities adding up to a total score of 52. The CMAS has shown good construct validity, interrater reliability and responsiveness in patients <18 years, making it a valid tool for assessment of physical function, muscle strength and endurance in children and adolescents (114). To use CMAS also in the adult population, however, was a controversial choice as it is not validated for this patient group. Among adult DM patients, the functional index 2 (FI 2) has been shown to be valid and reliable (125), and has recently (after this work started) been recommended as the choice of option for muscle function testing of adult IIM patients (98). As our cohort consisted
mainly of adult patients, this could have been an alternative test to use. However, when we planned visit 2, CMAS was already used for visit 1, making it reasonable to use this tool again for comparison reasons. Also, Sanner et al had previously shown correlations between CMAS and clinical measures such as MMT, DAS muscle, and MDI also in the adult population (76), while no studies exist on the FI2 in children. Finally, CMAS was included as one of four measures used to define inactive disease in the only validated criteria for this purpose (113). In visit 1 (PAPER I) CMAS was tested in both patients and controls, while in visit 2 (PAPER II and III) it was only tested in patients.

### 3.3.3.5 Patient Reported Outcome Measures (PROM)

During the last decades, there has been a growing focus on collaborative work between patients and health professionals in order to increase the patients’ autonomy when it comes to decision making about diseases affecting their lives (126). Thus, we have included patient reported outcomes in all three papers included in this thesis, to try to link scientific research to patient experiences.

**Short Form-36**: The SF-36 consists of a physical component summary (PCS) and a mental component summary (MCS). It assesses global health, not necessarily related to disease, and is not recommended for children (97). Thus, the SF-36 was used for both patients and controls ≥13y, and the Norwegian translation of SF-36 version 1 was used. It has been partly validated in adult myositis (97). Due to the focus on physical functioning, we chose to only focus on the physical domain for the purpose of this thesis.

**Childhood/Adult Health Assessment Questionnaire**: The cHAQ/HAQ are brief self-reporting questionnaires on the capability to perform activities of daily living (ADL) (97). They have traditionally been the choice of option for evaluating physical function in JDM. Both the HAQ and the cHAQ were originally developed to assess arthritis, but were successfully validated also for JDM. The HAQ is included in the activity core set measures of IMACS, and the cHAQ in both IMACS and PRINTO core set measures of disease activity. In our patient group, all patients <18y scored the cHAQ, and patients ≥18y scored the HAQ. The tools were then combined for statistical analyses.

**Parent/Patient Global Activity and Damage Assessment**: Parent/patient global assessment is included in the PRINTO and IMACS core set measures, however with slight differences between the two sets of measures (Table 3). The tools consist of a 10cm VAS scale in which the patient is supposed to score his or her perceived disease activity or disease damage. In visit 1, we had much missing data on patient/parent global activity and damage assessment, thus the variables were not included in PAPER I. In PAPER II and III (based on visit 2) we did have complete sets of patient/parent global activity and damage assessment, however, retrospectively we realized that PRINTO did not
operate with a division between activity and damage, only the patient’s overall wellbeing (116). As we experience great confusion among our patients and parents in the definitions of disease activity and damage, we chose to only use the global activity measure, and we believe that the PRINTO patient/parent global assessment would have been a better tool to use. Patient/parent global activity was included only in PAPER II.

3.3.3.6 Laboratory Measures:

Laboratory testing was performed by venous blood sampling for visit 1 and 2. General, inflammatory parameters and muscle enzymes (CK, LD, ASAT, ALAT) were measured in patients and controls as routine samples.

In addition to general biochemistry, additional blood sampling for freezing were sampled. This included serum and ethylenediaminetetra-acetic acid (EDTA) blood. These samples were stored at -80°C until further analyses.

3.3.3.7 Magnetic Imaging of Thigh Muscles

During the last decades, magnetic resonance imaging (MRI) has gained recognition as a useful, accurate tool in muscle evaluation in JDM (127). It is painless, assesses large volumes of muscle tissue simultaneously, and can be easily repeated without health-related hazards (127). However, the procedure is expensive and time consuming, and thus we chose to only perform MRI examinations of patients (not controls). MRI was performed using different equipment for visit 1 and 2. A 1.5T or 1.0T scanner (Siemens) was used for visit 1 (43), and a 1.5T scanner for visit 2 (detailed descriptions in PAPER III). Proximal thigh muscles were examined by a coronal T1-weighted spin-echo sequence and coronal and axial STIR sequences. Images were scored by two experienced radiologists blinded to patient information. If disagreement, consensus was made. MRI images were scored according to the presence/non-presence of muscle edema or calcinosis in muscle tissue, fascia, subcutis or cutis. Pathologic fatty infiltration was defined as perimysial fatty infiltration more than fatty streaks (76). For visit 2 the radiologists also measured the maximal cross-sectional area (CSA) of the anterior thigh compartment. A detailed description of this is given in PAPER III.

3.3.4 Examinations Exclusive to Visit 1

3.3.4.1 Submaximal Exercise Tests

Submaximal exercise tests are tests of physical function performed at a submaximal level of intensity, and can be divided into predictive and performance tests (128). Predictive submaximal exercise tests have been constructed to predict maximal aerobic capacity tested at a submaximal level. Some
patients with low physical functioning may even reach their maximal aerobic capacity at this submaximal intensity. Examples of this kind of submaximal exercise tests are the 6-minute walk test (6MWT), the 12-minute walk test, and the shuttle-walk test (128). Performance tests, however, assess basic mobility skills. Examples of mobility tests include the lift-and-reach test, the standing balance test and the Timed Up and Go (TUG) test (described below) (84, 128). Common to submaximal tests is the closer resemblance of activities of daily living (ADL) compared to maximal exercise tests.

6-Minute Walk Test

The 6-minute walk test (6MWT) is a predictive submaximal exercise test and a functional test commonly used in the evaluation of patients with various cardiopulmonary conditions (85, 129). It has later also been validated for certain rheumatologic diseases such as systemic sclerosis and juvenile idiopathic arthritis (130, 131). In 2016 (after we began our work) it was also proposed as a core set measure of submaximal aerobic fitness in JDM based on expert consensus (98) although no validation studies or other studies assessing the 6MWT as a major outcome exist for JDM. We used it as a functional test rather than a test to predict maximal aerobic capacity.

A detailed description of the 6MWT procedure used for our study is given in PAPER I.

Timed Up and Go Test

The Timed Up and Go (TUG) test is an objective version of the earlier subjective Get Up and Go from 1986 that evaluated falling tendency in elderly (19). The TUG test assesses basic mobility skills by measuring the total time to complete a set of everyday tasks (20). To our knowledge, the TUG test has never been systematically assessed in JDM, but was (like the 6MWT) recommended as a candidate core set measure of physical function to be studied in IIM (18).

A detailed description of the TUG test procedure used is given in PAPER I.

3.3.4.2 Lung Evaluation:

Pulmonary Function Tests: Pulmonary function tests (PFTs) are the preferred choice to diagnose restrictive lung disease (132). PFTs were performed using a computerized $V_{\text{max}}$ Pulmonary Function Unit (Viasys, Santa Ana, California, USA), and included spirometry, gas diffusion measurements, and body plethysmography. Spirometry variables included the forced vital capacity (FVC) and the forced expiratory volume in 1 s (FEV1). Gas diffusion variables (measured in duplicate) included DLCO, alveolar volume (VA) and the transfer coefficient (DLCO/VA). All diffusion variables were corrected for hemoglobin. The body plethysmography variables TLC and vital capacity (VC) were only tested in
individuals ≥9y. All lung function measurements were performed according to published guidelines (133-135). PFTs are presented in PAPER I.

**High Resolution Computed Tomography of the Thorax:**

High resolution computer tomography (HRCT) is the preferred method to detect interstitial lung disease (136). We performed HRCT scans of the thorax (included in PAPER I) using a LightSpeed 16 scanner (GE Healthcare, Milwaukee, Wisconsin, USA). Thin section CT images were obtained in the supine position during deep inspiration. No intravenous contrast was used. Supplementary scans in the prone position were obtained in all patients to differentiate between subpleural fine reticular fibrosis and dependent atelectasis. All CT examinations were done at our institution. In order to minimize the radiation dose, 120 kV, 40–200 mA, 0.8 s rotation time with 1.25 mm section thickness at 10 mm intervals were used, and tube current settings were adjusted to each patient’s age and weight.

The images were reviewed on a picture archiving and communication system screen in consensus and in random order by two specialist chest radiologists. Except for the diagnoses, the observers were blinded to patient history and lung function. The observers evaluated the presence and extent of interstitial lung disease (ILD) according to established criteria for CT evaluation of ILD (39). These findings included reticular pattern (fine intralobular fibrosis without evident cysts; microcystic; and macrocystic), ground-glass opacities, airspace consolidations, parenchymal bands, and subpleural curvilinear lines. The presence of traction bronchiectasies and traction bronchiolectasies was also assessed as well as chest wall calcinosis (43).

**3.3.4.3 Heart Evaluation**

For cardiac evaluation we used non-invasive examinations for the assessment of cardiac rhythm and structure as well as heart rate (HR) and blood pressure (BP) (PAPER I).

**Resting ECG:** A 12-channel ECG was used (73). Rhythm and ST segment were assessed, and PR, corrected QT interval (QTc) and QRS duration were measured. Left ventricular hypertrophy was assessed by Cornell voltage × QRS duration product (ECG parameter indicating left ventricular (LV) hypertrophy)(137); however, only validated in the adult population, it was not calculated in those <16 years. ECG was classified, blinded to clinical information and patient/control identity, as normal, borderline or pathological. Criteria for borderline ECG were: incomplete right bundle branch block, severe sinus arrhythmia, STT changes or Sokalow criterion >35 mm. Standard criteria for pathological ECG were used (138).
**Resting Echocardiography:** A two-dimensional, M-mode and Doppler echocardiography was performed using a Vivid 7 ultrasound scanner (GE—Vingmed Ultrasound, Horten, Norway). Systolic and diastolic cardiac function were assessed separately:

*Systolic cardiac assessment:* Systolic assessment was assessed using long axis strain. Long-axis strain was calculated as mitral annular displacement expressed as percentage of left ventricle end-diastolic length as previously described (80).

*Diastolic cardiac assessment:* Diastolic assessment was assessed using early diastolic transmitral flow (E), early diastolic tissue velocity (e’), and the E/e’ ratio as previously described (73).

**Blood Pressure:** Blood pressure (BP) was measured at rest using an automatic sphygmomanometer in all patients and controls.

### 3.3.5 Examinations Exclusive to Visit 2

#### 3.3.5.1 Cardiopulmonary Exercise Testing

Cardiopulmonary exercise testing (CPET) is the gold standard for measuring cardiorespiratory fitness (expressed as maximal oxygen uptake) during heavy exercise (VO$_{2max}$) (85). In JDM, CPET has shown good validity, reliability, and responsiveness in the few studies published on CRF (99, 139, 140).

During the test, a work load is increased from light to heavy, using either a bicycle or a treadmill. As the exercise intensity escalates, aerobic metabolism is gradually overcome by anaerobic metabolism until a point is reached when increased work load no longer increases oxygen uptake, and the VO$_{2max}$ is reached (Figure 6).

**Figure 6:**

![Figure 6: Oxygen consumption Relative to Exercise Intensity.](image-url)
The point at which anaerobic metabolism starts to replace aerobic metabolism is called the anaerobic or ventilatory threshold (VT) (85). Below this point there is a balance between O₂ uptake and CO₂ production, causing a stable pH, and exercise can continue for a long time. Above the threshold, however, CO₂ production increases more rapidly than O₂ uptake, causing a pH imbalance that eventually terminates the test (Figure 7). The VT is usually 50-60% of maximal oxygen consumption, but may be lowered by any process that would potentially produce a premature lactic acidosis, such as reduced oxygen delivery to muscle cells or alterations in muscle fiber recruitment (88).

Figure 7. Ventilatory threshold.

![Ventilatory threshold](image)

We performed CPET on treadmill (Woodway, Wursburg, Germany). A detailed description of the setup and procedure is given in PAPER II, and the setup is shown in picture 1. Not included in the paper are the potential indications used for exercise termination before physiologic limitation was reached (88). They included a) chest pain suggestive of ischemia, b) ECG changes of ischemia or pathologic arrhythmias, c) fall in systolic blood pressure >20mmHg from the highest value during the test, d) severe desaturation (<80%) when accompanied by symptoms and signs of severe hypoxia e) sudden pallor, f) loss of coordination, g) mental confusion, h) dizziness, i) signs of respiratory failure.
The CPET protocol was a version of the Balke protocol (141), modified according to self-reported physical activity levels. This is a different protocol than was suggested by an expert panel that newly proposed core set measures of muscle and endurance testing to be used in JDM (98). The proposals made by this panel were based on already existing studies on CPET in JDM, which all used the Bruce protocol when using treadmill prior to bicycle. The discussion did not, however, involve different protocols to be used on treadmill, but discussed the Bruce protocol versus bicycle protocols, favoring the Bruce protocol due to the high degree of muscle weakness in JDM. We believe, in accordance with the proposals, that treadmill prior to bicycle should be favored in order to reach the patients’ cardiorespiratory potential, but that the Balke protocol would be better than the Bruce protocol for JDM patients. While the Bruce protocol has an unequal duration of increment and variability in incremental size, the Balke protocol is performed at constant speed and an equal slope increase at regular intervals (Figure 8) (85). The uniformity of the Balke protocol enables the determination of slopes and thresholds needed for an extensive cardiorespiratory evaluation, procedures that uneven protocols like the Bruce protocol do not allow.
Figure 8. Examples of different CPET protocols.


All CPET analyses were based on printed reports. The ventilatory threshold (VT) was identified using the dual criteria method by two independent investigators (142). If difficulties determining the threshold, the v-slope method was attempted by plotting data from printed reports, however, if still not able to determine, the variable was excluded.
3.3.5.2 Testing of Muscle Strength and Muscular Endurance

In the newly proposed candidate core set for muscle strength testing, only the MMT-8 and handgrip-strength test (as well as CMAS for muscle endurance) met consensus and were included (98). However, it was advised that also other isometric, isokinetic, or isotonic tests be used for a better understanding of muscle strength, depending on available equipment and patient abilities. As we had already shown high scores of MMT-8 and CMAS in previous examinations of our own cohort (76), we wanted to examine muscle strength and endurance more objectively due to ceiling effects of MMT-8 and CMAS (97). We used a custom-made knee extension device (GYM 2000AS, Vikersund, Norway), to measure maximal isometric contractions and dynamic muscular endurance of the knee extensors. A detailed description of the setup and procedure is provided in PAPER III, and is depicted in photo 2.

Photo 2. Quadriceps muscle strength testing.

Nov 18, 2015. Consented by participants.

3.3.5.3 Physical Activity Measurements

Accelerometers have been regarded as the gold standard for measuring physical activity, and are more consistent than questionnaires regarding validity and reliability (143). In JDM, there has been found poor agreement between objective and subjective physical activity testing (article published
after we started our work) (144). The Actigraph GT3X accelerometer used in our study is a late version of the Actigraph GT1M which has been used to measure physical activity levels in children and adolescents (145). It accurately measures step counts across various ages, and the Actigraph series is said to be the most studied accelerometer brand (145).

The World Health Organization (WHO) base their recommendations of physical activity on accelerometer data, and propose ≥150 min/week of moderate to vigorous physical activity (MVPA) in bouts of at least 10 min in adults (MVPA bouts), and ≥60 min of daily MVPA in children <18y to achieve health benefits (146). We scored physical activity levels of our patients and controls according to these recommendations in order to compare with other studies and the general population.

A detailed procedure for the use of accelerometers for this thesis is given in PAPER II. In addition to accelerometers, we used a non-validated questionnaire of self-reported activities that may not be detected by accelerometry, such as bicycling, swimming and skiing. These data were not systematically reported in PAPER II and III, but were used for individual analyses of accelerometer data. Unfortunately, we lacked detailed reports on physical activity related to strength exercises, a limitation to the study.

### 3.3.5.4 Laboratory Tests Exclusive to Visit 2

Based on previously described knowledge on myokines in JDM (introduction, section 1.1.6.4), we measured serum myokines in our patients and controls. Myokines were analyzed according to standard protocols, either by luminex xmap technology (Luminex, Austin, TX) or ELISA kits (#EHDCN, Thermo Scientific, Frederick, MD, USA) and #DGDF80 and #DCP00, RnD systems, Minneapolis, MN, USA). Detailed descriptions of which myokines were examined by which methods are given in PAPER III.

After the CPET procedure, capillary blood was sampled for lactate analyses, approximately 1min after CPET termination. Separate oral consents were obtained for this examination.

### 3.3.5.5 Muscle Biopsies

Patients ≥ 18y were invited for a percutaneous needle muscle biopsy sample of the left vastus lateralis muscle. We chose to use a needle biopsy prior to an open biopsy to make the examination more gentle to the patients (147) (although not systematically assessed, several of our patients expressed traumatic experiences associated with open biopsies as children, and several complained of cosmetically bothersome scars still present). Needle biopsies are quickly obtained in patients using
local anesthetics, the patient can mobilize the muscle immediately after procedure, and the procedure itself leaves only a small scar. It has been shown useful in rheumatologic muscle evaluation although rarely used (148).

Muscle biopsies were performed using a Pelomi needle (Albertslund, Denmark) with manual suction. Detailed descriptions of the procedure of the biopsy sampling and immunohistochemical examination are given in PAPER III.

### 3.4 Statistical Considerations

All statistical analyses were performed as described in detail in PAPER I-III using different, updated versions (from 22 to 25) of IBM SPSS Statistics.

**Choice of Statistical Analyses:** Part of the objectives in PAPER I and II were to explore potential influence of cardiac, pulmonary or muscle involvement on reduced submaximal and maximal exercise capacity respectively. However, despite similar aims, different statistical approaches were used in the two articles which need mention.

PAPER I was based on data from visit 1. Prior to the PAPER I writing process, separate articles on cardiac, pulmonary, and muscle function from the same visit had already been published (43, 73, 76, 80). As the 6MWT itself is not designed to determine limiting factors to impairment, it was natural to incorporate data from the previous studies on organ involvement in the same JDM population (although pulmonary and cardiac function were tested at rest in contrast to the 6MWT) and use a linear, multivariate regression analysis to find significant associations.

PAPER II on the other hand was based solely on data from visit 2. As this was the first article produced from this visit, and due to the limited time frame, we did not have separate work on cardiac, pulmonary or muscle function (muscle function was assessed later) ready to perform linear regression analyses. We also wanted to save this data for future articles on specific organ involvement. However, as the CPET procedure in itself, unlike the 6MWT, involves the integrated assessment of separate organs involved in cardiorespiratory fitness, it was possible to use the direct data from the test to evaluate possible limiting factors to the CPET results (88). The strength of this method prior to a linear regression analysis was that all organs were tested simultaneously during the same physical exertion.

**Matching:** Another statistical matter that needs attention is the inconsistent use of pairing between patients and matched controls in the different articles; in PAPER I, we used unpaired statistical analyses between patients and controls as well as between patients with active and inactive disease.
For PAPER II and III, paired analyses were used between patients and controls, while unpaired analyses were used between patients with active and inactive disease.

Matching is a controversial topic, much debated amongst statisticians. While some define it strictly as one-to-one pairing to diminish confounders on an individual basis, others use it to create groups similar in distribution of the matching variables, without the individual matching of each case to a separate control (149). The latter is by some not regarded as matching, and should not involve paired statistics. In our selection of controls, we matched our patients 1:1 with a control in terms of age- and gender in order to account for gender differences across a large age dispersion. For PAPER I, we interpreted our age- and gender-matching as simply being used for distribution matters as described above, and hence we used unpaired statistics. Later, however, we redefined this interpretation as we, in fact, did have matching between patients and controls on a 1:1 basis, and changed to paired statistics.

Confounders: In PAPER I we chose to correct for self-reported physical activity levels when comparing the 6MWD and TUG time between patients and controls as we considered physical activity to be a confounder for the test results. Later, however, in learning about directed acyclic graphs (DAGs), we realized that physical activity was not a true confounder, but rather a modifier (Figure 9), not to be corrected for in comparative analyses (150, 151). Thus we chose not to correct for physical activity levels when dealing with cardiorespiratory fitness in PAPER II.

Figure 9: Directed Acyclic Graph (DAG) of physical activity as a mediator:

A: Model of a true confounder. B: The role of physical activity with regards to aerobic capacity in JDM.
Multiple Analyses

In all our articles we had numerous data presented in each table that in terms involved multiple statistical analyses. Multiple statistical analyses may predispose for type I errors, however, we chose not to correct for multiple analyses due to the hypothesis-generating character of our study (152).

3.5 Ethical Considerations

Both studies that this thesis is based on (visit 1 and 2) were approved by the Norwegian Regional Committee for Medical and Health Research Ethics (visit 1: S-05144, visit 2: 2013/1039). Visit 1 was approved by the Norwegian center for Research Data, whereas visit 2 was approved by the local ethics board at the Oslo University Hospital (no formal grant number available). All patients and controls (or guardians if < age 16 years) signed informed consents according to the World Medical Association of Helsinki (153); those who participated in both visits signed twice.

There are certain ethical aspects to the work that need some attention.

Controls

We chose to include controls from the general population. By performing a thorough clinical examination of mostly healthy people, however, poses a risk of discovering pathology, both serious and unserious, that could potentially create unnecessary worries. In for example one control we found cardiac arrhythmias during CPET that required further evaluation to rule out serious pathology.

Children

We chose to include children in our study, in order to get a representative cohort of the entire JDM population (although patients under 10 years of age were excluded due to complicated procedures). We found it important that all the children were able to sign the consent form in addition to their guardians if under 16 years of age in order to agree to participation. Some of the examinations were associated with painful procedures, such as blood sampling, which may cause children to resent clinical examinations. In order to minimize the discomfort, those who wanted it were offered topical anesthetics with Emla 5% cream. All were informed (also adults) about the possibility to withdraw from the study, without reason, at any time during the examinations.
**Exposure to Radiation**

We chose to examine our patients in visit 1 with HRCT scans despite not being part of routine examinations. Although this had been performed prior to the time scope of this thesis, the potential exposure to radiation should be mentioned.

Any examination involving x-rays involve some form of radiation. A normal HRCT scan exhibits radiation of approximately 2-3 milliSievert (mSv). Median CT dosage index in our project was 3.77, giving a median effective dosage of less than 1mSv. To put this in perspective, a classical thoracic x-ray examination exhibits 0.05-0.1mSv, the yearly Norwegian background radiation (although differing according to geographical area) approximately 3mSv, and a flight to the USA approximately 0.05mSv. Originally published in 1999, and then revised in 2001, the American Nuclear Society published a statement on health effects of low-level radiation, uttering that below 10 rem (same as 100mSv), the risks of health effects are either too small to be observed or are non-existent (154). This means that the radiation dose used for our project was relatively small. Still, for future research we must bear in mind the potential accumulative risk for each patients.
4 Summary of Results

4.1 PAPER I (Visit 1)

A total of 58/63 (92%) invited patients and 58 controls performed a 6-minute walk test and a Timed Up and Go Test after 16.8y disease duration. 29 (50%) patients had inactive disease (PRINTO). The total patient cohort walked a mean 57m (95%CI 28-86, <0.001) shorter than controls. Patients with active disease walked a mean 87m (95%CI 52-122, <0.001) shorter than controls and 60m (95%CI 20-100, <0.004) shorter than patients with inactive disease, while there was no significant difference in walking distance between patients with inactive disease and controls.

Patients in total used 0.8s (95%CI 0.1-1.5, p=0.036) longer than controls to complete the TUG test. All patients but one had a normal falling tendency scale. While there was no significant difference in TUG time between patients with inactive disease and controls, patients with active disease used 1.4s (95%CI 0.48-2.33, p=0.003) more than controls, and 1.2s (95%CI 0.14-2.28, p=0.028) longer than patients with inactive disease to complete the test.

In the total patient cohort, there was a strong, negative correlation between the six-minute walk distance (6MWD) and TUG time (r=-0.77, <0.001). Performing multivariate, linear regression analyses of the contribution of heart, lung, or muscle dysfunction on the 6MWD and TUG time in patients, both test outcomes were influenced by CMAS the most, followed by a low DLCO and HRCT pathology in the 6MWT and FVC in the TUG test. 6MWD and TUG time were also associated with patient reported health related quality of life and physical disability.

4.2 PAPER II (Visit 2)

CPET results were based on 45/72 (63%) invited patients and their 1:1 age- and gender-matched controls. Mean disease duration was 20.8y. VO\textsubscript{2max} was 11% lower in the total patient group, 10% lower in patients with inactive disease, and 12% lower in patients with active disease compared to respective controls. A low VO\textsubscript{2max} was found in 12 (27%) patients versus 2 (4%) controls (p=0.006); in 6/16 (38%) with active and 6/29 (21%) with inactive disease (p=0.222).

In patients with inactive disease, maximal ventilation (VE\textsubscript{max}) and oxygen pulse (O\textsubscript{2pulse}) were lower compared to controls, suggesting that VO\textsubscript{2max} may be reduced due to deconditioning. VO\textsubscript{2max} was negatively associated with disease damage. In patients with active disease, VE\textsubscript{max}, O\textsubscript{2pulse}, and maximal voluntary ventilation (MVV) were lower compared to controls, and VE\textsubscript{max} and MVV were lower than in patients with inactive disease, suggesting an impaired ventilatory capacity in this
patient group. This impairment may be due to reduced thoracic muscle force as VO$_{2\text{max}}$ was associated with muscle strength and function in this patient group.

### 4.3 PAPER III (Visit 2)

Results were based on 44/72 (61%) invited JDM patients participating in visit 2. Mean disease duration was 21.8y.

JDM patients had lower objectively measured knee extensor muscle strength (29 (95%CI 13-46) Nm lower peak torque, $p=0.001$) and endurance (Total work was 738 (565-1155) J in patients vs 1249 (815-1665) J in controls, $p<0.001$) compared to controls (also significantly lower in each patient group compared to respective controls). Patients with active disease had lower results than patients with inactive disease, but when correcting for muscle cross-sectional area (CSA), only muscular endurance remained significantly lower.

Clinically assessed muscle strength (MMT), endurance (CMAS), and muscle damage (MDI extent and severity scores), but not muscle disease activity (DAS), were higher in patients with active compared to inactive disease. Objective muscle tests of knee extensors correlated with clinical tests of strength and endurance only in patients with active disease.

MVPA correlated with torque only in patients with inactive disease.

MRI detected fatty infiltration was found in 21/44 (49%) of patients; 63% of these had active disease. Although not statistically tested, muscle edema and calcinosis appeared with similar frequency. Quadriceps CSA was significantly smaller in patients with active compared to inactive disease indicative of more muscle atrophy.

The total patient group had higher levels of the myokines decorin and IP-10 compared to controls, while there were no differences in myokine levels between active and inactive disease.

Muscle biopsy results indicated similar capillary density, but different muscle fiber composition between active and inactive disease although not statistically tested. There were no indications of muscle inflammation in either patients with active or inactive disease.
5 Discussion

This thesis focuses on different aspects of physical fitness in JDM after long-term disease duration; on physical function on a submaximal level resembling everyday activities (PAPER I), on cardiorespiratory fitness (PAPER II), and on structural and functional adaptations of skeletal muscle (PAPER III). The results revealed similar trends: after long-term disease duration the components of physical fitness were impaired in patient compared to controls, more pronounced in patients with active compared to patients with inactive disease.

5.1 Representativeness

The different cohort samples presented in PAPER I-III showed similar representativeness despite a different selection of patients for each article, although patients in visit 2 (PAPER II and III) were older, had a longer disease duration, and a smaller proportion with active disease compared to visit 1 (Table 4).

Table 4. Patient characteristics in PAPER I-III.

<table>
<thead>
<tr>
<th></th>
<th>PAPER I (n=59)</th>
<th>PAPER II (n=45)</th>
<th>PAPER III (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (n)</td>
<td>36 (61)</td>
<td>28 (62)</td>
<td>27 (61)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>25.2 (12.5)</td>
<td>28.9 (12.0)</td>
<td>30.1 (12.1)</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>16.9 (10.6)</td>
<td>20.8 (11.9)</td>
<td>21.8 (11.8)</td>
</tr>
<tr>
<td>DAS (0-20)↑</td>
<td>5 (3-6)</td>
<td>4.4 (2.6)</td>
<td>4.3 (2.5)</td>
</tr>
<tr>
<td>MDI (0-40)↑</td>
<td>3 (2-6)</td>
<td>3 (2-5)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td>PhyGloActVAS (0-10)↑</td>
<td>Not assessed</td>
<td>0.2 (0.0-0.7)</td>
<td>0.2 (0.0-0.6)</td>
</tr>
<tr>
<td>Active disease (n)</td>
<td>30 (51)</td>
<td>16 (36)</td>
<td>17 (39)</td>
</tr>
<tr>
<td>SF-36 PCS (0-100)</td>
<td>53.9 (46.4-58.2)</td>
<td>53.1 (47.8-59.6)</td>
<td>52.4 (46.6-59.0)</td>
</tr>
</tbody>
</table>

Values are presented as n(%), mean (SD), or median (IQR).

The female predominance in our cohort was similar to other JDM studies (7). Age was high and disease duration was long compared to other studies on JDM long-term outcome (67, 68). Disease damage was also relatively high, assumingly due to the long disease duration. Compared to a Brazilian JDM study that also used the original PRINTO criteria for inactive disease (after mean 6.7 (SD3.2) years disease duration), a larger proportions of our patients still had active disease (36-51% vs 11%) despite a longer disease duration (110).
Our controls were randomly drawn from the national registry in order to resemble the general population. However, we only had financial resources to assemble them from Oslo and its nearby county Akershus. Although the included area covers both urban and rural areas, patients were summoned nationwide, causing a potential bias especially regarding social habits. Particularly important to our work was the potential difference in exercise habits, however, when comparing exercise habits (one week of accelerometer data) of our controls ≥ age 18 years in paper II and III (from visit 2) with national records in the general population, these turned out to be comparable (155).

5.2 Primary Outcomes in the Total Cohort Compared to Literature

The major outcomes included 6MWD (m) and TUG time (s) in PAPER I, CRF (VO$_{2\text{max}}$, L*min$^{-1}$kg$^{-1}$) in PAPER II, and objectively tested muscle strength (Torque, Nm) and muscular endurance (work, W) in PAPER III (Table 5).

Table 5. The combined results of PAPER I-III.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>6MWD (m)</td>
<td>592 (81)</td>
<td>649 (79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TUG time (s)</td>
<td>13.1 (2.1)</td>
<td>12.3 (2.0)</td>
<td>0.036</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$</td>
<td>40.4 (9.5)</td>
<td>45.2 (9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak torque (Nm)</td>
<td>116.2 (38.8)</td>
<td>145.5 (46.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total work (J)</td>
<td>738 (565-1155)</td>
<td>1249 (815-1665)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as mean (SD) or median (IQR).

In PAPER I the 6MWD was lower, and the TUG time was higher compared to controls. There are no controlled studies on submaximal exercise tests in JDM to compare our results with. However, after we published PAPER I we discovered two articles published before and one article published simultaneously with our work on response to exercise in JDM; two addressing the 6MWT and one addressing the TUG test (but none using the 6MWT or TUG test as major outcomes)(101, 108, 156). Due to the lack of a control group in these articles, large age differences between the articles, and the use of different TUG test procedures, we can only compare our data to a Danish study assessing the 6MWT in 9 JDM patients in clinical remission with a mean age of 26y and median 17.5y since diagnosis (similar to our mean age of 25y and median disease duration of 16.8y). In this study the mean 6MWD before exercise intervention was comparable to the 6MWD in our patients with inactive disease (622m ± 23 vs 622m ± 76).
In PAPER II, CRF was reduced in patients compared to controls similar to other JDM studies (74, 99, 100), although our study showed smaller differences between patients and controls. As our patients also had physical activity levels comparable to a Danish cohort, but higher than a Brazilian cohort (74, 110), this may reflect that we had a more physically active patient group compared to other cohorts or might be explained by a gradual improvement in CRF with longer disease duration (PAPER II).

In PAPER III, objective muscle tests were also lower in patients compared to controls. Although there are studies including objective muscle strength testing in JDM, differences in test procedures and presentation of data made it difficult to compare our data to these other JDM studies. We have not identified any other articles assessing quadriceps muscle torque (measured in Nm) and work (measured in J) in JDM.

5.3 Differences between Patients with Active and Inactive Disease

We have not been able to find any other studies comparing CRF, objective muscle testing, or physical function tested by the 6MWT and the TUG test between JDM patients with active and inactive disease (PRINTO) (113). By doing this, we sought to investigate the impact of being in an active disease state on physical fitness; in all papers comparing the major outcomes in the patient groups against each other as well as against respective controls (assessed as groups in PAPER I and matched 1:1 in PAPER II and III). The results showed consistent differences between the two groups:

In patients classified as having inactive disease, there was a small reduction in aerobic exercise capacity compared to controls that only became visible on large physical exertion: While VO$_{2\text{max}}$ was slightly reduced compared to controls (PAPER II), there was no difference in 6MWD between patients with inactive disease and respective controls (PAPER I). There was also no difference in TUG time between patients with inactive disease and controls (PAPER I). CPET variables during the maximal testing suggested deconditioning as a possible reason for the reduced VO$_{2\text{max}}$ in this patient group rather than pathologic cardiac, pulmonary, or muscular processes (discussed in PAPER II). These findings were supported by the comparable results of the 6MWT and TUG test between patients with inactive disease and respective controls. Muscle torque and work were, however, lower in patients with inactive disease compared to respective controls (PAPER III). Although lacking sufficient data to conclude about underlying mechanisms, we did find disease damage such as MRI-detected fatty infiltration as well as lower physical activity compared to respective controls in patients with inactive disease. Thus we could speculate that the reduction might have been influenced by both deconditioning and disease damage in this patient group.
In patients with active disease, on the other hand, the picture was different. Submaximal and maximal exercise testing as well as objectively tested muscle strength and muscular endurance tests were all lower compared with respective controls, and all but VO\(_{2}\text{max}\) were lower compared to patients with inactive disease. During CPET (PAPER II), maximal voluntary ventilation (MVV), ventilation (VE), and oxygen pulse (O\(_2\text{pulse}\)) were lower in patients with active disease compared to controls and patients with inactive disease\(^2\), indicating a lower ventilatory capacity contributing to reduced VO\(_{2}\text{max}\) in patients with active disease (discussed in PAPER II).

The major hypothesis behind the lower ventilatory capacity was a reduced chest wall expansion possibly due to muscle weakness as VO\(_{2}\text{max}\) *kg\(^{-1}\) correlated moderately with MMT-8 and CMAS only in patients with active disease (discussed in PAPER II). This theory was supported by findings in PAPER I of associations between the 6MWD and DLCO\(_c\) and CMAS. A low DLCO\(_c\), but not DLCO corrected for alveolar volume (DLCO/VA), compared to controls had previously been found in our patients, indicating low lung volumes (43), which could be explained by a reduced strength to expand the thorax.

In PAPER III, results supported that there were differences in muscle characteristics between patients with active and inactive disease. Muscle strength and endurance, tested both clinically and objectively, were lower in active compared to inactive disease (PAPER III). Much of this difference could be explained by reduced muscle mass, as the quadriceps cross-sectional area (CSA) was lower in patients with active disease. However, muscular work remained lower in patients with active disease when accounting for this difference, suggesting other structural mechanisms in the muscle tissue contributing to the lower muscular endurance. MRI data showed numerically more fatty infiltration in patients with active disease, while fatty infiltration did not correlate with objective muscle testing, questioning its impact on muscular endurance (although we might have been underpowered). Nor was there any difference in myokine levels between patients with active and inactive disease. Muscle biopsies, however, revealed trends of different muscle fiber composition between patients with active and inactive disease. These changes could be suggestive of long-term physical inactivity, chronic disease activity (85), or hypoxia (159) (discussed thoroughly in PAPER III). We did not find differences in capillary density between patients with active and inactive disease,

\(^2\)In PAPER II we did not correct MVV for height in the comparison between patients with active and inactive disease although patients with inactive were significantly higher than patients with active disease. Several studies, however, do indicate strong correlations between MVV and height (157, 158). When retrospectively correcting for height, the difference between active and inactive disease became insignificant, while there was still a significant difference between patients with active disease (but not inactive disease) and their respective controls.
and there were no signs of muscle inflammation in any biopsy. A recent study of our own cohort (from visit 1), showed that nailfold capillary density was lower in patients with active compared to inactive disease (160). These results may suggest that our biopsy results were statistically underpowered, or there may be differences between nailfold and muscle capillaries. The lack of muscle capillary differences between active or inactive disease in muscle did not, however, tell us anything about potentially functional impairment of capillaries (159).

The results of the three papers presented in this thesis were based on examinations performed at two different time points with different samples of an open cohort. Still, the trends of differences in physical fitness between patients with active and inactive disease were consistent. This increases the robustness of our results. However, although we have dared to suggested possible hypotheses behind our results according to trends in our data sets, we should handle these interpretations carefully, as our study is an observational and hypothesis generating study only, and thus lacks the strength to conclude upon potential mechanisms.

5.4 Secondary Outcomes

Although secondary outcomes in PAPER I and III were treated primarily as explanation variables for the primary outcomes, some of the outcomes need mention as we believe that they represent novel contributions to clinical research.

Clinical Muscle Tests

Clinical muscle tests (MMT-8 and CMAS) were reduced in our patients compared to controls, similar to a Danish study of patients of mean 13.9y disease duration (74). However, compared to a multinational study of 490 patients with a mean 7.7y disease duration, more patients in our cohort had mild impairment, but fewer had serious impairment (discussed in PAPER III). This could indicate an improvement in muscle strength with disease duration, or the study population could represent a more physically fit patient group (more on physical activity below).

Magnetic Resonance Imaging

MRIs from visit 1 have previously been described in detail (76). MRIs from visit 2, however, were presented for the first time. Similar trends were found in the two visits despite a slightly different selection of patients: Patients with active disease had numerically more fatty infiltration although not statistically significant, while edema representing disease activity, and calcinosis were similar in frequency between patients with active and inactive disease.
**Myokines**

Myokines are known to be secreted from muscle tissue in response to exercise or inflammatory stimuli (94). In PAPER III (visit 2), we found higher levels of the myokines IP-10 and decorin in patients versus controls, similar to previous findings from visit 1 (77). IP-10 (also called CXCL10) was recently suggested as a biomarker for JDM (161), associated with disease activity. Although we defined active disease slightly different, we did not, however, find any difference in IP-10 between active and inactive disease. This may suggest that we were statistically underpowered, or that the myokine is less stable with time, as our patient cohort had a much longer disease duration. Further studies are needed to verify this hypothesis.

Decorin is known to be both antifibrotic and pro-inflammatory (162, 163). The antifibrotic effect is partly mediated by inactivation of both TGFβ and the skeletal muscle specific myostatin. We did not find differences in myostatin levels in patients vs controls, nor was any such difference found regarding TGFβ levels in the previous myokine study of our visit 1 cohort either (77). However, the higher circulating levels of decorin in patients might be due to the increase in visceral fat (VAT) depots, also previously found in our JDM-patients in visit 1 (164). VAT is a greater source of decorin than subcutaneous fat (165), and decorin in our patients could reflect the general inflammatory state of JDM rather than affecting muscle fibrosis/wasting through Myostatin.

**Muscle Fiber Composition**

To our knowledge, we were the first to describe muscle fiber composition in JDM patients (PAPER III). However, studies on long term adult DM showed a decreased proportion of type I muscle fibers in patients with chronic disease compared to untreated, newly diagnosed patients and healthy controls (166). The decreased proportion of type I muscle fibers in the chronic patients was similar to the proportion of type I fibers in our patients with active disease, perhaps indicating some connection between chronic JDM and muscle fiber types, consistent with other studies showing connections between chronic disease and a greater percentage of type II fibers (85). There has also been suggested that there is a switch from type I to type II fibers in response to anti-inflammatory DM treatment (166, 167). However, it was surprising given the numerically higher MVPA in patients with active compared to inactive disease, as exercise is found to increase the proportion of type I fibers in adult DM (103).

Unfortunately, fiber size was reported differently in the adult DM muscle fiber switch study and our study, making comparison difficult (166). Our results, however, showed trends of type I fibers being larger and type II fibers smaller in patients with active compared to inactive disease. Again, the
results were somewhat surprising as exercise in DM is also found to increase the size of type II fibers (103). Especially fiber size may be affected by sex and to a certain extent age (168), however, our groups were similar regarding these possible confounders.

**Physical Activity**

Our patients were less physically active than our controls and the general, Norwegian population (PAPER II and III). However, physical activity levels in our patients were comparable to a Danish JDM cohort (74) and higher than a Brazilian JDM cohort (110). Compared with the Brazilian cohort, a smaller proportion of patients had inactive disease (PRINTO) (70% vs 89%). Thus our patients presented relatively high levels of physical activity despite also a relatively high degree of disease activity. In Norway we have focused on physical activity in JDM rehabilitation since around the millennium, which might be a contributing factor to this.

**Patient Reported Outcomes**

In all papers included in the thesis, SF-36 was significantly lower in patients versus controls, and in patients with active compared to inactive disease. cHAQ/HAQ was higher in patients with active compared to inactive disease in PAPER I and II, while a different presentation of cHAQ/HAQ in PAPER III revealed comparable amounts of patients with value >0 in the two patient groups. In PAPER II, patients also reported higher self-experienced disease activity compared to patients with inactive disease.

In PAPER I, both the SF-36 and the cHAQ/HAQ correlated with 6MWD and TUG time in the direction of better results of both tests with increased physical function in the whole patient population. In PAPER II, cHAQ/HAQ also showed strong correlations with VO$_{2\text{max}}$, however, only in patients with active disease, while patients with inactive disease showed weak, significant correlations between VO$_{2\text{max}}$ and SF-36.

Together, these findings indicate that reduced cardiopulmonary fitness both at a maximal and submaximal level may not be subclinical, but may be experienced by the patients, and they support the link between disease activity, reduced physical function, and health related quality of life (HRQOL) in patients with active disease. Unfortunately, we did not correlate PROMs with muscle strength and muscular endurance in PAPER III.

5.5 **Active Disease or Disease Damage?**

The use of the PRINTO criteria of inactive disease resulted in our discovery that physical fitness in patients with inactive disease seemed very little affected by JDM. On the contrary, patients with
active disease, even after many years of disease duration, had impaired physical fitness not only during strenuous exercise, but also on a submaximal level affecting every day activities. Although much of the results pointed at muscle weakness being the key factor to this, also lung involvement was found to contribute. This would never have been found without the stratification into active and inactive disease and suggests that, although criticized for underestimating skin involvement (115), the PRINTO criteria could be useful in distinguishing JDM patients with physical impairment after long term disease duration from patients without.

What the stratification into active and inactive disease does not tell us, is whether the differences in physical fitness actually represent active inflammation or disease damage. The PRINTO criteria were originally validated in a JDM population with a short disease duration and little disease damage (113). The study even points out that there may be a challenge regarding these criteria when applied to long-term evaluation in which there may be a larger proportion of disease damage responsible for reductions in CMAS and MMT (113). A reduction in both these tests, regardless of the cause, means that a patient is automatically labelled “active”. Most of our patients with so-called “active disease” scored just outside the cut-off that defined inactive disease, perhaps due to a slightly low MMT or CMAS.

However, some results from previous studies support that the category of active disease does, in fact, represents active inflammation. After median 16.8y disease duration, MDAAT and DAS were higher (76), and nailfold capillary density lower (160) in patients with active compared to inactive disease. However, we have not found significant difference between the patient categories in terms of muscle enzymes (which are known to be poor biomarkers of JDM disease activity), inflammatory parameters, myokines, or MRI detected signs of activity inflammation. We also found significantly higher disease damage in patients with active compared to inactive disease, in terms of MDI, physician global damage and MRI-detected damage. Further research is needed to explore mechanisms of impairment within the PRINTO “active” category, as this could have an effect on the choice of treatment and follow-up after long-term disease.
6 Main Conclusions

- All assessed components of physical fitness (cardiorespiratory fitness (CRF), submaximal exercise testing, muscle strength, and muscular endurance) were reduced in JDM patients compared to controls after long-term JDM disease duration.
- All components of physical fitness apart from CRF were more impaired in patients with active compared to inactive disease (according to PRINTO criteria of inactive disease).
- Also patients with inactive disease had impaired physical fitness compared to respective controls, however, only visible during high exercise intensity and muscle testing.
- There seemed to be different reasons for reduced physical fitness in patients with active and inactive disease. Muscle impairment was central in patients with active disease and was associated with disease activity, disease damage, and perhaps also muscle fiber composition, and muscle impairment may have contributed to reduced thoracic expansion and hence low ventilation during exercise in these patients. Impairment in patients with inactive disease, on the other hand, seemed to a large extent related to deconditioning as well as disease damage.
- Throughout the work represented in this thesis, there was a distinct difference between patients labelled with active and inactive disease according to the PRINTO criteria of inactive disease. However, it is uncertain whether the classification truly distinguishes between inflammation or disease damage after very long-term disease. Further studies are needed to verify this.
7 Clinical Implications and Future Perspectives

Our results reveal new insights into differences in physical fitness between patients with active and inactive disease after long-term disease duration; that patients with inactive disease have almost normalized physical fitness compared to the general population, while patients with active disease have muscle weakness and reduced muscular endurance, perhaps especially in the thoracic region, that may prohibit them from reaching their physical goals. One could argue that because MMT and CMAS are included in the criteria to define active or inactive disease, these findings are obvious as the patients to a large extent are grouped based on clinical muscle performance. However, we do believe that, regardless of whether the difference represents disease activity/damage or physical impairment, these two groups may require different attention when it comes to physical rehabilitation.

During the last decades there has been a growing focus on the importance of exercise training in JDM (108), also after recovering from the disease (101). It was earlier believed that exercise could increase inflammation, however, research has shown that exercise training in JDM is safe (156). The first randomized, controlled trial on this used a training program incorporating interval and strength training with increasing intensity and number of repetitions over a 12-week period (108). This program increased VO$_{2\text{max}}$, muscle function, and functional ability, however, not directly focused on thoracic muscles. We believe that our patients, regardless of whether they are classified with active or inactive disease, should perform regular exercise, both aerobic exercise and strength training. However, to date, there are no exercise studies in JDM dividing patients into active or inactive disease. In our data from PAPER II, patients with active disease had significantly higher physical activity levels compared to patients with inactive disease, and still VO$_{2\text{max}}$ was lower. Future studies are needed to explore our suggested mechanisms behind reduced CRF in active and inactive disease, assess whether the two patient groups react to physical activity in the same way, and explore whether they require different exercise strategies according to different mechanisms behind reduced physical function.
8 References


69

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Functional and Structural Adaptations of Skeletal Muscle in Long-Term Juvenile Dermatomyositis; a Controlled Cross-Sectional Study

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ABSTRACT

Objective: To compare muscle strength/endurance of knee extensors between a) patients with long-term juvenile dermatomyositis (JDM) and controls and b) patients with active and inactive disease. In patients; to explore associations between strength/endurance and i) clinical parameters ii) physical activity, and iii) humoral or structural adaptation in skeletal muscle.

Methods: In a cross-sectional study, we tested isometric muscle strength (peak torque) and dynamic muscular endurance (total work) of knee extensors, physical activity (accelerometers), and myokines (ELISA) in 44 patients and 44 age and sex matched controls. Patients were examined by validated tools (clinical muscle tests, disease activity/damage and inactive disease) and magnetic resonance imaging of thigh muscles including cross-sectional area (CSA). Needle biopsies of m. vastus lateralis (n=12, age ≥18y) were assessed by immunohistochemistry.

Results: After mean 21.8±11.8y disease duration, peak torque and total work were lower in patients vs. controls, and in active vs. inactive disease. When controlling for quadriceps CSA, only work remained lower in active vs inactive disease. Torque and work correlated with clinical muscle tests in patients with active, but not inactive disease. Muscle biopsy results indicated different fiber type composition but similar capillary density between active and inactive disease.

Conclusions: In long-term JDM, muscle strength/endurance of knee extensors were lower compared to controls, and in active vs inactive disease. Impaired muscular endurance in active disease may be influenced by structural and functional adaptations of muscle tissue independent of muscle size. Our results indicate a need for more sensitive muscle tests in this clinical setting. [Word count: 249]
INTRODUCTION

Reduced muscle strength and endurance are major clinical manifestations of juvenile dermatomyositis (JDM), the most common idiopathic inflammatory myopathy (IIM) of childhood (1). Muscle involvement often comprises proximal muscles of extremities as well as truncal muscles including neck flexors (2).

During the early phase of JDM, decreased muscle function can be severe and is related to active myositis (3). Autoimmune-like mechanisms are believed to contribute to perivascular inflammation and vasculopathy, resulting in ischemic muscle fiber damage, perifascicular atrophy and reduced capillary density (4). However, also non-immune mechanisms related to reduced blood flow seem to be involved in impaired muscle function (3, 5). Both immune and non-immune processes may induce the release of myokines; cytokines derived from muscle tissue (6). Myokines are associated with muscle inflammation, but are also believed to mediate anti-inflammatory effects related to exercise (6).

With treatment, muscle strength and endurance gradually improve. Yet, long-term outcome studies have shown persistent, mild muscle weakness and decreased muscle endurance tested clinically by the unilateral manual muscle test (MMT-8) and the childhood myositis assessment scale (CMAS) (7-9), more pronounced in patients with active than inactive disease (10). Severe impairment is rare (7). However, a challenge related to MMT and CMAS, is their frequently observed ceiling effects (11). Mild, but functionally important, muscle weakness may therefore be difficult to detect, especially in patients with inactive disease (10).

Testing of isometric strength and dynamic muscular endurance could provide a more objective and sensitive testing of muscle function in JDM patients with mild muscle
impairment (12). Thigh muscles including knee extensors are among the most commonly involved muscles in JDM (13), and is the muscle group most frequently examined by biopsies (14) and magnetic resonance imaging (MRI) (15). Therefore, knee extensors could serve as a representative test localization for proximal muscle function in JDM.

After long-term disease MMT-8 or CMAS have been found to be associated with disease activity (9, 16), disease damage (16), and elevated serum myokines (17). Despite the association with active inflammation, myokines may also be involved in persistent muscle weakness in treatment-suppressed, non-inflamed muscle (6). However, the association between objective muscle tests and these parameters in long-term JDM is not known.

To our knowledge, no studies exist on muscle fiber composition after long-term JDM. A study on adult DM found altered muscle fiber composition in patients with chronic disease (18). Muscle fiber composition is dynamic, with size and proportion of slow-twitch oxidative type I and fast-twitch fiber type II muscle fibers changing according to numerous factors including age, sex, and exercise (19, 20). Exercise was found to increase the proportion of type I muscle fibers in adult DM (21).

We aimed to compare isometric muscle strength and dynamic muscular endurance of knee extensors measured by sensitive, objective methods between patients with long-term JDM and controls, and between patients with active and inactive disease. Furthermore, in patients to explore if changes in thigh muscle strength/endurance were associated with i) disease parameters including commonly used clinical muscle tests, ii) physical activity, and iii) structural or humoral adaptation of skeletal muscle.
PATIENTS AND METHODS

We used a controlled, cross-sectional design. The study was part of a larger project on physical fitness in JDM patients at Oslo University Hospital (OUS) and the Norwegian School of Sport Sciences (NIH) between 2013 and 2015. We re-invited patients from an already established JDM cohort (22). In addition, eight patients from a prospective JDM cohort at OUS and three additional patients were invited.

Patient inclusion criteria were a) diagnosis of JDM after 1970, b) a definite or probable dermatomyositis according to Peter and Bohan criteria (23), c) diagnosis < age 18y, and d) age ≥ 10y at examination. Patients were excluded from data analyses if not completing tests of isometric muscle strength and muscular endurance or a muscle biopsy. Patients were scored retrospectively according to the 2017 EULAR/ ACR classification criteria (24).

Controls were randomly drawn from the National Registry, and were age- and sex-matched 1:1 with the patients. Exclusion criteria were a) mobility problems, b) inflammatory rheumatic disease, c) other active, autoimmune disease, d) other autoimmune disease treated with immunosuppressive agents, e) serious lung or heart disease, and f) exclusion of matched patient.

All participants (or guardians if age <16 y) signed informed consents according to the Declaration of Helsinki (25). The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics (2013/1039).

Clinical examination

In patients, we used the Disease Activity Score (DAS) (0-20) and the Physician Global Activity Visual analogue Scale (VAS) (0-10) to assess global disease activity,
and the Myositis Damage Index (MDI) (0-40) and the Physician Global Damage VAS (0-10) to assess global disease damage (11). We used the MMT-8 (0-80) (including the separate MMT knee extensor component (0-10) and the CMAS (0-52) to clinically assess muscle strength and endurance (11). We defined MMT/CMAS score <64/35 as severe impairment (7). We used the DAS muscle component (0-11) to assess disease activity in muscle (26), and the MDI muscle damage extent (0-3) and severity (VAS) (0-10) scores to assess disease damage in muscle (27). We divided patients into active or inactive disease by the original PRINTO criteria for inactive disease (28).

**Self-reported health**

To evaluate physical function, we used the Norwegian version of the 36-Item Short Form Survey (SF-36) physical component score (PCS) (0-100) in patients and controls ≥14y old, and the Childhood/Adult Health Assessment Questionnaire (cHAQ/HAQ) (0-3) </≥18y old in patients (11).

**Physical activity**

As previously described we measured physical activity by waist-borne accelerometers in patients and controls for seven consecutive days (29). We defined counts/min (CPM) divided by valid assessment days <100 as sedentary, 100-1999 as light physical, and ≥2000 as moderate to vigorous exercise (MVPA) (30). MVPA bouts were defined as the average daily time of MVPA of at least 10min duration.

**Objective muscle testing of patients and controls**

We used maximal voluntary isometric contraction (MVC) force of knee extension, expressed as peak torque (Nm), to measure muscle strength, and dynamic knee
extensions, expressed as total work (J), to measure muscular endurance (collectively referred to as objective muscle tests). A custom-made knee extension device (GYM 2000AS, Vikersund, Norway), was set up as previously described (31). Following a warm-up protocol, participants performed three consecutive unilateral maximal isometric contractions of knee extensors lasting 5s under strong verbal encouragement, separated by 60s rests. We processed data via a LabVIEW software (National Instruments Corporation, Austin, TX, USA), and used the average of the maximum force for each leg for statistical analyses. We calculated peak torque as follows: Peak torque (Nm) = Force (N) x lever arm length (m).

To measure muscular endurance, a resistance mass of 30% of the MVC force was attached to the knee extension device. Guided by a metronome paced at 1 Hz, participants performed rounds of full knee extension and 90° flexion until exhaustion (incapability to fully extend). The average between the right and left leg’s maximal number of extensions was used for statistical analyses. We calculated total work as follows: Total work= 30% of peak torque (Nm) x sin (90°) x number of repetitions.

**MRI**

Patients underwent MRIs of thigh muscles using a 1.5T scanner (Siemens, Erlangen, Germany) with phased array body coils, including transversal T1 turbo spin echo (TSE) and short tau inversion recovery (STIR) sequences. Three MRIs performed at local hospitals were summoned and scored collectively with the remaining cohort. Two experienced musculoskeletal radiologists (EK and EM) scored the presence of edema and pathologic fatty infiltration in muscle and calcinosis in soft tissue layers as previously described (9). They also measured the maximal cross-sectional area...
(CSA) of the anterior thigh compartment (quadriceps femoris) (mm²) separately for each leg (31). We used the average of both legs for statistical analyses.

**Muscle enzymes and myokines in blood**

We obtained serum through venous blood sampling, and performed all procedures mentioned below according to manufacturers’ protocols.

In patients and controls, we analyzed creatine kinase (CK), lactate dehydrogenase (LD), and aspartate amino transferase (ASAT) in the hospital’s routine laboratory. We analyzed circulating levels of IL-6, IL-8, IL-15, IFN-γ, IP-10, CCL5, and TNF-α by Luminex Xmap technology (Luminex, Austin, TX), using the Bio-Plex Pro™ Human Cytokine 27-plex Assay (#M500KCAF0Y, Bio-Rad, Hercules, CA). The assay included a high-sensitive standard curve in order to detect very low concentrations of cytokines. We used ELISA kits to measure levels of decorin (#EHDCN, Thermo Scientific, Frederick, MD, USA), myostatin, and MCP-1 (#DGDF80 and #DCP00, RnD systems, Minneapolis, MN, USA).

**Muscle biopsies**

We invited patients ≥18y for a percutaneous needle muscle biopsy (a more gentle procedure compared to open biopsy as it was not intended for clinical purposes (32)) of the left vastus lateralis muscle. With the patient in a supine position, in local anesthesia (Xylocain 10mg/mL + Adrenaline 5µg/mL) and sterile procedure, we used a 6mm Pelomi needle (Albertslund, Denmark) with manual suction to obtain the biopsy (TR). We obtained 30-40 mg tissue for immunohistochemistry. Following excision, samples were frozen in an optimal cutting temperature medium (OCT).
(CellPath, Newtown Powys, UK) dispersed in isopentane at freezing point, before storage at -80°C until further analyses.

**Immunohistochemical analyses:**

We thawed the frozen biopsy samples to -20°C and cut serial 8-μm thick sections using a microtome (CM 1860 UV, Leica, Nussloch, Germany), before mounting them on microscopic slides (Superfrost Plus, Thermo Scientific, MA, USA). We performed immunohistochemical analyses as described by Paulsen et al., using primary antibodies against myosin heavy chain type 1 (MHC1), dystrophin, and CD31 to evaluate muscle fibers and capillaries (33), and CD68, tenascin C, and embryonic MHC to evaluate active inflammation and regeneration (34) (details about primary and secondary antibodies are listed in supplementary table 1). We identified fiber type distribution, fiber cross-sectional area, and capillaries by TEMA software (CheckVision, Hadsund, Denmark). We expressed capillarization as capillaries per fiber (CF, total number of capillaries/total number of fibers), capillaries around each fiber (CAF) and CAF related to fiber area (CAFA) for type 1 and type 2 fibers.

**Statistical analyses**

We used IBM SPSS statistics version 25. To compare patients and controls, we used the paired sample T-test, the Wilcoxon signed rank test, or the McNemar’s test as appropriate. To compare patients with active vs inactive disease, we used the independent sample T test, the Mann Whitney U test, or the Chi-squared test as appropriate. Values are presented as mean±SD, median (IQR), or n (%). We performed correlations using Pearson’s R or Spearman’s rho as appropriate, defined as weak $r < 0.3$, moderate $0.3 < r < 0.69$, or strong $r \geq 0.7$. P-values <0.05 were
considered statistically significant. We did not correct for multiple analyses because of the hypothesis generating nature of our study, nor did we perform statistical analyses of muscle biopsy results due to a small n.

RESULTS

Patient participation

Of 72 invited patients, 45/53 (85%) accepting participation fulfilled the inclusion criteria. One patient was later excluded due to change of diagnosis. We sampled biopsies from 17/37 (46%) patients ≥18y. All patients but one fulfilled EULAR/ACR classification criteria for JDM, the last patients fulfilled the criteria for IIM, but lacked the classic presentation of rashes.

General characteristics of patients and controls

Among patients, 17/44 (39%) had active disease (14/17 (82%) were >18y old) and 27/44 (61%) had inactive disease (23/27 (85%) were >18y old) (Table 1). Regarding physical activity, patients had 13.2 (95%CI 4.6-21.8, p=0.003) min/d less MVPA than controls. While there was no significant difference in MVPA between patients with active disease and respective controls, nor between the control groups of active/inactive disease (data not shown), patients with inactive disease had 16.4 (95%CI 6.5-26.2, p=0.002) min/d less MVPA than respective controls. In patients <18y old (n=6) MVPA was 58.5±34.3 min/d. In adults ≥18y old (n=37), CPM were 341.8±131.6. DAS >0 was found in 42/44 (98%) patients, mean MDI score was 3.3±2.4, and 27/44 (61%) had MDI VAS global >0.2 cm. In the whole patient group there were no correlations between age and any accelerometer variables.

Muscle characteristics in patients and controls
Patients had 29 (95%CI 13-46) Nm lower peak torque than controls (p=0.001) (Table 2). Total work was 738 (565-1155) J in patients vs 1249 (815-1665) J in controls (p<0.001). Peak torque and total work were also lower in patients with active and inactive disease compared to respective controls (p’s<0.034, data not shown), and in patients with active compared to inactive disease (p’s 0.016 and 0.019 respectively, Table 2). When normalized to quadriceps femoris area, only total work/CSA remained lower between the patient groups (p=0.027).

In the total patient group, 38 (86%) had MMT-8 <80, and 27 (61%) had CMAS <52; only 1 (2.5%) had MMT <64 and CMAS <35 (severe impairment). MDI muscle dysfunction was found in 9 (21%); muscle weakness in 31 (71%); and muscle atrophy in 5 (11%). MMT and CMAS (included in the definition of active/inactive disease) were lower and the muscle damage extent (MDI muscle) and severity (MDI VAS muscle) scores higher in patients with active versus inactive disease (p’s<0.005) (Table 2).

The total patient group had higher levels of decorin and IP-10 (myokines related to inflammation) compared to controls, while there were no significant differences in myokine levels between active and inactive disease (Table 2). No myokine correlated with DAS total/muscle.

MRI-assessed variables showed no significant differences between active and inactive disease, but a numerically larger proportion of patients with active disease had pathologic perimysial fatty infiltration compared to patients with inactive disease (Table 2). None had fatty infiltration of more than 50%. The quadriceps CSA was smaller in patients with active compared to inactive disease (p=0.017) (Table 2).
**Associations between objective muscle tests and disease variables/physical activity measures**

In patients with active disease, peak torque and total work of knee extensors showed moderate to strong correlations with MMT-8, MMT knee extensor component, and CMAS (Table 3, figure 1). There were no significant correlations between clinical and objective tests of muscle strength or endurance in patients with inactive disease (Table 3). Peak torque correlated weakly with MVPA in patients with inactive disease. Both peak torque and total work correlated with DAS muscle in patients with active and inactive disease. In the whole patient group, peak torque correlated negatively with the MDI muscle score. No significant correlations were found between peak torque or total work and MDI muscle scores or MRI findings in the patient subgroups. In patients with active disease, peak torque showed a moderate, positive correlation with IL-6 (Table 3).

**Muscle biopsy characteristics in patients**

Twelve out of 17 (71%) muscle biopsies had adequate quality for muscle fiber assessment; 11/17 (65%) for capillary assessment. The samples with inadequate quality had tissue resembling muscle tissue, but with a texture unsuitable for slicing (2/5 of patients with inadequate biopsies had MRI-detected perimysial fatty infiltration, none had calcinosis or edema). Among patients with adequate biopsy quality, median time from clinical examination to muscle biopsy was 11.0 (9.0-16.3) months, and none reported major changes in life style or disease activity during this time.
General characteristics of the 12 presented biopsied patients showed no significant differences from the remaining cohort ≥18y (supplementary table 2). 7/12 (58%) had active, and 5/12 (42%) had inactive disease, with comparable age and sex distribution between the groups. None of the biopsied patients used anti-inflammatory medication and 8/12 (67%) patients had MRI-assessed fatty infiltration (2 of these with 50% distribution, the rest with pathologic streaks).

Table 4 presents biopsy results. One patient with active disease had abnormally large muscle fibers and showed signs of perifascicular atrophy. None of the patients had inflammatory infiltrates (accumulation of CD68+ cells) or the inflammatory markers Tenascin C or embryonic myosin heavy chain (MHC). There were numerical trends of patients with active disease having a larger area of type I compared to type II muscle fibers. There were also trends of these patients having a larger area of type I muscle fibers compared patients with inactive disease. In patients with inactive disease, this was reversed, with a larger area of type II fibers both compared to type I fibers as well as type II fibers in patients with active disease. There was also a numerically smaller proportion of type I fibers in patients with active compared to inactive disease (Figure 2). There was no indication of differences in capillary features between active and inactive disease.

DISCUSSION

In our study on long-term skeletal muscle outcomes in JDM, we found lower muscle strength and muscular endurance in knee extensors of patients than controls; patients with active disease had lower values than those with inactive disease. Corrected for muscle size (CSA), only muscular endurance remained significantly lower in active versus inactive disease. Clinically assessed muscle damage was
higher in patients with active compared to inactive disease. Objective muscle tests of knee extensors correlated with clinical tests of strength and endurance only in patients with active disease. Muscle biopsy results indicated similar capillary density, but different muscle fiber composition between active and inactive disease. To our knowledge, this is the first long-term study to assess functional, laboratory, serologic, radiographic and histologic muscle outcomes simultaneously in JDM.

Our patients were older, had longer disease duration, and comparable sex distribution compared to other JDM outcome studies (7, 8). More patients had DAS >0, a comparable proportion had MDI global VAS >0.2, but MDI scores were higher compared to the other studies (7, 8). Physical activity levels of our patients were similar to a Danish cohort and higher than a Brazilian cohort (35, 36). Based on these results, our patients presented quite high levels of physical activity despite a relatively high frequency of disease activity and damage.

Controls were randomly selected from the National Registry, which is a strength of our study. They were age- and sex-matched with our patients to handle large age dispersion and exclude age-related confounders to our main results. Physical activity levels of adult controls resembled those of the general, Norwegian, adult population (37), supporting the control group representativeness.

Isometric strength and muscular endurance of knee extensors, MMT-8, and CMAS were lower in patients with active compared to inactive disease, but these clinical and objective muscle tests correlated only in patients with active disease. Although the objective muscle tests were only assessed in knee extensors, peak torque also correlated with MMT knee component only in patients with active disease. Together, these results support the ceiling effects of MMT-8 and CMAS. A precise scoring of
mild muscle weakness and dysfunction, especially in patients with inactive disease, may therefore require more objective and sensitive muscle testing.

MMT and CMAS were mildly reduced (median 77.5 and 50.0 respectively), similar to a Danish study of JDM patients assessed after 13.9y disease duration with mean values of 78.0 and 48.8 respectively (8). MMT <80 and CMAS <52 were, however, more frequent in our study than a multinational outcome study of 490 JDM patients with a mean 7.7y disease duration (86% and 61% vs 41% and 53% respectively) (7). Severe muscle weakness/dysfunction, on the other hand, was rare; only one patient (2.5%) had serious muscle weakness and dysfunction (MMT <64 and CMAS <35) compared to 7% and 8% respectively in the multinational study. A longer disease duration in our study could be the reason for this. Even though CMAS has been used in mixed pediatric/adult JDM populations (7-9), it has not been validated for adults with JDM. However, our group has shown moderate correlations between CMAS and disease measures (MMT, DAS muscle and MDI) also in patients aged >18y (9), supporting the use of the tool also in this age group.

Objective muscle strength and muscular endurance were lower in patients (both with active and inactive disease) compared to controls. Multiple factors may contribute to these results (38), including exercise habits and disease related features (age/sex effects were controlled for by matching). We did not systematically collect data on strength training habits, a limitation to our study. However, only patients with inactive disease had lower MVPA compared to controls, as well as correlations between MVPA and torque. This suggests that deconditioning may play a larger role in explaining lower muscle strength in these patients compared to patients with active
disease although muscle disease activity and damage were present also in this patient group.

Patients with active disease had higher muscle damage (MDI muscle and MDI VAS muscle as well as numerical values of MRI-detected damage including fatty infiltration), but not muscle activity (DAS muscle, muscle enzymes, or MRI-detected edema) compared to patients with inactive disease. However, muscle damage scores did not correlate with isometric muscle strength or muscular endurance in either patient group. This suggests that the difference in peak torque and total work between active and inactive JDM may be due to other disease related factors than classic JDM measures of disease damage.

Muscle cross-sectional area (CSA) was lower in patients with active compared to inactive disease, and this could represent a larger volume reduction due to muscle atrophy in patients with active disease. CSA correlated with objective muscle tests in both patient groups. However, when correcting muscle strength and muscular endurance for CSA, only total work remained lower in active compared to inactive disease. This may indicate that muscle endurance associated with active disease is influenced by structural or functional differences within the muscle tissue independent of muscle size. This hypothesis was supported by muscle biopsy results. We found no signs of muscle inflammation in the biopsies. However, although not statistically tested, there were numerical trends of different muscle fiber composition in patients with active and inactive disease. In patients with active disease type I fibers were relatively larger and type II fibers were relatively smaller compared to patients with inactive disease, and patients with active disease had lower proportion of type I fibers. Given the numerically higher MVPA in patients with active disease, this was
unexpected as exercise is found to increase the size of type II fibers and the proportion of type I fibers in adult DM (21). However, there is evidence that long-term physical inactivity or chronic disease can cause a greater percentage of type II fibers (39).

Hypoxia has been suggested as a possible mechanism for reduced muscle endurance in JDM due to difficulty of increasing blood flow in response to exercise (40). In severe chronic obstructive pulmonary disease (COPD) causing chronic hypoxia, type I muscle fiber proportion has been found decreased together with an increase in type II fiber area, similar to our patients with active disease (41). We found similar histological capillary density to studies on healthy populations (42), and no differences between patients with active and inactive disease. However, the presented biopsy results do not tell us anything about potentially functional impairment of capillaries (40).

Myokines are known to be secreted from muscle tissue in response to exercise or inflammatory stimuli (6). We found higher levels of IP-10 in patients versus controls, similar to previous data of our own cohort (17), supporting that this myokine is upregulated even after long-term disease. IP-10 was recently validated as a strong, reliable, and sensitive biomarker for active JDM (43). We did not, however, find significant differences in IP-10 levels between active and inactive disease suggesting that we may be underpowered, or that the myokine may be a less stable marker of disease activity with time. Our patients had higher circulating levels of decorin compared to controls. Decorin is known to be both antifibrotic and pro-inflammatory (44, 45). Higher levels may be due to increased visceral fat (VAT) depots described earlier (46) (VAT is a greater source of decorin than subcutaneous fat (47)), and may
reflect the inflammatory state of JDM. Surprisingly, we found a positive association between IL-6 and torque in patients with active disease. IL-6 is known as a biomarker for active inflammation in JDM (48). Interestingly, it was also recently found to be both expressed and secreted from type 1 muscle fibers in mice (49), while torque was associated with type 1 fibers in female athletes (50). Thus we could speculate that the association between IL-6 and torque is related to an increase in the area of type 1 fibers in patients with active disease.

Of limitations to our study not already mentioned, the time delay between muscle biopsies and other examinations may have affected the interpretation of biopsy results although none reported life-style changes including physical activity habits. The small number of patients in each group of active and inactive disease may have created type II errors when performing statistical analyses. For the lowest n, therefore, we chose not to perform statistical analyses, but rather describe numerical differences. We also isolated the muscle sub-scores of the validated tools DAS and MDI, and the knee extensor component of MMT-8, although these sub-scores have not been validated separately.

Conclusion: After 20y disease duration, objectively measured muscle strength and endurance of knee extensors were lower in JDM patients compared to controls, and in patients with active compared to inactive disease. Objective and clinical muscle tests correlated in patients with active disease only, suggesting the need for more objective and sensitive muscle tests in this clinical setting. From our study, we can hypothesize that impaired muscular endurance of knee extensors in patients with active disease may be influenced by structural and functional adaptions of muscle tissue independent of muscle size, but this should be studied further.
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FIGURE LEGENDS:

Figure 1: Correlations between objective and clinical (both general and knee extensor) muscle tests in patients in total, and in patients with active and inactive disease. MMT-8=Manual Muscle Test of 8 muscle groups (unilateral); MMT knee=Knee extensor component of the MMT-8; CMAS=Childhood Myositis Assessment Scale; J=joules; Nm=Newton meter.

Figure 2: A 2x2 table visualizing muscle fiber composition and relative size in patients with active and inactive disease. Not drawn according to scale. Type I=Muscle fiber type I; type II=Muscle fiber type II.
Table 1. General characteristics, physical activity measures, and disease variables of juvenile dermatomyositis patient and controls.

<table>
<thead>
<tr>
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<th>JDM patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>Active (n=17)</td>
<td>Inactive (n=27)</td>
</tr>
<tr>
<td></td>
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<tr>
<td><strong>General characteristics</strong></td>
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<tr>
<td>Age (years)</td>
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</tr>
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<td>Female (n)</td>
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<td>Height (cm)</td>
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<tr>
<td></td>
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<td>Group 2</td>
</tr>
<tr>
<td>---------------------------</td>
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</tr>
<tr>
<td>LPA (min/d)</td>
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<td>48.3 (28.0)</td>
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<td>CPM (counts/min)</td>
<td>389.4 (177.2)</td>
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**Disease variables**

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<td>Disease duration (y)</td>
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<td>21.8 (11.8)</td>
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<td>On JDM med (n)</td>
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<td>6 (22)</td>
<td>10 (23)</td>
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<td>DAS (0-20)**</td>
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<td>4.3 (2.5)</td>
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<td>MDI (0-40)**</td>
<td>5.0 (2.0-5.5)</td>
<td>2.0 (1.0-4.0)</td>
<td>3.0 (1.0-5.0)</td>
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<td>PGA (0-10)*</td>
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<td>PGD (0-10)**</td>
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**Self-reported physical health**

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<tr>
<th>SF-36 PCS (0-100)↓</th>
<th>49.4 (33.2-53.2)**</th>
<th>56.3 (48.9-60.3)</th>
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<tbody>
<tr>
<td>cHAQ/HAQ &gt;0 (n)</td>
<td>9 (53)</td>
<td>9 (33)</td>
<td>18 (41)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values represent mean (SD), median (IQR) or n (%). *p<0.050, ** p<0.010, ***p<0.001 between patients and controls or between patients with active and inactive disease. Active/inactive=Disease inactivity defined according to PRINTO criteria. LPA=Light Physical Activity; MVPA=Moderate to Vigorous Physical Activity; CPM=counts per minute; JDM=Juvenile Dermatomyositis; DAS=Disease Activity Score; MDI=myositis damage index; PGA=Physician Global Activity Assessment; PGD=Physician Global Damage Assessment; SF-36 PCS=Short Form 36 Physical Component Summary; cHAQ/HAQ= child/adult Health Assessment Questionnaire. ↓=lower score denotes more impairment/worse function; ↑=higher score denotes more impairment/worse function.
Table 2. Muscle characteristics in JDM patients and controls

<table>
<thead>
<tr>
<th></th>
<th>JDM patients</th>
<th></th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active (n=17)</td>
<td>Inactive (n=27)</td>
<td>Total (n=44)</td>
</tr>
<tr>
<td><strong>Objective muscle test variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Torque (Nm)</td>
<td>98.0 (37.8)*</td>
<td>127.0 (35.8)</td>
<td>116.2 (38.8)**</td>
</tr>
<tr>
<td>Repetitions (n)</td>
<td>22.2 (8.7)</td>
<td>26.2 (7.9)</td>
<td>24.7 (8.3)**</td>
</tr>
<tr>
<td>Work (J)</td>
<td>565 (350-1032)*</td>
<td>994 (651-1175)</td>
<td>738 (565-1155)***</td>
</tr>
<tr>
<td>Torque/CSA (Nm/mm²)</td>
<td>2.0 (1.6-2.2)</td>
<td>2.1 (2.0-2.2)</td>
<td>2.0 (1.8-2.2)</td>
</tr>
<tr>
<td>Work/CSA (J/mm²)</td>
<td>10.0 (8.3-14.3)*</td>
<td>16.5 (12.4-18.9)</td>
<td>14.1 (9.3-18.5)</td>
</tr>
<tr>
<td><strong>Clinical variables</strong></td>
<td></td>
<td></td>
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</tbody>
</table>

29
<table>
<thead>
<tr>
<th>Measure</th>
<th>Lower Limit (Range)</th>
<th>Median (Range)</th>
<th>Upper Limit (Range)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMT-8 (0-80)↓</td>
<td>75.0 (73.0-77.0)**</td>
<td>78.0 (77.0-79.0)</td>
<td>77.5 (74.3-79.0)</td>
<td>NA</td>
</tr>
<tr>
<td>CMAS (0-52)↓</td>
<td>48.0 (45.0-51.5)*</td>
<td>50.5 (49.0-52.0)</td>
<td>50.0 (48.0-52.0)</td>
<td>NA</td>
</tr>
<tr>
<td>DAS muscle (0-11)↑</td>
<td>2.0 (1.0-4.5)</td>
<td>1.0 (1.0-2.0)</td>
<td>1.8 (1.0-2.5)</td>
<td>NA</td>
</tr>
<tr>
<td>MDI muscle (0-3)↑</td>
<td>1.5 (0.7)**</td>
<td>0.7 (0.6)</td>
<td>1.0 (0.7)</td>
<td>NA</td>
</tr>
<tr>
<td>MDI VAS muscle (0-10)↑</td>
<td>0.6 (0.3-1.6)**</td>
<td>0.2 (0.0-0.4)</td>
<td>0.3 (0.0-1.0)</td>
<td>NA</td>
</tr>
<tr>
<td>MDI VAS muscle &gt;0.2 (n)</td>
<td>13 (77)*</td>
<td>11 (41)</td>
<td>24 (55)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Lab measures**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Lower Limit (Range)</th>
<th>Median (Range)</th>
<th>Upper Limit (Range)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (U/L)</td>
<td>179 (84-266)</td>
<td>108 (67-131)</td>
<td>118 (79-180)</td>
<td>116 (86-182)</td>
</tr>
<tr>
<td>LD (U/L)</td>
<td>181 (40)</td>
<td>160 (19)</td>
<td>168 (30)</td>
<td>170 (37)</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>29 (24-31)</td>
<td>24.0 (22-33)</td>
<td>26 (22-31)</td>
<td>23 (20-29)</td>
</tr>
<tr>
<td>Decorin (pg/ml)</td>
<td>177.2 (155.6-194.9)</td>
<td>191.4 (156.2-252.2)</td>
<td>182.0 (156.2-238.1)*</td>
<td>157.3 (135.4-184.4)</td>
</tr>
<tr>
<td>Molecule</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>IP-10 (pg/ml)</td>
<td>579.1 (460.5-582.8)</td>
<td>308.8 (110.0)</td>
<td>2446.3 (908.2)</td>
<td>6529.7 (1609.8)</td>
</tr>
<tr>
<td>MCP1 (pg/ml)</td>
<td>799.1 (530.5-1174.8)</td>
<td>327.4 (167.7)</td>
<td>1843.5 (628.5)</td>
<td>6122.1 (1822.3)</td>
</tr>
<tr>
<td>Myostatin (pg/ml)</td>
<td>863.4 (547.3-1174.8)</td>
<td>290.3 (125.1)</td>
<td>1983.2 (855.3)</td>
<td>308.8 (110.0)</td>
</tr>
<tr>
<td>CCL5 (pg/ml)</td>
<td>765.3 (517.9-1476.5)</td>
<td>1872.5 (719.6)</td>
<td>1046.3 (2057.7)</td>
<td>6046.3 (2057.7)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>374.6 (212.9)</td>
<td>1824.2 (2071.5)</td>
<td>6.0 (0.4-1.1)</td>
<td>1.0 (0.5-1.4)</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>375.6 (212.9)</td>
<td>224.2 (2071.5)</td>
<td>6.0 (0.4-1.1)</td>
<td>0.9 (0.4-1.4)</td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>6224.2 (212.9)</td>
<td>6424.2 (2071.5)</td>
<td>8.0 (0.4-1.1)</td>
<td>1.0 (0.5-1.4)</td>
</tr>
<tr>
<td>MRI muscle Edema (n)†</td>
<td>19.9 (11.3)</td>
<td>19.9 (11.3)</td>
<td>19.9 (11.3)</td>
<td>19.9 (11.3)</td>
</tr>
<tr>
<td>MRI muscle Fatty infiltration (n)</td>
<td>2 (7)</td>
<td>2 (7)</td>
<td>2 (7)</td>
<td>2 (7)</td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
<td>Active Disease</td>
<td>Inactive Disease</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Calcification</strong></td>
<td>1 (6)</td>
<td>3 (11)</td>
<td>4 (9)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Quadriceps CSA (mm²)</strong></td>
<td>48.5 (39.8-58.7)*</td>
<td>56.7 (50.6-62.5)</td>
<td>53.2 (45.0-62.3)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values represent mean (SD), median (IQR) or n (%). *p<0.050, ** p<0.010, ***p<0.001 between patients and controls or between patients with active and inactive disease. DAS=Disease Activity Score; MDI=Myositis Damage Index (muscle damage extent); MDI VAS= MDI Visual Analogue Scale (muscle damage severity); MMT-8=Unilateral Manual Muscle Testing of 8 muscle groups; CMAS=Childhood Myositis Assessment Scale; CK=Creatine Kinase; LD=Lactate Dehydrogenase; MRI=Magnetic Resonance Imaging. Fatty infiltration=the presence of pathologic fatty infiltration; CSA= cross sectional area; Torque/CSA and Work/CSA=Peak torque and total work per maximal quadriceps CSA. ↓=lower score denotes more impairment/worse function; ↑=higher score denotes more impairment/worse function. a: n=12, b: n=16, c: n=26. †Due to small numbers, data on edema and calcinosis are presented without statistical analyses.
Table 3. Correlations between peak torque or total work of knee extensors and general, disease related, and muscle characteristics in patients.

<table>
<thead>
<tr>
<th></th>
<th>Peak torque†</th>
<th></th>
<th>Total work</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active</td>
<td>Inactive</td>
<td>Total</td>
<td>Active</td>
</tr>
<tr>
<td><strong>General characteristics/physical activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height†</td>
<td>0.662**</td>
<td>0.602**</td>
<td>0.650***</td>
<td>0.735**</td>
</tr>
<tr>
<td>Weight†</td>
<td>0.360</td>
<td>0.532**</td>
<td>0.464**</td>
<td>0.206</td>
</tr>
<tr>
<td>MVPA†</td>
<td>0.092</td>
<td>0.395*</td>
<td>0.227</td>
<td>0.066</td>
</tr>
<tr>
<td>MVPA bouts</td>
<td>0.260</td>
<td>0.203</td>
<td>0.133</td>
<td>0.254</td>
</tr>
<tr>
<td><strong>Disease and muscle variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration†</td>
<td>0.272</td>
<td>0.150</td>
<td>0.165</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>MMT-8</td>
<td>CMAS</td>
<td>DAS muscle</td>
<td>MDI muscle†</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.770***</td>
<td>0.574*</td>
<td>-0.667**</td>
<td>-0.144</td>
</tr>
<tr>
<td><strong>Correlation</strong></td>
<td>0.270</td>
<td>0.140</td>
<td>-0.436*</td>
<td>-0.292</td>
</tr>
<tr>
<td><strong>Coefficient</strong></td>
<td>0.521***</td>
<td>0.456**</td>
<td>-0.605***</td>
<td>-0.359*</td>
</tr>
<tr>
<td></td>
<td>0.838***</td>
<td>0.574*</td>
<td>-0.706**</td>
<td>-0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.533***</td>
<td>-0.385*</td>
<td>-0.182</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-0.576***</td>
<td>-0.228</td>
</tr>
<tr>
<td></td>
<td>IL-6</td>
<td>IL-8</td>
<td>TNFα†</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.536*</td>
<td>-0.053</td>
<td>0.203</td>
<td>0.105</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.096</td>
<td>-0.277</td>
<td>-0.098</td>
<td>0.063</td>
</tr>
<tr>
<td>TNFα†</td>
<td>0.466</td>
<td>0.113</td>
<td>0.236</td>
<td>0.014</td>
</tr>
</tbody>
</table>

“Active” refers to patients with active disease, and “inactive” to patients with inactive disease. ***p<0.001; **p<0.010; *p<0.050.
†=normally distributed variables. Pearson R and Spearman’s rho have been used as appropriate. MVPA=moderate to vigorous physical activity; MVPA bouts=average time (min) of MVPA spent in bouts of 10 min; DAS=disease activity score; MDI=Myositis Damage Index; MMT-8=the unilateral manual muscle test; CMAS=Childhood Myositis Assessment Scale; MRI=Magnetic Resonance Imaging; CSA=Quadriceps cross sectional area. The myokines presented in this table were selected based on associations seen in the present and previous studies of myokines in JDM.
Table 4. Biopsy results between active and inactive disease:

<table>
<thead>
<tr>
<th></th>
<th>Active</th>
<th>Inactive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=5</td>
<td>(n=12)</td>
</tr>
<tr>
<td>Type 1 area</td>
<td>4497 (3143-5387)</td>
<td>4045 (3073-4478)</td>
<td>4212 (3233-4600)</td>
</tr>
<tr>
<td>Type 2 area</td>
<td>4030 (2922-4720)</td>
<td>5081 (2655-5713)</td>
<td>4255 (2939-5434)</td>
</tr>
<tr>
<td>Type 1 percentage</td>
<td>39 (33-64)</td>
<td>47 (33-52)</td>
<td>43 (33-53)</td>
</tr>
<tr>
<td>CF</td>
<td>1.7 (1.1-1.9)</td>
<td>1.8 (1.5-2.0)</td>
<td>1.7 (1.4-2.0)</td>
</tr>
<tr>
<td>CAF1</td>
<td>4.3 (3.3-4.7)</td>
<td>4.0 (3.7-4.4)</td>
<td>4.2 (3.7-4.5)</td>
</tr>
<tr>
<td>CAF2</td>
<td>3.6 (3.4-3.9)</td>
<td>3.7 (3.0-4.5)</td>
<td>3.6 (3.3-4.1)</td>
</tr>
<tr>
<td>CAFA1</td>
<td>0.9 (0.8-1.2)</td>
<td>0.9 (0.8-1.2)</td>
<td>0.9 (0.8-1.2)</td>
</tr>
<tr>
<td>CAFA2</td>
<td>1.0 (0.7-1.3)</td>
<td>0.9 (0.6-1.1)</td>
<td>1.0 (0.7-1.3)</td>
</tr>
</tbody>
</table>

Type 1 percentage=Percentage of number of fibers; CF=Total number of extracellular nuclei/total number of fibers; CAF=Capillaries/fiber; CAFA=Capillaries/fiber/muscle area. 1 and 2 refers to muscle fiber type. Capillary data of inactive disease.
represent n=4; capillary data of the total patient group represent n=11. No statistical analyses have been performed due to the small patient samples.
Patients with active disease (n=7)

Type I

Patients with inactive disease (n=5)

Type II
<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Antigen</th>
<th>Identifies</th>
<th>Product number</th>
<th>Producer</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin heavy chain 1*</td>
<td>Myosin heavy chain 1</td>
<td>Muscle fibre type 1*</td>
<td>BA-D5</td>
<td>DSHB</td>
<td>1:500</td>
</tr>
<tr>
<td>CD31</td>
<td>Capillaries</td>
<td>M0823</td>
<td>Dako</td>
<td>1:200</td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophages</td>
<td>M0718</td>
<td>Dako</td>
<td>1:300</td>
<td></td>
</tr>
<tr>
<td>Tenascin C</td>
<td>Tenascin C</td>
<td>MA5-1606</td>
<td>Invitrogen</td>
<td>1:100</td>
<td></td>
</tr>
<tr>
<td>Embryonic myosin heavy chain</td>
<td>Embryonic myosin heavy chain</td>
<td>F1.652</td>
<td>DSHB</td>
<td>1:500</td>
<td></td>
</tr>
</tbody>
</table>
Dystrophin  Dystrophin/cell membrane  Ab15277  Abcam  1:500

**Secondary antibodies**

Goat anti-mouse alexa fluor  A11001  Invitrogen  1:200
488 conjugated

Goat anti-rabbit alexa fluor  A11012  Invitrogen  1:200
594 conjugated

Goat anti-rabbit alexa fluor  A11011  Invitrogen  1:200
568 conjugated

*Muscle fiber type 2 was identified at non-stained muscle fibers (black)*
Supplementary table 2: General characteristics of biopsied versus not biopsied patients ≥18

<table>
<thead>
<tr>
<th>General variables</th>
<th>Biopsied patients</th>
<th>Not biopsied patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active (n=7)</td>
<td>Inactive (n=5)</td>
</tr>
<tr>
<td>Active disease (n)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age (y)</td>
<td>37.4 (12.8)</td>
<td>32.1 (8.0)</td>
</tr>
<tr>
<td>Female (n)</td>
<td>4 (57)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.3 (6.9)</td>
<td>171.6 (6.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.1 (13.7)</td>
<td>64.7 (7.2)</td>
</tr>
</tbody>
</table>
**Accelerometer**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary time (min/day)</td>
<td>578.8 (84.4)</td>
<td>548.5 (63.3)</td>
<td>566.2 (74.8)</td>
<td>569.7 (68.6)</td>
</tr>
<tr>
<td>Light exercise (min/day)</td>
<td>163.7 (65.1)</td>
<td>205.5 (90.1)</td>
<td>181.1 (75.9)</td>
<td>158.8 (49.4)</td>
</tr>
<tr>
<td>MVPA (min/day)</td>
<td>49.5 (21.2)</td>
<td>44.9 (31.7)</td>
<td>47.6 (24.8)</td>
<td>39.7 (20.8)</td>
</tr>
</tbody>
</table>

**Disease variables**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration (y)</td>
<td>26.7 (10.5)</td>
<td>24.0 (11.1)</td>
<td>25.6 (10.4)</td>
<td>24.0 (11.3)</td>
</tr>
<tr>
<td>DAS (0-20)↑</td>
<td>5.1 (2.5)</td>
<td>4.6 (1.5)</td>
<td>4.9 (2.1)</td>
<td>4.4 (2.9)</td>
</tr>
<tr>
<td>MDI (0-40)↑</td>
<td>5.0 (2.0-6.0)</td>
<td>2.0 (1.0-5.5)</td>
<td>4.0 (2.0-5.8)</td>
<td>3.0 (1.5-5.0)</td>
</tr>
<tr>
<td>PGA (0-10)↑</td>
<td>0.2 (0.0-0.6)</td>
<td>0.0 (0.0-0.6)</td>
<td>0.2 (0.0-0.5)</td>
<td>0.1 (0.0-0.6)</td>
</tr>
<tr>
<td>Measure</td>
<td>Value 1 (Range)</td>
<td>Value 2 (Range)</td>
<td>Value 3 (Range)</td>
<td>Value 4 (Range)</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>PGD (0-10)↑</td>
<td>1.4 (0.2-3.3)</td>
<td>0.3 (0.3-2.0)</td>
<td>1.0 (0.3-2.3)</td>
<td>1.1 (0.3-2.3)</td>
</tr>
<tr>
<td>SF-36 PCS (0-100)↓</td>
<td>52.7 (34.6-55.4)</td>
<td>56.3 (50.1-60.1)</td>
<td>54.5 (46.4-56.5)</td>
<td>51.0 (48.2-59.6)</td>
</tr>
<tr>
<td>cHAQ/HAQ &gt; 0 (n)</td>
<td>4 (57)</td>
<td>0 (0)</td>
<td>4 (33)</td>
<td>11 (44)</td>
</tr>
</tbody>
</table>

**Aerobic capacity and muscle variables**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value 1 (Range)</th>
<th>Value 2 (Range)</th>
<th>Value 3 (Range)</th>
<th>Value 4 (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torque (Nm)</td>
<td>111.5 (90.5-152.6)</td>
<td>116.6 (108.0-144.8)</td>
<td>116.6 (96.2-141.6)</td>
<td>113.9 (94.7-156.5)</td>
</tr>
<tr>
<td>Work (J)</td>
<td>860.0 (520.8-1316.3)</td>
<td>994.0 (938.3-1076.8)</td>
<td>994 (564-1155)</td>
<td>738 (598-1256)</td>
</tr>
<tr>
<td>Quadriceps CSA (mm²)</td>
<td>5382 (3910-6729)</td>
<td>5372 (5187-5838)</td>
<td>5377 (5091-6273)</td>
<td>5687 (4824-7376)</td>
</tr>
<tr>
<td>Peak torque/area (Nm/mm²)</td>
<td>2.1 (1.7-2.4)</td>
<td>2.2 (2.0-2.6)</td>
<td>2.2 (0.3)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td>Total work/area</td>
<td>14.0 (9.7-21.9)</td>
<td>18.5 (16.8-19.8)</td>
<td>16.6 (4.7)</td>
<td>13.9 (5.4)</td>
</tr>
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</tr>
</tbody>
</table>

(\text{J/mm}^2)

Values represent mean (SD), median (IQR), or n (%). *p<0.050, ** p<0.010, ***p<0.001 between biopsied and not biopsied patients.

Statistical analyses not performed between active and inactive disease due to a small patient sample. MVPA daily=Moderate to Vigorous Physical Activity measured in minutes/day; DAS=Disease Activity Score; MDI=myositis damage index; PGA=Physician Global Activity Assessment; PGD=Physician Global Damage Assessment; SF-36 PCS=Short Form 36 Physical Component Summary; cHAQ/HAQ= child/adult Health Assessment Questionnaire; CSA=cross sectional area. ↓=lower score denotes more impairment/worse function; ↑=higher score denotes more impairment/worse function.