Muscle weakness correlates with muscle atrophy and precedes the development of inclusion body or rimmed vacuoles in the mouse model of DMRV/hIBM

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Submitted 12 April 2008; accepted in final form 7 July 2008

Malicdan MC, Noguchi S, Hayashi YK, Nishino I. Muscle weakness correlates with muscle atrophy and precedes the development of inclusion body or rimmed vacuoles in the mouse model of DMRV/hIBM. Physiol Genomics 35: 106–115, 2008. First published July 15, 2008; doi:10.1152/physiolgenomics.90219.2008.—Distal myopathy with rimmed vacuoles (DMRV), also called hereditary inclusion body myopathy (hIBM), is characterized clinically by weakness and atrophy that initially involves the distal muscles and pathologically by the presence of rimmed vacuoles (RVs) or intracellular protein deposits in myofibers. It is caused by mutations in the UDP-N-acetylgalactosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene that is important in sialic acid synthesis. Recently, we generated a mouse model (Gne–/–hGNE176VTg) that exhibits muscle weakness and pathological changes similar to DMRV patients. To gain better understanding of the pathomechanism of DMRV, we determined temporal changes in the overall motor performance of this model mouse for DMRV in correlation with the structure and function of isolated skeletal muscles and muscle pathology. These DMRV mice exhibited muscle weakness, decreased whole muscle mass and cross-sectional area (CSA), and reduced contractile power in an age-related manner. Single-fiber CSA further supported the finding of muscle atrophy that involved both type I and type II fibers. These results suggest that atrophy is highly correlated with reduced production of force at young age, both in vivo and ex vivo, thereby implicating the important role of atrophy in the pathomechanism of DMRV. In older age, and particularly in gastrocnemius muscles, RVs and intracellular inclusions were seen in type IIA fibers, further aggravating reduction of force and specific increase in twitch-tetanus ratio.

Distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy; skeletal muscle force; amyloid

DISTAL MYOPATHY WITH RIMMED VACUOLES (DMRV) OR HEREDITARY INCORPORATION BODY MYOPATHY (hIBM) IS AN AUTOSOMAL RECESSIVE DISORDER CAUSED BY MUTATIONS IN THE UDP-N-ACETYLGLUCOSAMINE 2-EPIMERASE/N-ACETYLGLUCOSAMINE KINASE (GNE) GENE (9, 12, 21). THIS GENE ENCODES THE BIFUNCTIONAL ENZYME CATALYZING THE TWO CRITICAL STEPS IN SIALIC ACID SYNTHESIS.

Because DMRV and hIBM are the same disorder, these terms are used synonymously here. DMRV predominantly affects distal muscles at the initial stages but also involves proximal muscles during the progression of the disease. This condition has been reported as quadriceps-sparing myopathy because the quadriceps muscles are relatively spared during the early stages of the disease (3). The skeletal muscles are primarily affected, but other organs, including cardiac muscles, were affected in a group of patients as well (20). The term used in the nosology of this condition partly arises from observations in pathological studies. In skeletal muscles, rimmed vacuoles (RVs) are seen in some fibers, in addition to the finding of scattered atrophic fibers and intracellular congophilic deposits that are immunoreactive to amyloid and tau, among other various proteins. Endomysial fibrosis, necrotic and regenerating processes, and inflammatory cell infiltrates are not commonly seen but have been demonstrated in anecdotal reports. Although speculations and hypotheses abound regarding the pathomechanism to explain how these RVs are formed and how they could lead to muscle weakness (1, 2, 18, 22, 23, 25, 31, 32), precise information is not fully elucidated at this time.

Muscle weakness in DMRV has been attributed to several events. RV formation was generally believed to trigger a downstream cascade that ultimately leads to muscle fiber degeneration and atrophy. Deposition of inclusion bodies within myofibrils could physically interfere with the contractile apparatus, or could instigate a process of myofiber degeneration. RV formation in skeletal muscles, which indicates impaired autophagic process (15, 20, 23), is associated with reduced clearance of cytosolic proteins through basal autophagy; the resulting accumulation of autophagic vacuoles may interfere with the function of skeletal muscle. The contribution of each phenomenon to muscle weakness and how these events relate to each other have not been fully verified primarily because of the lack of a detailed time-course study, which is rather difficult to accomplish in patients with gradually progressive illnesses.

We recently generated the first mouse model for this myopathy that resembles the phenotype in humans (16). Gne–/–hGNE176VL-Tg mice, which we refer to here as “DMRV mice,” exhibited hyposialylation of serum and various organs, muscle weakness, and mild to moderate serum creatine kinase elevation from 30 wk of age, a time during which only subtle changes were seen in skeletal and cardiac muscles in addition to intracellular deposition of amyloid in a few fibers. From 40 wk onward, RVs were seen in scattered fibers (15).

The primary pathogenesis in most murine models for muscular dystrophy can be traced to a common defect on the dystrophin-glycoprotein complex (DGC) that initiates a sequence of events that eventually lead to necrosis or apoptosis partly due to increased intracellular calcium (14). In DMRV
mice, however, we have excluded at least the “leaky hypothesis,” because they have intact DGC complex, and this is supported by the paucity of necrotic and regenerating process. The absence of definite sarcolemmal defect, nevertheless, is also seen in other murine models of muscular dystrophies (8, 11, 26), making the origin of loss of force generation in these murine models far from being understood. Unfortunately, very few studies have addressed the mechanism of muscle weakness in nondystrophic states, including chronic myopathies.

Our previous results prompted us to work on several hypotheses. First, we hypothesize that factors other than the presence of pathological hallmarks play an important role in the pathogenesis of muscle weakness, because we have seen generalized body weakness at the age when there were no obvious pathological findings. Second, we think that the mechanism underlying muscle weakness is distinct from the theories established in other muscular dystrophies. Finally, because some muscles are relatively spared from the formation of RVs and intracellular inclusion, the degree of effect is most likely not the same among different muscles. Thus a study focusing on the structure and function of the muscles of these DMRV mice is appropriate, and could help us discover further clues that we could use in developing methods useful for evaluating treatment strategies for this debilitating myopathy.

METHODS

**Ethical approval.** All animal experiments conducted in this study were approved by and carried out in accordance with the rules and regulations of the Ethical Review Committee on the Care and Use of Rodents in the National Institute for Neuroscience, National Center of Neurology and Psychiatry (NCNP). These policies are based on the “Guidelines for Animal Experimentation,” as sanctioned by the Council of the Japanese Association of Laboratory Animal Science.
m/min every minute until the mouse was exhausted and could no longer run. Exhaustion was defined as the inability of the mouse to reengage the treadmill belt after 10 s of staying on the shock bars despite prodding. The time of exhaustion was used to calculate the distance the mouse covered during the exercise. The endurance exercise consisted of a 30-min treadmill run at 30 m/min with a 7° incline. During the test the total number of beam breaks was recorded, and this was inversely proportional to the ability of the mouse to sustain workload. A digital video camera was positioned above the treadmill to record each test; video recordings were used for analysis. Both tests were done three times, with a 3- to 4-day period of rest in between.

Contractile properties. Measurement of muscle contractile properties was performed according to previous protocols (6, 14), with some modifications. All materials used for in vitro measurement of force were acquired from Nihon Kohden (Tokyo, Japan). We analyzed the following muscles: gastrocnemius muscle, which is the preferentially involved muscle in terms of pathology; tibialis anterior (TA) muscle, which had no RVs even among aged DMRV mice; and quadriceps femoris (QF) muscle, because DMRV was initially known to be “quadriceps sparing.” The mice were weighed and deeply anesthetized with pentobarbital sodium (40 mg/kg) intraperitoneally, with supplemental doses as necessary to maintain adequate anesthesia, which was judged by the absence of response to tactile stimuli.

The entire muscle was isolated, removed, and secured with a 4-0 silk suture at the distal muscle tendon and proximal bone of origin, after which the mice were killed by cervical dislocation. Subsequently, the muscle was mounted in a vertical chamber, connected to a force-displacement transducer (TB-652T for gastrocnemius and QF, TB-653T for TA), and positioned between a pair of platinum electrodes that delivered electrical stimulus. Throughout the analysis, the muscle was bathed in a physiological solution consisting of (in mM) 150 NaCl, 2 KCl, 1 CaCl2, 1 MgCl2, 5.6 glucose, 5 NaH2PO4 (pH 7.4), and 0.02 μM tubocurarine, maintained at a temperature of 20°C, and perfused continuously with a mixture of 95% O2-5% CO2 to facilitate acquisition of maximum level of force contraction as previously reported (6, 14). This supranormal oxygen level is nevertheless nonphysiological, because it has been shown to produce oxidative insult (10). Square wave pulses 0.2 ms in duration were generated by a stimulator (SEN-3301) and amplified (PP-106H), and subsequently muscle length was adjusted to the length (Lo) that resulted in maximal twitch force (Pt). With the muscle held at Lo and the duration changed to 3 ms, the force developed during trains of stimulation pulses (10–200 Hz) was recorded.

Stimulation frequency was increased until the maximum absolute tetanic force (P0) was achieved. For TA muscles 300-ms trains of pulses were used, while 600-ms trains were used for gastrocnemius. Data obtained were digitized and analyzed with a Leg-1000 polygraph system equipped with QP-111H software. Absolute force was normalized with the physiological cross-sectional area (CSA), which was computed as the product of the ratio of muscle weight and Lo and the density for mammalian skeletal muscle, 1.066 mg/mm3, to obtain specific force (P/CSA and P0/CSA). After analysis of force generation, the muscles were removed from the chamber, blotted dry, and weighed.

Pathological and morphological analysis. Muscle tissues were processed for pathological analysis as previously reported (16). Serial cryosections were stained with hematoxylin and eosin, modified Gomori trichrome, and acid phosphatase according to standard procedures. Stained sections were visualized on a microscope (Olympus

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**Fig. 3.** Muscle mass and cross-sectional area (CSA). A: gastrocnemius muscle weight. B: gastrocnemius muscle CSA. C: tibialis anterior (TA) muscle weight. D: TA muscle CSA. In A and C, with the increasing body weight of control littermates, the masses of gastrocnemius (A) and TA (C) muscles show increase by 10–20% from 10 to 31 wk of age. From 40 to 50 wk of age, however, there is a very slight increase in the gastrocnemius muscles, while a slight decrease to no change was observed in the TA muscles. For the DMRV mice, muscles do not show any appreciable increase in weight from 21 wk of age and instead demonstrate a steadily decreasing muscle mass of at least 10–20% from 31 wk of age. In B and D, the CSA of both gastrocnemius (B) and TA (D) muscles in DMRV mice exhibit a delay in increase in CSA with age compared with control mice; as a result, the CSA is 10–15% less than that in control mice from 21 wk of age and decreases up to 40–50% by 50 wk. Values are expressed as means; error bars represent SE. *P < 0.05.
BX51, Olympus, Melville, NY), and digitized images (DP70, Olympus) were acquired for pathological analysis. Congo red staining, visualized by fluorescence light, was likewise used to probe for intracellular inclusions (4).

Immunohistochemical analysis. For single-fiber CSA, sections were probed with β-dystroglycan (rabbit polyclonal antibody, a gift from Dr. Ejiro Ozawa, NIN, NCNP Tokyo, Japan) followed by appropriate secondary antibody. Images from six random areas of the muscles were captured at 200 magnification. From these images, individual fiber diameter was measured from 600 – 800 fibers with J-image software (National Institutes of Health, http://rsb.info.nih.gov/ij/), taking note of the shortest diameter. Histological CSA was plotted and analyzed according to mouse group. Mouse monoclonal antibodies against myosin heavy chain (MHC) fast type and slow type (Novocastra, Newcastle upon Tyne, UK), BF-F3 (S. Schiaffino, ATCC) for MHC 2B, and SC-71 (S. Schiaffino, ATCC) for MHC 2A were used for muscle fiber type analysis.

Statistical analysis. All data are presented as means ± SE. For muscle mass and muscle contractile properties, repeated-measures (mixed model) ANOVA was used to determine the primary effects of age and genotype. Post hoc comparison by Bonferroni test was used to compare replicate rows. All statistical tests were considered to be significant when the error was <5% (P < 0.05). Our analysis showed that overall the effects of genotype on body weight, muscle weight, and contractile properties were not different for muscles of male and female mice (data not shown), but for clarity of presentation data from male and female mice are displayed separately and asterisks in Figs. 2–5 indicate only genotype differences, i.e., between DMRV and littermate mice. Among DMRV mice, a gradual decline is seen after 30 wk of age, with remarkable reduction after 40 wk of age; P0 production seems to be more affected, because significant differences between DMRV and littermates are seen in all ages. C and D: force normalized by CSA shows similar pattern, except that remarkable differences are noted from 31 wk. Values are expressed as means. Error bars represent SE. *P < 0.05.

Table 1. Contractile properties of DMRV muscles compared with control

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<tr>
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<th>31–40 wk</th>
<th>41–50 wk</th>
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<td>Pt-to-P0 ratio</td>
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<td>97.74</td>
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DMRV, distal myopathy with rimmed vacuoles; P0, isometric twitch force; Pt, tetanic force.

Fig. 4. Contractile properties of gastrocnemius muscle. A: peak isometric twitch (Pt). B: maximum tetanic force (P0). C: Pt normalized by CSA, or specific Pt. D: P0 normalized by CSA, or specific P0. A and B: among littermates, Pt and P0 follow a steady increase from 10 to 40 wk and gradual decrease thereafter, except in female mice. Among DMRV mice, a gradual decline is seen after 30 wk of age, with remarkable reduction after 40 wk of age; P0 production seems to be more affected, because significant differences between DMRV and littermates are seen in all ages. C and D: force normalized by CSA shows similar pattern, except that remarkable differences are noted from 31 wk. Values are expressed as means. Error bars represent SE. *P < 0.05.
RESULTS

Gross morphology of mice. The DMRV mice weighed less, and this difference in weight became more remarkable with age (Fig. 1). Body masses of both male and female control littermates increased 35–40% between 10 and 31 wk of age, with barely appreciable changes from 41 to 50 wk. In contrast, DMRV mice were at least 5–10% smaller at 10 wk of age and only showed small increases of body mass with age. Weight among these mice reached a plateau at around 30 wk of age, but decreased considerably from 41 wk.

DMRV mouse impaired motor performance and reduced endurance by treadmill analysis. When subjected to increasing workload, the DMRV mice performed worse than their littermates. The total distance they were able to run before total exhaustion was significantly less than that of control mice, and this was evident from the age of 21 wk in both males and females (Fig. 2A; data of female mice not shown). When given a consistent workload, the DMRV mice had greater beam breaks, reflecting less ability for endurance, although significant differences were only noted at 31 wk among male mice and at 41 wk among female mice. Error bars represent SE. *P < 0.05.

Decreased muscle weight and CSA contribute to weight loss in DMRV mice. Both the gastrocnemius and TA muscles of DMRV mice weighed less compared with control mice (Fig. 3, A and C). More remarkable and more significant statistical differences were noted in the older age groups. In addition, the muscles did not show any appreciable increases in weight from 21 wk of age and instead demonstrated a steadily decreasing muscle mass of at least 10–20% from 31 wk of age, with more remarkable decrease in muscle mass of gastrocnemius from 41 wk of age.

Compatible with the pattern of muscle mass with age, the whole muscle CSA of both muscles in DMRV mice rather exhibited a delay in increase in CSA with age compared with control (Fig. 3, B and D); as a result, the CSA is 10–15% less than control mice from 21 wk of age and decreases by 40–50% more with age than that of control mice by 50 wk.

The quadriceps muscles of the DMRV mice were also affected, but at a much later age. From 31 wk of age, the QF muscles were lighter and had lower CSA compared with control (Supplemental Fig. S1).1

Muscle contractile properties. In DMRV gastrocnemius muscles, P_t and P_0 showed gradual decrements with age (Fig. 4, A and B) compared with control: 90% by 10 wk, 80% by 21 wk, 70% by 31 wk, and 50% by 41 wk. Of note, the P_0 values were markedly reduced after 41 wk of age (Fig. 4B); thus when the twitch-tetanic ratio is computed, it is significantly higher in both male and female DMRV mice compared with control mice (Table 1). Specific P_t and P_0 values showed similar temporal pattern of force reduction in DMRV mice (Fig. 4, C and D), except that significant differences between DMRV and control mice were only seen from the age of 31 wk.

In DMRV TA muscles, P_t showed a different pattern with age, in contrast to control (Fig. 5, A and B), slightly increasing from 10 to 20 wk and then gradually decreasing from 21 wk

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1 The online version of this article contains supplemental material.
onward. Furthermore, Pt was notably lower in all age groups, despite the absence of any noted abnormality in pathology. P0 values in these model mice were likewise lower than in control mice in all age groups, similar to the gastrocnemius, but with statistical significance at 21 wk of age in male mice and at 31 wk of age in female mice. Similar to the gastrocnemius muscles, the specific Pt values of TA muscles of DMRV mice were nearly normal during early age; remarkable reduction was only seen from 31 wk (Fig. 5, C and D). Table 1 further summarizes the contractile properties of the gastrocnemius and TA muscles of the DMRV mice compared with littermates. Overall, ~10–50% deficit in generation of force is observed in these muscles.

The QF muscles of the DMRV mice showed reduction of both P0 and Pt from 31 wk of age, but no differences with respect to control mice were seen when these values were normalized with CSA (Supplemental Fig. S2).

Atrophic changes are noted before development of pathological hallmarks in DMRV muscle. From 10 to 20 wk of age the muscles from the DMRV mice appear morphologically unremarkable on light microscopy, except for minimal variation in fiber size in the gastrocnemius (Fig. 6A), TA (Fig. 7A), and QF (Supplemental Fig. S3A) muscles. For both gastrocnemius and TA muscles, the number of small-sized fibers increases from 21 to 30 wk of age, contributing to the variation in fiber size. From 31 to 40 wk of age, scattered

Fig. 6. Gastrocnemius muscle: pathological findings and single-fiber CSA. A: hematoxylin and eosin (H&E) and modified Gomori trichrome (mGT) sections show only mild variation in fiber size in gastrocnemius muscle of DMRV mice at 10–20 wk, almost indistinguishable from control. No endomyosial fibrosis or inflammation is seen. Small atrophic fibers are seen randomly (black arrows) and are observed by 21–30 wk, and the number of such fibers tends to increase by 31–40 wk, making the variation in fiber size more remarkable. By 41–50 wk, rimmed vacuoles (RVs) (red arrows) are noted to be scattered in the muscle. These RVs are more highlighted in mGT and are stained in acid phosphatase, indicating upregulation of the lysosomal system. Double arrows show intracellular inclusions. Bars, 50 μm. B: single-fiber CSA shows the variation in fiber size, which is more remarkable in DMRV mice as they age. Note that myofibers of DMRV mice are generally smaller at all ages, implying gradual decrease in the caliber of fibers. Values are expressed as means; error bars represent SD.
small angular fibers are observed. At this age, at least in the gastrocnemius, there are few intracellular inclusions, which are Congo red positive (data not shown) and immunoreactive to various antibodies to amyloid B as we demonstrated previously (16). RVs are noted from 42 wk and in older mice in the gastrocnemius muscles, while none is seen in the TA. Interestingly, the same changes were seen in the QF (Supplemental Fig. S3A), although the onset of changes notably occurred at a later age; the presence of small atrophic fibers was noted from 30 wk; few Congo red-positive inclusions were seen after 40 wk; and some RVs were visible after 50 wk.

By measuring the diameter of fibers in both gastrocnemius and TA muscles, we found that fiber size in DMRV mice is almost normal and comparable to littermates from 10 to 20 wk of age. After 20 wk of age, however, remarkable variations in fiber size were seen in both gastrocnemius and TA muscles (Figs. 6B and 7B), and the frequency of smaller-sized fibers increased with age, shifting the histogram to the left (Supplemental Fig. S4) and providing further evidence of atrophy. Similarly, the fibers of QF muscles also appear smaller, but the variation is much more evident after 40 wk of age (Supplemental Fig. S5).

Muscle atrophy affects both fiber type I and type II. In terms of fiber type effect, we did not find fiber type predominance in DMRV muscles (data not shown). At least in gastrocnemius (Fig. 8A, top), TA (Fig. 8A, bottom), and QF (Supplemental Fig. S4) muscle, we noted reduction in individual fiber CSA in
both fast and slow fibers (quantitative data not shown). Interestingly, almost all fibers that had either RVs or intracellular depositions were type II fibers (Fig. 8, B and C) and were fast oxidative type (Fig. 8, D and E).

**DISCUSSION**

Through the analysis of structure and function of the muscles of DMRV mice, we found that skeletal muscles exhibit atrophy and that this phenomenon is well-correlated with reduction in force generation and, consequently, development of muscle weakness. More importantly, the onset of muscle atrophy predated the pathological hallmarks of DMRV, which include intracellular inclusion body and RV formation, and may be regarded to have a greater contribution to the development of muscle symptoms than RV formation per se.

The overall muscle fiber size is determined by the balance between synthesis and degradation of intracellular components. Muscle atrophy occurs when the protein degradation rate is higher than the synthesis rate and is noted in several situations like disuse, fasting, aging, and a number of disease states. Because of the variety of conditions in which muscle atrophy is evident, different signaling pathways and molecular triggers are thought to determine the activation of target systems responsible for decreased protein synthesis or increased proteolysis.

Three proteolytic systems that have been implicated in muscle wasting are the ubiquitin-proteasome (UPS) (5, 27), lysosomal (13, 30), and calpain (7) systems. The activity of UPS is markedly increased in atrophying muscles, mainly because of the transcriptional activation of two muscle-specific ubiquitin ligases, namely, atrogin-1 and Murf-1, among other genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes. The expression of these ubiquitin ligases is mainly activated by two major signaling pathways, including FoxO genes.

Fig. 8. Muscle atrophy involves both type I and type II fibers. A: representative sections from gastrocnemius (top) and TA (bottom) muscles stained with myosin heavy chain (MHC) fast fiber type and MHC slow fiber type. Compared with control, both type I and type II fibers in DMRV muscles are smaller. B: muscle cryosections from the gastrocnemius muscles (44-wk-old female DMRV mouse) were stained with H&E showing myofibers with RVs (arrows) and inclusion bodies (arrowheads), in addition to marked variation in fiber size. C: MHC slow type for type I (slow) fibers. D: MHC fast type for type II (fast) fibers. E: BF-F3, which recognizes type 2B (fast glycolytic) fibers. Note that fibers with RVs and inclusions are virtually type IIA (fast oxidative) fibers, as shown by positive staining for SC-71 (D). Bars, 50 μm.

Estating to see whether the same mechanism is involved in DMRV, because activation of both the UPS and lysosomal systems have been demonstrated in this myopathy. Such systems, however, are thought to be stimulated as a response to accumulation of various proteins in the myofibers (29). This, however, could not entirely explain the phenomenon of muscle atrophy in the young DMRV mice, in which there are virtually no abnormal intracellular protein accumulations that could trigger these proteolytic systems. Thus it is more likely that it could involve the activation of certain upstream molecular signals that may initiate myofibrillar proteolysis; this notion is worth exploring to get other clues for understanding how muscles atrophy in DMRV.

The relationship of fiber type involvement and pathological changes in DMRV has not been fully clarified, although it has been suggested that type II fibers are predominantly affected (25). The preferential involvement of type II fibers in the gastrocnemius muscles of DMRV mice, in terms of RV formation and intracellular protein deposition, is not clear at the moment. The predominant involvement of type II muscle fibers in other murine models of muscular dystrophy and myopathies (24) has been presumed to be due to the increased susceptibility of these muscles to eccentric contraction-induced damage (8, 33). In the DMRV mice, this is intriguing since the mechanism underlying the disease is remarkably different from these other murine models. However, in a transgenic mouse overexpressing β-amyloid precursor protein (β-APP) intracellular amyloid deposition has been noted predominantly in type II fibers (28). This could suggest that the involvement of fast-type fibers may be secondary to poor endocytic trafficking and vesicular fusion, characteristics that have been attributed to fast-type fibers. Moreover, in a mouse overexpressing β-APP in type II fibers, an increase in resting calcium and relative membrane depolarization in muscle fibers have been observed and are thought to represent a mechanism relating β-APP
mismetabolism to altered calcium homeostasis and clinical weakness (19). Because intracellular amyloid depositions are seen in muscles of DMRV mice, this topic may be of interest for future investigations.

By analyzing physiological properties of the muscle in DMRV mice, we have seen that as the mice age the difference in force production becomes more remarkable. At least in the gastrocnemius muscle, the reduction of force can be attributed to muscle atrophy during the earlier ages. From 31 to 40 wk of age, the presence of intracellular deposits may interfere with the function of myofibrils. From 41 to 50 wk, the remarkable reduction in force generation can be attributed to RV formation and muscle degeneration, which subsequently lead to myofibrillar disorganization and interfere with sarcromeric contracture.

It is of particular interest, however, that isometric twitches are particularly affected at 31–40 wk of age, the age at which inclusion bodies start to appear, while tetanic forces are predominantly affected at the age of RV formation. This may imply that other signaling pathways in skeletal muscle contraction can be affected as well. The fact that the P1-to-P0 ratio is increased from 41 wk would additionally suggest that the contribution of RV formation to weakness is greater than the influence of the presence of intracellular inclusions. The same deduction, however, cannot be applied to the DMRV TA muscles, in which both P1 and P0 values follow a gradual reduction in an age-related manner, but where P0 and P0/CSA values are markedly reduced at 41–50 wk. It is tempting to speculate that the P1-to-P0 ratio is actually maintained because of the absence of structural pathological changes in these muscles, but this would need further studies.

The analysis of the physiological properties of muscles in DMRV mice allowed us to demonstrate that atrophy is indeed evident in the muscles of these DMRV mice, and this seems to play a major role in the reduced generation of muscle force, especially in the early ages before the appearance of RVs and/or inclusion bodies. Furthermore, our data suggest that RV formation is most likely a downstream event in the pathogenesis of DMRV. But because the most constant finding that we have seen in all age groups is hyposialylation (16) long before the development of any muscle phenotype, further studies to elucidate how decreased sialylation triggers the pathological pathways leading to atrophy may give further clues on disease pathomechanism.

GRANTS

This study was supported partly by the “Research on Psychiatric and Neurological Diseases and Mental Health” from the Japanese Health Sciences Foundation, partly by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), partly by the “Research Grant (17A-10, 19A-7) for Nervous and Mental Disorders” from the Ministry of Health Labour and Welfare, partly by the Kato Memorial Trust for Nambyo Research, and partly by the Neuromuscular Disease Foundation.

ACKNOWLEDGMENTS

The authors acknowledge the valuable assistance offered by Ryuta Hoshi, Dr. Kazunari Momma, and Yu Matsuda in the motor performance evaluation of mice.

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