Sarcomeric dysfunction contributes to muscle weakness in facioscapulohumeral muscular dystrophy

Saskia Lassche, MD
Ger J.M. Stienen, PhD
Tom C. Irving, PhD
Silvère M. van der Maarel,
PhD
Nicol C. Voermans, MD,
PhD
George W. Padberg, MD,
PhD
Henk Granzier, PhD
Baziel G.M. van Engelen,
MD, PhD*
Coen A.C. Ottenheijm,
PhD*

Correspondence to
Dr. Lassche:
s.lassche@neuro.umcn.nl

ABSTRACT

Objective: To investigate whether sarcomeric dysfunction contributes to muscle weakness in facioscapulohumeral muscular dystrophy (FSHD).

Methods: Sarcomeric function was evaluated by contractile studies on demembranated single muscle fibers obtained from quadriceps muscle biopsies of 4 patients with FSHD and 4 healthy controls. The sarcomere length dependency of force was determined together with measurements of thin filament length using immunofluorescence confocal scanning laser microscopy. X-ray diffraction techniques were used to study myofilament lattice spacing.

Results: FSHD muscle fibers produced only 70% of active force compared to healthy controls, a reduction which was exclusive to type II muscle fibers. Changes in force were not due to changes in thin filament length or sarcomere length. Passive force was increased 5- to 12-fold in both fiber types, with increased calcium sensitivity of force generation and decreased myofilament lattice spacing, indicating compensation by the sarcomeric protein titin.

Conclusions: We have demonstrated a reduction in sarcomeric force in type II FSHD muscle fibers, and suggest compensatory mechanisms through titin stiffening. Based on these findings, we propose that sarcomeric dysfunction plays a critical role in the development of muscle weakness in FSHD. *Neurology*® 2013;80:733-737

GLOSSARY

FSHD = facioscapulohumeral muscular dystrophy.

Although muscle weakness is the hallmark feature of facioscapulohumeral muscular dystrophy (FSHD), the molecular mechanisms underlying weakness remain largely unknown. Before treatment options can be pursued, more insight into the pathophysiologic mechanisms of muscle weakness in FSHD is needed.

To understand why FSHD muscles are weak, we can take a clue from the genetics of the disease. FSHD1, the most common type of FSHD, is caused by a contraction of D4Z4, a 3.3-kb macrosatellite repeat located on chromosome 4q35. This contraction changes chromatin configuration, which is hypothesized to permit transcription of otherwise epigenetically silenced genes.¹ Of the candidate genes currently under investigation, some are muscle-specific and encode proteins involved in myogenesis and the development of the sarcomere—the smallest contractile unit in muscle. Gene expression profiling studies in FSHD muscle biopsies have shown dysregulation of several sarcomeric proteins.² Overexpression of *DUX4*, the leading FSHD candidate gene, has been shown to activate pathways involved in sarcomeric protein degradation.³

Despite evidence pointing toward changes on the level of the sarcomere, no studies have examined whether sarcomeric dysfunction contributes to muscle weakness in FSHD. This study is the first to report on sarcomeric function in FSHD.

*These authors contributed equally to this work.

From the Department of Neurology (S.L., N.C.V., G.W.P., B.G.M.v.E.), Neuromuscular Centre Nijmegen, Radboud University Nijmegen Medical Centre, Nijmegen; Department of Physiology, Institute for Cardiovascular Research (G.J.M.S., C.A.C.O.), and Department of Physics and Astronomy (G.J.M.S.), VU University Medical Center, Amsterdam, the Netherlands; Department of Biological and Chemical Sciences (T.C.I.), Illinois Institute of Technology, Chicago; Department of Human Genetics (S.M.v.d.M.), Leiden University Medical Centre, Leiden, the Netherlands; and Department of Physiology (H.G., C.A.C.O.), University of Arizona, Tucson.

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METHODS Muscle biopsies. Muscle biopsies were obtained from 4 patients with FSHD and 4 healthy control subjects without relevant medical history or neuromuscular symptoms. FSHD severity and genetic and histologic features are described in the table. Average age at biopsy was 41 years for patients with FSHD and 38 years for controls.

Standard protocol approvals, registrations, and patient consents. For patients with FSHD, biopsies that were collected for diagnostic purposes were used. For control subjects, the local medical ethics committee approved the collection of muscle biopsies. Informed consent was obtained.

Muscle fiber preparation. Single muscle fibers were isolated from the muscle biopsies and demembrated for 20 minutes in a relaxing solution containing 1% Triton X-100 at ~4°C.^{4,5} Triton permeabilizes all membranous structures, allowing for activation of the sarcomere with exogenous Ca²⁺ and enabling study of sarcomeric function in isolation. On average, 9 fibers were prepared per biopsy.

Contractile measurements. Experimental details have been described previously.^{4,5} In brief, isolated single muscle fibers were mounted between a force transducer and a length motor. Maximum force-generating capacity was measured at a sarcomere length of 2.5 μm by activating the fibers in a saturating [Ca²⁺] solution.^{4,5} Force was normalized to muscle fiber cross-sectional area. Various submaximal Ca²⁺ concentrations were used to assess the Ca²⁺ sensitivity of force generation.^{4,5} We measured passive force at a sarcomere length of 2.5 μm by imposing length changes on relaxed muscle fibers.⁵ Myosin heavy chain isoform composition was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis.⁴

Thin filament length measurements. Thin filament length was determined using confocal light microscopy with fluorescently labeled actin-binding proteins.⁴

X-ray diffraction studies. Myofilament lattice spacing was determined by small-angle x-ray diffraction experiments at the Advanced Photon Source at Argonne National Laboratory, Lemont, IL.⁵ In brief, single muscle fibers were mounted on a small angle x-ray diffraction setup. Sarcomere length and myofilament lattice spacing were measured simultaneously. Separation of the 1,0 equatorial reflections from the diffraction pattern were converted to d1,0 lattice spacing via Bragg's law.

Statistical analysis. Statistical analyses were performed by *t* test; *p* < 0.05 was considered statistically significant. Data are presented as means ± SEM.

RESULTS Maximum force-generating capacity is reduced in type II FSHD muscle fibers. We observed a marked reduction in contractile strength in type II (fast-twitch) FSHD muscle fibers. FSHD type II fibers generated 59.8 ± 6.0 mN/mm² compared to 87.4 ± 5.0 mN/mm² in controls (*p* = 0.0012), whereas FSHD type I (slow-twitch) fibers generated 66.7 ± 6.5 mN/mm² compared to 71.4 ± 7.9 mN/mm² in controls (*p* = 0.7006, figure 1A).

The normalized force–sarcomere length relation of FSHD fibers closely overlapped that of controls (figure 1B). No difference in thin filament length was observed between FSHD and control fibers (figure 1C). These findings indicate that muscle fiber weakness in FSHD is not caused by alterations in thin filament length and is independent of sarcomere length.

FSHD muscle fibers produce markedly higher passive forces. In patients with FSHD, passive force (i.e., force in the absence of Ca²⁺) was increased, with the most profound increase in type II fibers. Type I FSHD fibers produced 17.3 ± 4.9 mN/mm² compared to 3.4 ± 1.4 mN/mm² in control tissue (*p* = 0.026924), whereas type II FSHD fibers produced 24.2 ± 5.1 mN/mm² compared to 1.9 ± 0.3 mN/mm² in controls (*p* < 0.0001) (figure 2A).

In demembrated fibers, passive force is generated mainly by titin, a giant sarcomeric protein. Titin-based passive force has been shown to affect submaximal active force generation of muscle fibers by modulating their calcium sensitivity, presumably due to titin's effect on myofilament lattice spacing (explained in figure 2D).⁶ In accordance with the increased passive force, our x-ray diffraction studies demonstrated that as sarcomere length increases, myofilament lattice spacing decreases more in FSHD muscle fibers than in control fibers (figure 2B). The force–pCa curve was shifted leftward compared to control fibers, indicating an increased calcium sensitivity of force generation (figure 2C).

Table Patient and muscle biopsy characteristics ^a							
Patient with FSHD	Disease severity: Ricci CSS score ¹² (age-adjusted CSS)	Genetics		Histology			
		Fragment length, kb	Haplotype	Biopsy site	Myopathic features	Inflammatory abnormalities	Mitochondrial abnormalities
1	4 (25)	14	4A161	QF	+	–	–
2	0 (0)	14 (30%); 70 (70%)	4A161	QF	–	–	+
3	6 (35)	30	Unknown ^b	TA	+++	+	–
4	7 (40)	21	4A161	QF	++	–	+

Abbreviations: CSS = Clinical Severity Scale; FSHD = facioscapulohumeral muscular dystrophy; QF = quadriceps femoris muscle; TA = tibialis anterior muscle.

^aMyopathy: – none; + mild; ++ moderate; +++ severe.

^bNo haplotype data available.

Figure 1 Reduced maximum force-generating capacity in type II facioscapulohumeral muscular dystrophy muscle fibers

(A) Reduced maximum force generation—at a sarcomere length of 2.5 μm —in type II facioscapulohumeral muscular dystrophy (FSHD) muscle fibers compared to healthy controls. (B) Normalized force-sarcomere length relationships of control and FSHD muscle fibers overlap, indicating that sarcomeric weakness is not dependent on sarcomere length. (C) Confocal light microscopy with fluorescently labeled actin-staining antibodies demonstrates preserved myofibrillar structure and thin (actin) filament length. Line scan shows intensity of actin staining; highest intensity corresponds with the Z line, moderate intensity corresponds with the actin filaments, lowest intensity corresponds with the actin filament bare zone. Inset shows 2 $\times$ thin filament length.

DISCUSSION The force-generating capacity of sarcomeres is significantly impaired in FSHD. Sarcomeric weakness was restricted to type II muscle fibers, in which maximum force generation was only 70% of normal strength. In contrast to active force measurements, a 5- to 12-fold increase in passive force was seen in type I and type II fibers, respectively, indicating stiffening of titin molecules. These findings are corroborated by the observed decrease in myofilament lattice spacing and the increase in calcium sensitivity, both physiologic consequences of titin stiffening.⁶

The observed type II specific sarcomeric weakness suggests that the pathologic changes in FSHD affect these fibers in particular. A previous study also suggested

type II specific pathology, either through impaired generation of type II fibers or through increased susceptibility of these fibers to early apoptotic death signaling.² Furthermore, type II muscle fibers are more susceptible to oxidative stress,⁷ which has been shown to play a role in the pathophysiology of FSHD.³

The increased passive force provides indirect evidence for titin stiffening, which is modulated by alternative splicing of the titin gene and by post-translational modifications of titin molecules. Expression profiling studies in FSHD have revealed changes in sarcomeric proteins, including titin.² We hypothesize that titin stiffening is a mechanism to compensate for muscle weakness in FSHD by augmenting the responsiveness of the sarcomere to submaximal calcium concentrations.

(A) Increased passive force in type I and type II facioscapulohumeral muscular dystrophy (FSHD) muscle fibers compared to healthy controls. (B) X-ray diffraction studies show that as sarcomere length increases, myofilament lattice spacing decreases more in FSHD than in control fibers. (C) Leftward shift of force-pCa relations of FSHD compared to control fibers, indicating increased calcium sensitivity. Inset shows pCa_{50} , the pCa at which 50% of maximum force is generated. (D) Schematic diagram illustrates the effect of titin stiffening on myofilament lattice spacing. Due to titin's oblique attachment to the thin and thick filaments, titin stiffening pulls the filaments closer together, reducing myofilament lattice spacing. This facilitates crossbridge formation, increasing the sensitivity of fibers to calcium.

In myotonic dystrophy, and during conditions of muscle disuse, sarcomeric force generation is diminished in both fiber types, in particular in type I fibers.^{8,9} In inflammatory myopathies, no abnormalities were found.¹⁰ Therefore, the changes in sarcomeric function observed here are not the consequence of muscular dystrophy, inflammation, or muscle disuse in general, and appear to be specific for FSHD.

In our experiments, the use of demembranated muscle fibers eliminated any influences on the level of the sarcolemma, meaning that the results presented

here are solely the effect of weakness on the level of the sarcomere. However, in addition to sarcomeric dysfunction, muscle weakness in FSHD is likely to be further augmented by the compromised integrity of the sarcolemma demonstrated previously.¹¹ This hampers the lateral transmission of force produced by sarcomeres to the surrounding extramuscular structures.

We have shown sarcomeric dysfunction in type II muscle fibers from patients with FSHD, and suggest compensatory mechanisms through titin stiffening. Our findings are based on a small group of patients

and controls, and more extensive studies are needed to confirm these data. Restoring sarcomeric function might be a potential treatment approach.

AUTHOR CONTRIBUTIONS

Dr. Lassche was responsible for acquisition, analysis, and interpretation of the data, and drafted and revised the manuscript. Dr. Stienen was involved in revision of the manuscript. Dr. Irving was involved in revision of the manuscript. Dr. van der Maarel was involved in interpretation of the data and revision of the manuscript. Dr. Voermans was involved in revision of the manuscript. Dr. Padberg was involved in interpretation of the data and revision of the manuscript. Dr. Granzier was involved in revision of the manuscript. Dr. van Engelen conceived the study, obtained funding, and was involved in interpretation of the data and drafting and revision of the manuscript. Dr. Ottenheijm conceived the study, obtained funding, and was involved in acquisition, analysis, and interpretation of the data, and drafting and revision of the manuscript.

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