Muscle fiber dysfunction contributes to weakness in inclusion body myositis

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Abstract

Atrophy and fatty infiltration are important causes of muscle weakness in inclusion body myositis (IBM). Muscle weakness can also be caused by reduced specific force; i.e. the amount of force generated per unit of residual muscle tissue. This study investigates *in vivo* specific force of the quadriceps and *ex vivo* specific force of single muscle fibers in patients with IBM. We included 8 participants with IBM and 12 healthy controls, who all underwent quantitative muscle testing, quantitative MRI of the quadriceps and paired muscle biopsies of the quadriceps and tibialis anterior. Single muscle fibers were isolated to measure muscle fiber specific force and contractile properties. Both *in vivo* quadriceps specific force and *ex vivo* muscle fiber specific force were reduced. Muscle fiber dysfunction was accompanied by reduced active stiffness, which reflects a decrease in the number of attached actin-myosin cross-bridges during activation. Myosin concentration was reduced in IBM fibers. Because reduced specific force contributes to muscle weakness in patients with IBM, therapeutic strategies that augment muscle fiber strength may provide benefit to patients with IBM.
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Keywords: Inclusion body myositis; Muscle weakness; MRI.

1. Introduction

Sporadic inclusion body myositis (IBM) is one of the most common acquired muscle disorders in adults over 50 years old [1,2]. Progressive disease is characterized by atrophy and fatty infiltration of muscle tissue, resulting in muscle weakness [3–5]. In early disease, the quadriceps, deep finger flexors and the pharyngeal muscles are most frequently affected, restricting functional ability and quality of life [6–8]. IBM is the result of a complex and only partially understood interplay between muscle inflammation, degeneration and impaired proteostasis which results in the accumulation of proteins in rimmed vacuoles [9].

No curative treatment options are currently available for IBM. The use of bimagrumab, a myostatin inhibitor which aims to ameliorate muscle atrophy by promoting muscle growth, resulted in increased thigh muscle volume in a small proof of concept trial [10]. However, a subsequent

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double-blind placebo controlled trial did not meet its primary endpoint [data not yet published]. A conference abstract on rapamycin treatment reported reduced fatty replacement and increased contractile cross-sectional area, but detected no change in quadriceps muscle strength [11]. This suggests that merely increasing the amount of muscle tissue is not sufficient to increase muscle strength in patients with IBM.

Besides loss of muscle mass due to atrophy and fatty infiltration, muscle weakness can also be caused by a reduction in specific force, i.e. the amount of force generated per unit of residual muscle tissue. An important contributor to *in vivo* specific force is muscle fiber strength. In IBM, muscle fiber strength may potentially be affected by inflammation, which may trigger and sustain cell stress, resulting in sarcomeric protein degradation, accumulation of unwanted proteins and irreversible muscle fiber damage [12–14].

This study investigates *in vivo* specific force of the quadriceps and *ex vivo* specific force of single muscle fibers to determine whether muscle fiber dysfunction contributes muscle weakness in patients with IBM.

2. Patients and methods

2.1. Participants

Patients with IBM fulfilling the 2010 modified Griggs criteria were recruited from the Radboud university medical center [15]. Healthy individuals without a history of neuromuscular disease were recruited as controls. Exclusion criteria for all participants were: age <18 or ≥65 years, diabetes mellitus, chronic obstructive pulmonary disease, chronic heart failure, current malignancy, previous treatment with chemotherapy and/or radiation therapy, use of corticosteroids during more than two weeks in the past 5 years, current use of statins, being wheelchair bound, contra-indications for MRI or muscle biopsy. Participants were age-matched on the group level. All participants underwent a neurologic examination and creatine kinase (CK) was measured. In IBM patients anti-cN-1A reactivity was established using ELISA [16]. The Medical Ethics Review Committee region Arnhem-Nijmegen approved this study (no. 2011/181). Informed consent was obtained from each participant.

2.2. Quantitative force studies

Maximum voluntary contraction (MVC) of the quadriceps was measured with a custom-built setup as described previously [17]. The right leg was examined, except in the presence of asymmetrical muscle weakness as measured by MRC score in which case the weakest leg was tested. Subsequent imaging and muscle biopsy were performed in the same leg. MVC was measured with the hip angle at 90° and the knee angle set at 120° Participants were strapped at the hips and upper body to prevent compensatory movements. Participants were asked to perform a MVC of the quadriceps during 3s. Mean force from 3 contractions was used to represent MVC [18].

2.3. Quantitative muscle imaging

Transversal T1 weighted, multi-echo T2 and Turbo Inversion Recovery Magnitude (TIRM) images of the upper and lower leg were acquired on a 3 Tesla MRI system (Tim TRIO, Siemens, Erlangen, Germany). Upper leg MRI images were obtained at 1/3 of the distance between anterior superior iliac spine and patella, this level also corresponded to the approximate site of vastus lateralis muscle biopsy. Lower leg images were obtained at the level that corresponded to the approximate site of the tibialis anterior muscle biopsy. Muscle fraction and fat fraction were quantified from the multi-echo T2 images [19]. Total muscle cross-sectional area (TCSA) was determined by manually tracing the outline of the quadriceps using ImageJ. The amount of fatty infiltration (FCSA) was determined by multiplying the fat fraction with the TCSA. Residual quadriceps contractile cross-sectional area (CCSA) was determined by multiplying the muscle fraction with the TSCA. *In vivo* specific force was calculated by dividing the quadriceps MVC by the quadriceps CCSA.

2.4. Muscle biopsies

A needle biopsy of both the vastus lateralis and of the tibialis anterior was obtained. The vastus lateralis – part of the quadriceps – was chosen because this muscle is involved early and severely in patients with IBM. The tibialis anterior was included to obtain additional tissue from a less severely affected muscle in the lower limb. Biopsy specimens were snap-frozen in isopentane and stored at −80 °C for histological analysis and deposited in a solution containing half glycerol and half relaxing solution and stored at −20 °C for single fiber studies (the composition of this solution is described elsewhere [20]).

2.5. Histopathological analysis

Frozen sections underwent HPhlox and ATPase staining to evaluate fiber size and type distribution. HPhlox was used to evaluate variability in fiber size, extent of central nucleation, necrosis and regeneration, interstitial fibrosis and inflammation. Severity was graded as normal (0), mild (1), moderate (2) or severe (3) for each of these parameters and scores were added to provide a cumulative histopathological severity score [21]. Inflammation was graded separately. All histopathology and inflammation severity scores were assigned by an experienced neuropathologist who was not aware whether a biopsy belonged to the control or IBM group. Morphometric analysis was performed to establish the percentages of each fiber type by a final-year pathology resident and randomly checked by an experienced pathology analist.

2.6. Single muscle fiber studies

2.6.1. Muscle fiber preparation

Biopsy material was placed in a relaxing solution containing 1% Triton X-100 and kept at 4 °C during isolation of single muscle fibers [22]. Triton permeabilizes the plasma membranes, resulting in 'skinned' muscle fibers. This procedure permits studies of sarcomeric function. Protease inhibitors were added to the solution to prevent protein degradation. Skinned single muscle fibers were isolated and fiber ends were attached to aluminum t-clips. The clips were mounted between a length motor on one end, and a force transducer on the other. Sarcomere length was set at 2.5 μm for measurement of maximum force, cross-bridge cycling kinetics, and calcium sensitivity. After adjusting sarcomere length, fiber length and cross-sectional area were measured.

2.6.2. Muscle fiber specific force and contractile properties

We determined the maximum force generation by activating the fibers with a saturating Ca^{2+} solution (pCa 4.5). Specific force was determined by dividing the generated maximum force by the fiber cross-sectional area, thus reflecting the force generated by the sarcomeres. After peak force was reached, a rapid unloaded shortening and restretching of the muscle fiber was used to determine the rate constant of force redevelopment (ktr). Ktr depends on the attachment and detachment rate of actin-myosin cross-bridges during activation, and thereby reflects *the fraction of strongly bound cross-bridges*. After peak force is reached a second time, slight length perturbations (−0.9, −0.6, −0.3, 0.3, 0.6 and 0.9%) are imposed on the muscle fiber to determine the active stiffness, which is dependent on *the number of attached cross-bridges during activation*. The tension/stiffness ratio reflects *the amount of force generated per cross-bridge* (assuming the myofibrils are intact).

After measurement of maximum force and cross-bridge cycling kinetics, the fiber is rested for 5 min and sarcomere length is verified and adjusted if necessary. Next, calcium sensitivity of force generation was measured by transfer of the muscle fiber to solutions with increasing concentrations of Ca^{2+}. Force-pCa data were fitted to the Hill equation to provide the pCa_{50}, which is the pCa at which 50% of maximal active tension is reached.

2.6.3. Muscle fiber protein analysis

After contractile experiments, individual fibers were stored in 25 μl of SDS sample buffer until MHC isoforms analysis. MHC isoform composition and concentration of isolated single fibers was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE) [17]. Sample volumes of 8 μl were loaded per lane. Gels were run for 24 h at 15 °C and a constant voltage of 275 V. The composition of the sample buffer and stacking gel is described elsewhere [23]. In hybrid fibers (9% of fibers in both the control and IBM group), fiber type was assigned as type 1 or type 2 based on the predominant MHC isoform. Because there were only a limited amount of type 2X fibers ($N=5$) these were analyzed together with type 2A fibers.

2.7. Statistics

Statistical analysis was performed with IBM SPSS Statistics 22. We used sex as a covariate where appropriate to account for the overrepresentation of men in the IBM group. Continuous data were analyzed using independent samples *t*-test. Ordinal data were analyzed using Pearson Chi-square. Single fiber measurements were analyzed with linear mixed models. A random intercept was modeled for individual biopsies and individual subjects, using a "Variance components" covariance structure. Data are mean ± standard error of the mean (SEM) unless otherwise specified.

2.8. Data availability

A study protocol has been published previously [17]. Anonymized data are available on request.

3. Results

3.1. Participants and biopsies

We included 8 IBM patients aged 49–64 years (7M/1F) and 12 healthy control subjects aged 42–65 years (6M/6F). Two IBM patients fulfilled the 2010 modified Griggs criteria for pathologically defined IBM, four fulfilled the criteria for clinically defined IBM, and one patient fulfilled the criteria for possible IBM. We were unable to find enough female IBM patients that fulfilled the inclusion criteria, which is why a higher number of female controls participated. To account for the overrepresentation of men in the IBM group we used sex as a covariate in appropriate statistical analyses. Muscle biopsies of both the vastus lateralis and tibialis anterior were obtained from all participants, except in two. In one IBM participant, advanced fatty infiltration prohibited vastus lateralis muscle biopsy. Another IBM participant declined a muscle biopsy of the tibialis anterior. For each muscle biopsy, 8–20 single muscle fibers were measured (median 12 fibers per biopsy). We measured a total of 299 control muscle fibers (type 1 muscle fiber: 50.2%, type 2 muscle fiber: 49.8%) and 189 IBM muscle fibers (type 1: 69.8, type 2: 30.2%). Additional information about participants, laboratory findings and muscle biopsy characteristics is provided in Table 1.

3.2. In vivo *quadriceps specific force*

3.2.1. Quantitative force studies

Quadriceps MVC was significantly decreased in IBM patients (MVC: 217 ± 58N in IBM vs. 572 ± 53N in controls, $p < .001$, Fig. 1A).

3.2.2. Quantitative muscle MRI

To determine the amount of atrophy and fatty infiltration we determined the TCSA and FCSA relative to control
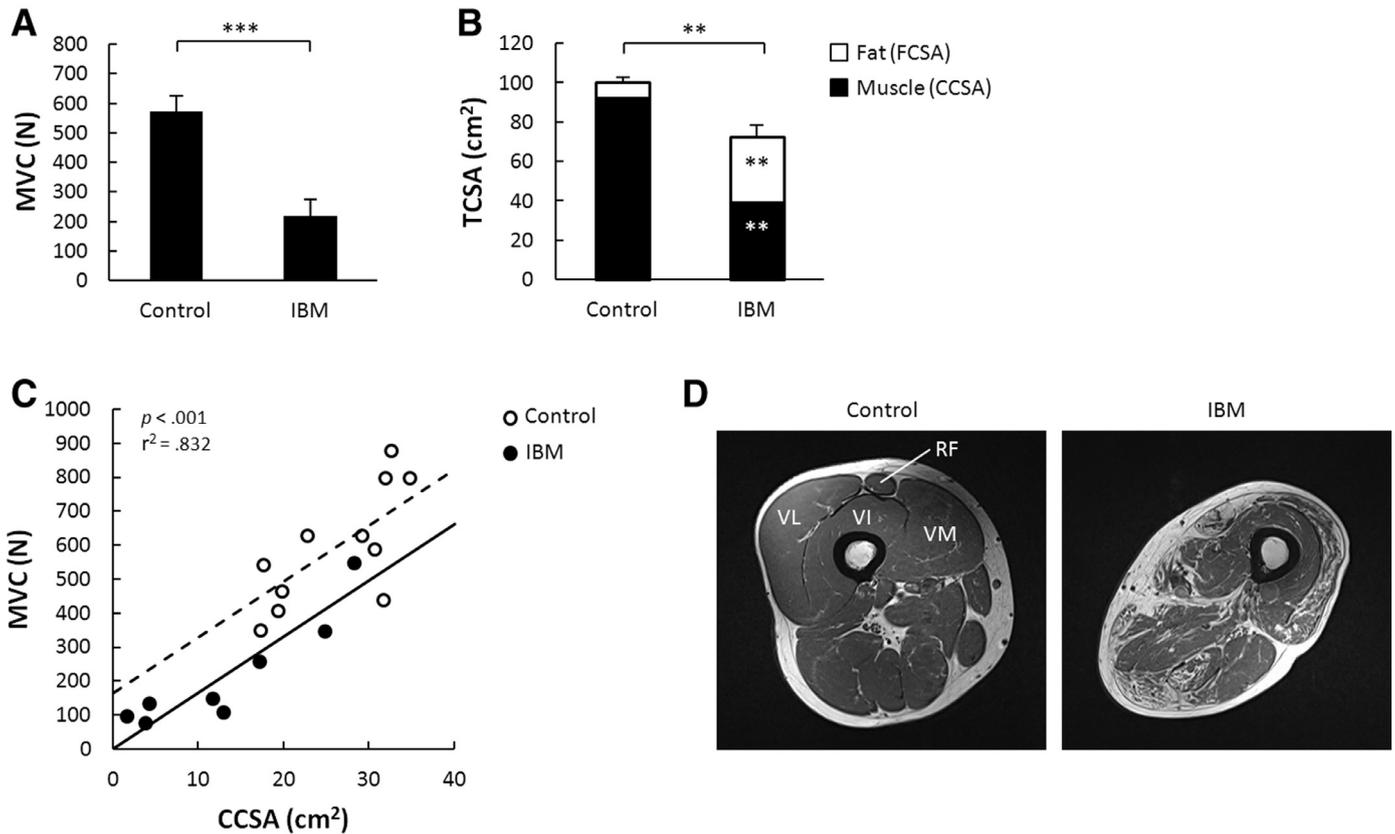
Fig. 1. *Quadriceps muscle strength*. A. Maximum voluntary contractile force of the quadriceps is reduced in IBM patients compared to healthy controls. B. Reduced TCSA, indicating muscle atrophy, and increased FCSA, indicating fatty replacement of muscle tissue, in IBM patients result in a reduced amount of available contractile tissue, or CCSA. Stars indicate *p*-value for TCSA (line above bars), FCSA (within bar chart), and CCSA (within bar chart). C. Specific force is significantly reduced in IBM patients compared to healthy controls. D. Upper leg MRI for a control and IBM participant. Individual muscles of the quadriceps are indicated in the control image. Participant C4, right leg: TCSA 98%, FCSA 4%, CCSA 94%. Participant I2, left leg: TCSA 65%, FCSA 31%, CCSA 34%.

Abbreviations: CCSA = contractile cross-sectional area. IBM = inclusion body myositis. MVC = maximum voluntary contraction. RF = rectus femoris. VI = vastus intermedius. VM = vastus medialis. VL = vastus lateralis. $p < .05$ is considered significant, * $p < .05$, ** $p < .01$, *** $p < .001$.

muscles (Fig. 1B). TCSA was reduced by 28% in IBM patients (TCSA: $72.3 \pm 5.9\%$ in IBM vs. $100 \pm 2.5\%$ in controls, $p = .002$, Fig. 1B). Of the remaining muscle tissue, 33% was replaced by fat (FCSA: $32.9 \pm 6.5\%$ in IBM vs. $7.7 \pm 2.0\%$ in controls, $p = .005$, Fig. 1B). As a result of atrophy and fatty replacement of muscle tissue, CCSA was reduced by 53% in IBM patients (CCSA: $39.4 \pm 10\%$ in IBM vs. $92.3 \pm 2.4\%$ in controls, $p = .001$, Fig. 1B).

3.2.3. Quadriceps specific force

We calculated *in vivo* quadriceps specific force (MVC / CCSA) to determine whether reduced contractile quality of remaining muscle tissue also contributed to quadriceps muscle weakness. There was a linear relationship between quadriceps MVC and CCSA ($r^2 = 0.832$, $p < .001$, Fig. 1C). Specific force was reduced in IBM patients compared to control individuals, which indicates dysfunction of residual muscle tissue ($p = .003$).

3.3. Ex vivo *single muscle fiber studies*

3.3.1. Vastus lateralis single muscle fiber specific force

We isolated single muscle fibers from vastus lateralis muscle biopsies to determine whether reduced *in vivo*

quadriceps specific force was caused by reduced muscle fiber specific force (Fig. 2A). Single muscle fiber specific force was reduced in IBM (type 1 specific force: 151.4 ± 5.9 mN/mm^2 in IBM vs. 181.9 ± 4.4 mN/mm^2 in controls; type 2 specific force: 179.5 ± 7.9 mN/mm^2 in IBM vs. 215.3 ± 6.7 mN/mm^2 in controls, $p = .003$, Fig. 2B).

3.3.2. Vastus lateralis single muscle fiber contractile properties

In permeabilized single muscle fibers, specific force depends largely on (1) the fraction of strongly bound actin-myosin cross-bridges, (2) the number of available cross-bridges, and (3) the force per cross-bridge [24,25]. Reduced force generation should be accompanied by a change in one or more of these parameters.

The rate constant of force redevelopment (ktr) represents cross-bridge attachment and detachment rates during activation and provides information on the fraction of strongly bound cross-bridges. Ktr was not decreased in IBM muscle fibers (type 1 Ktr: 5.9 ± 0.9 s^{-1} in IBM vs. 4.9 ± 0.3 s^{-1} in controls, $p = .380$; type 2 Ktr: 12.6 ± 0.8 s^{-1} in IBM vs. 13.2 ± 0.5 s^{-1} in controls, $p = .743$, Fig. 2C).
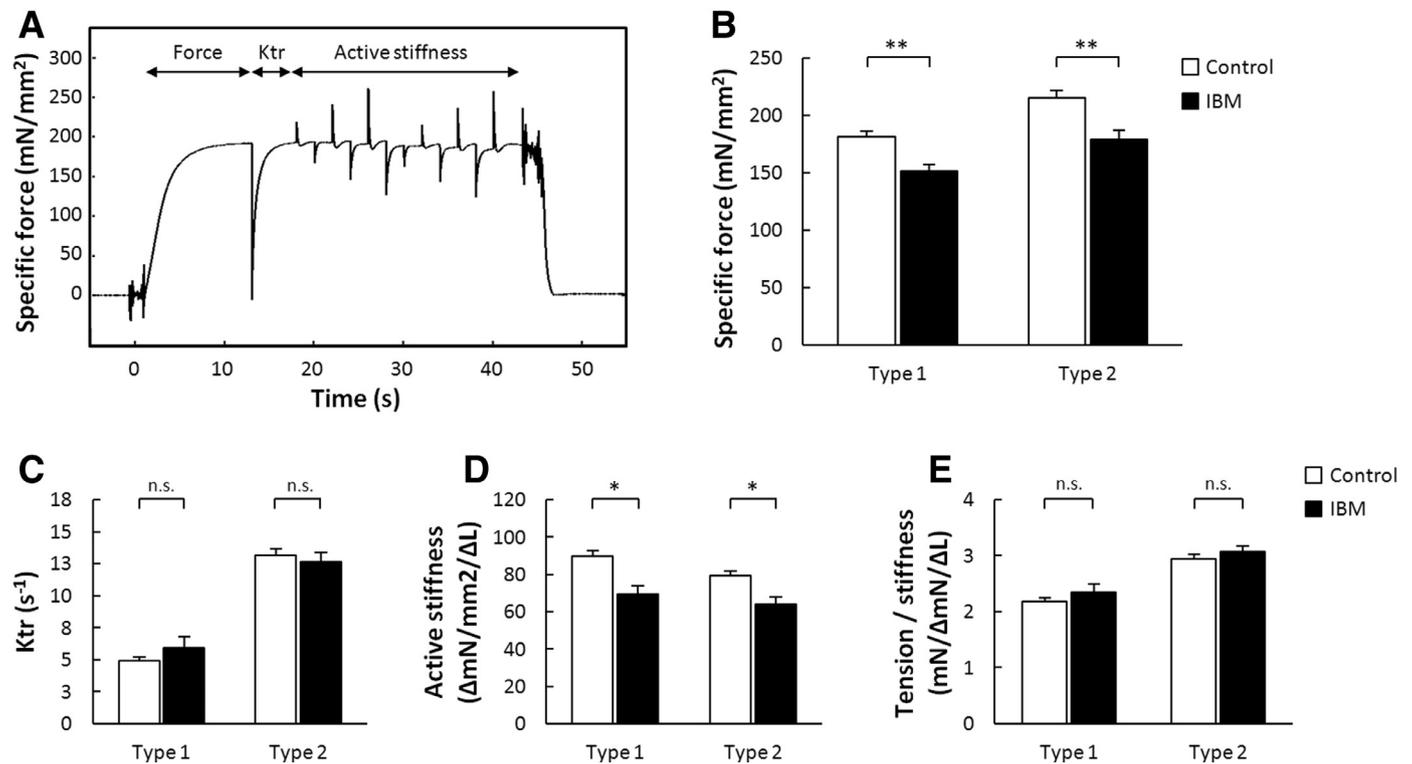
Fig. 2. *Reduced specific force and altered actin-myosin interactions in vastus lateralis single muscle fibers.* A. Representative force tracing showing transfer of the muscle fiber to a saturating Ca^{2+} solution, followed by generation of force. After peak force is reached, a rapid unloaded shortening and restretching of the muscle fiber is used to determine the Ktr, which is an estimate of the attachment and detachment rate of actin-myosin cross-bridges during activation. After peak force is reached a second time, slight length perturbations (−0.3, −0.6, −0.9, 0.3, 0.6 and 0.9%) are imposed on the muscle fiber to determine the active stiffness, which is an estimate of the number of attached cross-bridges during activation. B. Specific force is reduced in vastus lateralis IBM muscle fibers. C. Ktr is not changed in IBM fibers, reflecting preserved attachment and detachment rate of actin-myosin cross-bridges during activation. D. Active stiffness is reduced in IBM fibers, indicating a reduced number of cross-bridges during activation. E. No reduction in the tension / stiffness ratio, reflecting preserved force generated per cross-bridge. $p < .05$ is considered significant, * $p < .05$, ** $p < .01$, *** $p < .001$.

Active stiffness, which reflects the number of attached cross-bridges during activation, was reduced in IBM fibers of both fiber types (type 1 active stiffness: 69.6 ± 4.5 mN/mm^2/ΔL in IBM vs. 89.6 ± 3.4 mN/mm^2/ΔL in controls; type 2 active stiffness: 64.3 ± 3.8 mN/mm^2/ΔL in IBM vs. 79.3 ± 2.6 mN/mm^2/ΔL in controls, $p=.015$, Fig. 2D).

Assuming a situation in which all myofibrils within a muscle fiber are intact, the tension/stiffness ratio represents the amount of force generated per cross-bridge. The tension/stiffness ratio was not different in IBM patients (type 1 tension/stiffness ratio: 2.4 ± 0.2 in IBM vs. 2.2 ± 0.1 in controls; type 2 tension/stiffness ratio: 3.1 ± 0.1 in IBM vs. 2.95 ± 0.1 in controls, $p=.300$, Fig. 2E).

To summarize, our results indicate that reduced specific force in IBM fibers is caused by a decrease in the number of attached actin-myosin cross-bridges during activation.

3.3.3. Tibialis anterior single muscle fiber specific force
Specific force in single muscle fibers obtained from tibialis anterior biopsies was lower in IBM, but not significantly different from control fibers (type 1 specific force: 162.1 ± 5.4 mN/mm^2 in IBM vs. 175.1 ± 5.5 mN/mm^2
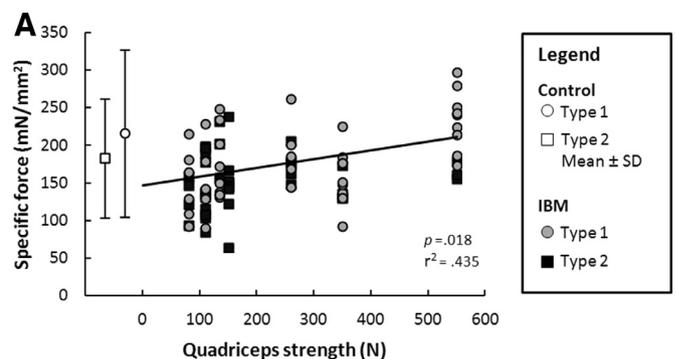

Fig. 3. *Correlation between single fiber specific force and quadriceps strength.* Ex vivo single muscle fiber specific force correlates with in vivo quadriceps quantitative strength.

in controls; type 2 specific force: 182.8 ± 17.8 mN/mm^2 in IBM vs. 212.0 ± 5.3 mN/mm^2 in controls, $p=.195$).

3.3.4. Correlation with disease severity
Vastus lateralis single muscle fiber specific force correlated reasonably well with in vivo quadriceps strength ($p=.018$, Fig. 3). We found no significant correlation with disease

Table 1
Participants and muscle biopsy characteristics.

	Control	IBM	p
Participants			
N	12	8	–
Age (years)	53.8 ± 1.7	58.5 ± 1.8	.081
Sex	6 M/6F	7 M/1F	.085
BMI (cm/m^2)	27.3 ± 1.5	26.6 ± 1.6	.752
Disease duration (years)	N/A	6.4 ± 1.3	–
IBM criteria			
Pathologically defined	N/A	2	–
Clinically defined	N/A	4	–
Possible	N/A	1	–
Laboratory findings			
CK (U/L)	158 ± 41	772 ± 261	**.045**
cN-1A reactivity (N)	N/A	2/8	–
Muscle and biopsy characteristics			
Vastus lateralis			
MRC score (quadriceps)	5.0 ± 0.0	4.0 ± 0.9	**.005**
MRI			
Fatty infiltration (%)	8.3 ± 2.5	51.6 ± 12.1	**.009**
Inflammation or edema (N)	0/12	3/8	**.021**
Histopathology			
Histopathology severity score	1.0 ± 1.0	7.0 ± 6.0	**.021**
Inflammation score	0.0 ± 0.0	2.0 ± 0.3	**<0.001**
Fiber type distribution (type 1%)	57.6 ± 4.0	57.7 ± 9.1	.997
Tibialis anterior			
MRC score	5.0 ± 0.0	4.5 ± 0.8	**.005**
MRI			
Fatty infiltration (%)	3.9 ± 0.8	29.6 ± 10.2	**.006**
Inflammation or edema (N)	0/12	2/8	.068
Histopathology			
Histopathology severity score	2.0 ± 1.0	6.0 ± 6.0	**.037**
Inflammation score	0.0 ± 0.0	2.0 ± 1.0	**.005**
Fiber type distribution (type 1%)	68.3 ± 20.5	76.9 ± 5.2	.343

Data are reported as mean ± SEM for continuous data, and median ± IQR for ordinal data.
Abbreviations: CK: creatinine kinase. IBM: inclusion body myositis. MRC: Medical Research Council.

Fig. 4. *Calcium sensitivity of force in IBM muscle fibers.* A. Representative force tracing showing transfer of the muscle fiber to solutions with increasing concentrations of Ca^{2+}, resulting in incremental force generation. After peak force is reached at a saturating Ca^{2+} solution (pCa 4.5) the fiber is transferred to a relaxing solution. B. Specific force-pCa curve for type 1 and type 2 fibers. There is no difference in pCa$_{50}$, the pCa at which 50% of maximum force is reached, between groups.

duration ($p=.159$), MRI fat percentage ($p=.548$) or histopathology severity score ($p=.476$).

3.3.5. Calcium sensitivity

To investigate other potential causes of muscle fiber weakness we evaluated calcium sensitivity of force generation (Fig. 3A). The pCa$_{50}$, which represents the calcium concentration at which 50% of maximum force is reached, did not differ between affected IBM and control fibers (type 1 pCa$_{50}$: 5.82 ± 0.01 in IBM vs. 5.77 ± 0.01 in controls; type 2 pCa$_{50}$: 5.82 ± 0.02 in IBM vs. 5.79 ± 0.01 in controls, $p=.073$). The Hill coefficient, a measure of myofilament cooperativity, was also unchanged in IBM fibers (type 1 n$_H$: 2.9 ± 0.1 in IBM vs. 3.0 ± 0.1 in controls; type 2 n$_H$: 3.5 ± 0.1 in IBM vs. 3.6 ± 0.1 in controls, $p=.845$) (Fig. 4). These results indicate that the amount of force generated in response to a specific calcium concentration does not differ between fibers of IBM patients and healthy controls.

3.3.6. Myosin heavy chain concentration

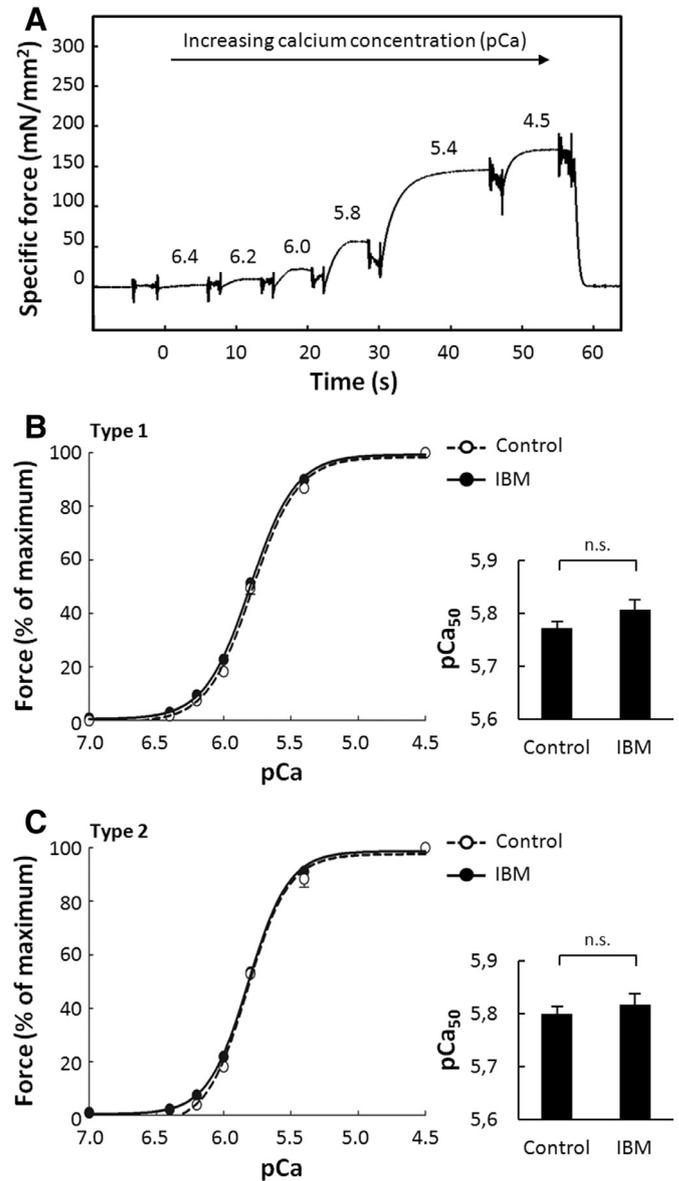Reduced single muscle fiber specific force due to a decreased number of attached cross-bridges during activation may be caused by impaired actin-myosin interactions or myosin loss. After force studies, MHC concentration was determined in a subset of single muscle fibers ($N=90$). Myosin concentration was significantly reduced in IBM type 1 fibers (type 1: $63.3 \pm 5.7 \mu g/\mu l$ in IBM vs. 106.6 ± 8.2 in controls $\mu g/\mu l$, $p=.034$) (Fig. 5). Myosin concentration was also reduced in type 2 fibers, however this finding failed to reach significance, most likely due to the low number of type 2 fibers ($N=6$) that was encountered due to type 1 predominance in the IBM fibers (type 2: 61.84 ± 12.3 in IBM vs. 84.4 ± 4.85 in controls, $p=.108$).
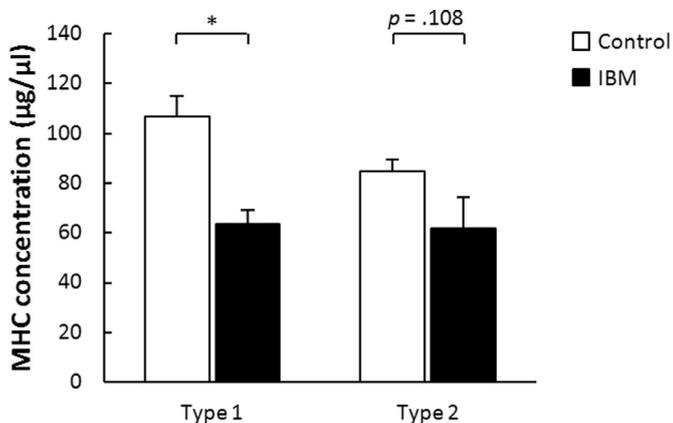
Fig. 5. *Single muscle fiber MHC concentration.* Single muscle fiber MHC concentration is reduced in IBM type 1 fibers. A reduction is also present in type 2 fibers, however this failed to reach significance probably due to a lower number of type 2 fibers in this subset.

4. Discussion

Atrophy and fatty infiltration of the quadriceps are hallmark features of IBM and reduce the amount of muscle tissue available for contraction, resulting in quadriceps muscle weakness. This study demonstrates that quadriceps strength in patients with IBM is further decreased due to reduced specific force. An important contributor to *in vivo* specific force is muscle fiber strength, which is reduced by 17% due to a decreased number of attached cross-bridges during activation, suggesting myosin loss or impaired actin-myosin interaction.

A previous study on contractile function in IBM muscle fibers obtained from biceps and vastus lateralis has previously reported preserved contractile function [26]. However, this study included a small number of patients ($N = 5$ biopsies, $N = 115$ IBM fibers) with relatively short disease duration (1–5 years). IBM is a progressive disease and biopsies obtained at an early stage may fail to show characteristic muscle abnormalities [27]. Our cohort included more patients and a longer disease duration of 2–10 years ($N = 14$ biopsies, $N = 236$ IBM fibers). Discrepancies between our results and the previous study may also be related to confounding factors such as age, lifestyle and comorbid disease that are difficult to control for in human studies. Advanced cell models, for example skeletal muscle-on-a-chip platforms that allow contractile studies in terminally differentiated striated muscle fibers, may complement studies that used patient-derived muscle tissue.

We observed that muscle fiber specific force was reduced in vastus lateralis, but not in tibialis anterior. In IBM, the quadriceps is affected early in the disease course. This was reflected in the current study: the quadriceps had a lower MRC grade, more severe fatty infiltration and higher histopathology sumscores compared to tibialis anterior. Furthermore, *ex vivo* vastus lateralis single muscle fiber specific force correlated with *in vivo* quadriceps quantitative strength. These findings suggest that reduced muscle fiber

specific force in IBM is a consequence of progressive disease. We found no correlation between single muscle fiber specific force and disease duration or fatty infiltration, most likely because rates of disease progression may differ significantly between IBM patients, and because extensive inflammation may be present in biopsies from clinically and radiologically spared muscles [28,29].

In single muscle fiber preparations, the plasma membranes are eliminated using a 'skinning' solution which enables the study of sarcomeric function without the confounding influence of endomysial connective tissue, membranous structures and endoplasmatic reticulum calcium handling. As a consequence, reduced single muscle fiber specific force reflects dysfunction of the sarcomere. The mechanisms that result in sarcomeric weakness may be disease specific and include loss of structural proteins and impaired interaction between sarcomeric proteins [30]. A potential cause of reduced muscle fiber specific force as seen in our patient with IBM is myosin loss.

Myosin concentration was indeed reduced in IBM muscle fibers in this study. Due to myosin loss, less actin-myosins crossbridges can be formed resulting in muscle fiber weakness. Reduced muscle fiber specific force due to myosin loss is also observed in patients with ICU-acquired weakness, and in these patients is caused by upregulation of the ubiquitin-proteasome pathway [31]. Some studies have suggested that this pathway is also disrupted in IBM [32]. Sustained cell stress due to inflammation may also result in sarcomeric protein degradation and muscle fiber damage [12–14]. Loss of sarcomeric proteins has been reported in IBM, with more severe depletion of type 2 structural proteins including myosin heavy chain [33].

Reduced muscle fiber specific force due to impaired actin-myosin interaction has also been reported in nemaline myopathy due to mutations in *NEB*, *ACTA1*, *TPM2* and *TPM3* [34]. Mutations in these genes all induce structural changes in sarcomeric proteins that are components of the actin-based thin filament, resulting in contractile abnormalities due to impaired actin-myosin interaction. Whole exome sequencing study found no abnormalities in nemaline myopathy-related genes in 30 Finnish IBM patients [35]. However, we can't exclude the possibility that IBM and nemaline myopathy share common pathways in the development of muscle weakness.

Studies on single muscle fiber contractile function in other muscle disorders are limited. Reduced single muscle fiber specific force was reported in small studies including muscle biopsies from patients with Duchenne muscular dystrophy ($N = 4$), myotonic dystrophy ($N = 2$) and facioscapulohumeral muscular dystrophy ($N = 4$) [36–38]. This suggests that reduced muscle fiber specific force may be a general consequence of muscle disease. However, these studies are small and our experience in larger datasets of muscle fibers from facioscapulohumeral ($N = 28$) and oculopharyngeal muscular dystrophy ($N = 23$) muscle biopsies is that progressive muscle pathology is not generally associated with reduced muscle fiber specific force

(unpublished data). Furthermore, muscle degeneration in mobility-limited older adults was not associated with reduced single muscle fiber specific force [39].

Recent therapeutic trials in IBM resulted in an increased amount of muscle mass, but did not improve functional performance or quadriceps strength [10,11]. Reduced specific force due to muscle fiber dysfunction may explain why merely promoting muscle growth or reducing fatty infiltration may not result in clinical improvement. Therapeutic strategies that augment muscle fiber contractile strength may provide benefit to patients with IBM, either alone or combined with therapeutic agents that increase or preserve muscle mass. Our findings also underline the importance of functional outcome measures, as muscle imaging will not reflect all components of muscle weakness.

We included a relatively small number of IBM patients and were unable to include enough women that fulfilled the inclusion criteria. Hence, we used sex as a covariate in statistical analysis where appropriate to account for the overrepresentation of men in the IBM group. There were no significant differences between men and women.

Quadriceps muscle strength was assessed by voluntary muscle contraction, which is influenced by pain, fatigue or lack of motivation. However, we do not expect these factors to have negatively influenced voluntary force generation in IBM participants. Five out of eight IBM patients reported no pain, and experimental protocols included resting periods to negate the influence of fatigue.

Skinned single muscle fiber preparations are, by design, limited to evaluation of the contractile apparatus of the muscle fiber. Hence, our experiments do not address upstream mechanisms of force generation such as sarcoplasmic reticulum Ca^{2+} handling or mitochondrial abnormalities, which require intact muscle fibers that cannot be obtained from routine muscle biopsies.

5. Conclusions

Specific force is reduced in IBM single muscle fibers and contributes to *in vivo* reduced specific force of the quadriceps. Muscle fiber weakness is caused by a decreased number of actin-myosin cross-bridges during activation, which is caused by myosin loss. Therapeutic strategies that augment muscle fiber strength may provide benefit to patients with IBM.

Ethics approval and consent to participate

The Medical Ethics Review Committee region Arnhem-Nijmegen approved this study (no. 2011/181). Informed consent was obtained from each participant.

Availability of data and material

The anonymized datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.03.001.

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