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[2]*Neuromuscular Centre Nijmegen, Institute of Neurology, Radboud University Nijmegen Medical Centre, Nijmegen; and* [3]*Institute of Neurology and Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*

Blijham, P. J., H. J. ter Laak, H. J. Schelhaas, B. G. M. van Engelen, D. F. Stegeman, and M. J. Zwarts. Relation between muscle fiber conduction velocity and fiber size in neuromuscular disorders. *J Appl Physiol* 100: 1837–1841, 2006. First published January 19, 2006; doi:10.1152/japplphysiol.01009.2005.—To determine the relation between muscle fiber conduction velocity (MFCV) and muscle fiber diameter (MFD) in pathological conditions, we correlated invasively measured MFCV values with MFD data obtained from muscle needle biopsies in 96 patients with various neuromuscular disorders. MFCV was significantly correlated with MFD and independent of the underlying disorder. Pathological diameter changes were fiber-type dependent, with corresponding MFCVs. A linear equation expresses the relation well: MFCV (m/s) = 0.043·MFD (μm) + 0.83. We conclude that fiber diameter determines MFCV largely independent of the underlying neuromuscular disorders studied.

electromyogram; muscle biopsy; muscle fiber potentials; propagation velocity

THE PROPAGATION VELOCITY OF action potentials along nerve and muscle fibers increases with increasing fiber diameter. In frog muscle, a linear relationship between muscle fiber diameter (MFD) and muscle fiber conduction velocity (MFCV) was found by Håkansson (5). For healthy human muscle fibers, the relationship between MFCV and MFD was estimated from (separate) literature data on fiber diameters and velocities as MFCV (m/s) = 0.05·MFD (μm) + 0.95 (8). In various neuromuscular disorders, MFCV is abnormal (1, 3, 14), which is generally ascribed to an increased MFD variability (10), although sarcolemmal lesions and fiber splitting may also influence MFCV (4). To date, no studies on the intrasubject relation between MFCV and MFD in either individual patients or healthy subjects are available.

The aim of the present study was to determine how MFCV and MFD are related in subjects with various neuromuscular diseases, possibly dependent on the pathophysiological characteristics of the disease. A dependence on (a specific) disease is a starting point for further investigating underlying causes, whereas invariance would yield a method for predicting fiber size from MFCV measurements in neuromuscular patients and in muscle waste, e.g., in the elderly.

METHODS

Subjects. Patients with a clinical indication for a muscle biopsy were asked to participate in this study. All subjects were seen by a neurologist with neuromuscular expertise within the Neuromuscular Centre Nijmegen before inclusion. After providing their informed consent, they underwent a routine diagnostic workup, including laboratory investigation, routine electromyogram (EMG), and muscle biopsy. The final diagnosis, made by the neurologist, was based on the clinical findings and the results of the diagnostic workup. Three disease subgroups were defined, namely various noninflammatory myopathies, inflammatory myopathies, and neurogenic diseases. The study was approved by the University Hospital's ethic committee and was in adherence with the principles of the Declaration of Helsinki.

The generally preferred site for MFCV measurements was the brachial biceps muscle, and for the biopsy it was the quadriceps muscle. For the purpose of the study, the MFCV measurements and the biopsy study were performed in the same affected muscle (brachial biceps or quadriceps muscle) when clinical symptoms were significantly different between the proximal leg and the arm muscles.

Measurement of MFCV. MFCV was measured with a Synergy EMG system (Oxford Instruments, Surrey, UK), using a modified invasive technique (1, 13). In short, a bundle of muscle fibers was stimulated directly (stimulus strength, 2–10 mA; duration, 0.05 ms; rate, 1 Hz), using a monopolar EMG needle electrode as cathode, inserted at some distance from the end-plate zone along the fiber direction, and a surface electrode as anode. A complex of propagating single muscle fiber action potentials from a small bundle of stimulated fibers was picked up with a concentric needle electrode (filter settings 500–10,000 Hz), inserted 45–55 mm proximal to the stimulating needle electrode. Identical muscle fiber responses to five consecutive stimuli were required to ensure reproducibility and to identify any interfering responses caused by voluntary contraction (Fig. 1A). Latencies were measured to the positive peaks of a spike exceeding at least 20 μV in amplitude. Each latency value was transformed into a velocity, using the distance, measured at the skin, between the points of insertion of the stimulating and the recording electrode as estimate of the propagated distance. MFCV was measured in at least five different sites in the muscle, and the total number of muscle fiber action potentials to be recorded in a patient was at least 25.

Muscle biopsy. After the MFCV measurements, three muscle specimens (cylindrical in shape with a length of 1 cm and a radius of 0.5 cm maximally) were taken by a Bergstrom needle (length 15 cm) biopsy from the superficial part of the biceps brachii or quadriceps muscle (depth from skin to puncture place 3 cm). If the same muscle was used for MFCV recording and biopsy, care was taken to insert the biopsy needle at a site between the stimulation site and recording site of the MFCV study. Specimens mostly contained ~200 muscle fibers. One specimen per patient was taken for muscle fiber histochemistry and enzyme histochemistry. These specimens were quickly frozen in isopentane cooled by liquid nitrogen. Transverse sections (10 μm thick) were cut at minus 20°C in a cryostat and were stained for fiber

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Fig. 1. *A*: muscle fiber conduction velocity study of a inflammatory myopathy patient. Five traces are superimposed, showing an identical response of several individual muscle fiber potentials to 5 subsequent stimuli. The fastest conduction velocity is 4.1 m/s (L1) and slowest is 1.7 m/s (L2), illustrating a wide range of velocities (distance of propagation: 5 cm). *B*: detail of the quadriceps muscle biopsy from the same patient, showing a severe increase of muscle fiber diameter variability (from extremely atrophic to normal). Bar: 50 µm (hematoxylin and phloxine-stained frozen section).
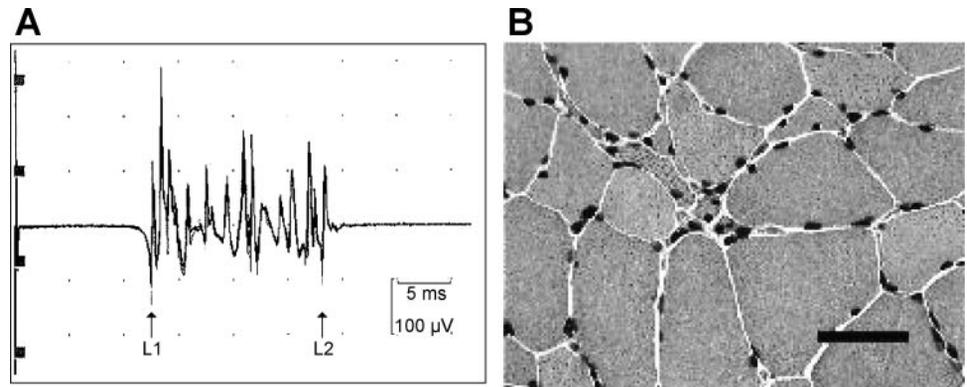
typing (myofibrillar ATPase after preincubation at pH 4.2) so that type I fibers (darkly stained) and type II fibers (lightly stained) could be discerned.

Both the smallest and largest type I and type II fibers were drawn using a drawing microscope. Each drawn fiber area was transformed into a circle by hand using a template with several circular diameters. The matching diameter served as the measure for fiber size (12). Thus four muscle fibers per patient were measured, providing the smallest and largest diameter for type I and type II fibers.

Analysis. For each patient, the slowest and fastest MFCV of the population of fibers measured and the smallest and largest MFD were used for the present analysis. Correlation between MFD and MFCV values and the significance of the correlations were calculated by linear regression analysis. The validity of a linear relation was confirmed by counting runs, testing a significant deviation of the regression line from linearity (using GraphPad Instat version 3.05).

Such linear regression analysis was performed for the whole data set and for each subgroup separately, for data sets obtained from the same muscle, and for data sets taken from a different muscle. As a next step, the analysis was performed using the diameters of type I fibers and of type II fibers separately. The significance of any difference between the subgroups, between the measurements of the same muscle, and between different muscles was determined by multiple regression analysis. A linear contrast of a regression model with the diameters of fiber type I and type II was constructed to determine fiber-type influence on the measurements. A *P* value < 0.05 was considered significant.

RESULTS

Ninety-six patients older than 18 yr were included. Of these subjects, 35 had a myopathy (4 patients had limb girdle syndrome, 5 dystrophic myopathy, 2 steroid myopathy, 3 nemaline rod myopathy, 5 mitochondrial myopathy, 3 acid maltase deficiency, 1 phosphorylase deficiency, 1 calcium-ATPase deficiency, 11 aspecific myopathy), 21 had an inflammatory myopathy (13 patients had polymyositis, 5 dermatomyositis, 3 inclusion body myositis), 1 had a neuromuscular junction disorder, and 15 had a neurogenic disorder (10 patients had motoneuron disease, 1 chronic inflammatory demyelinating polyneuropathy, 1 spastic paraparesis, 3 unclassified neuropathy). On the basis of the routine diagnostic workup, one-fourth of the subjects (24) turned out not to have a neuromuscular disorder. The male-to-female ratio was 1:2 in the myopathies group and 1:1 in the other groups. Mean age was 49 ± 16 yr in the myopathies group, 56 ± 4 yr in the inflammatory myopathies group, and 56 ± 16 yr in the neurogenic group.

MFCV studies and biopsies were performed in the same muscle in 53 subjects (24 in the brachial biceps and 29 in the quadriceps muscle). In 43 subjects, MFCV was measured in the brachial biceps, and the biopsy was taken in a different muscle (39 in the quadriceps muscle, 1 in the deltoid, 1 in the gastrocnemius, and 2 in the anterior tibial muscle). An example of the MFCV measurements and a biopsy sample of the same subject is given in Fig. 1.

The correlations between the smallest MFD and the slowest MFCV, and between the largest MFD and fastest MFCV, are given in Table 1. The correlations in the disease groups were all significant, except for that for the largest MFD and the fastest MFCV in the neurogenic group. The correlation between the smallest MFD and the slowest MFCV was significantly higher ($P = 0.05$) in the neurogenic subgroup than in the myopathies subgroup. The scatterplots for the smallest MFD and the slowest MFCV are presented in Fig. 2 for all subjects, including the cases without a neuromuscular disease (*A*), and separately for the myopathies group (*B*), the inflammatory myopathies group (*C*), and the neurogenic group (*D*). Equivalent scatterplots for the largest MFD and the fastest MFCV are presented in Fig. 3. The data of the largest MFD and the fastest MFCV in the myopathies group (Fig. 3*B*) contained one outlier. The correlation between the largest MFD and the fastest MFCV was significant in the whole group ($P = 0.001$), but not in the myopathies group, when this outlier was removed from the analysis. None of the regressions deviated

Table 1. *Correlation coefficients for the association between the smallest muscle fiber diameter and the slowest muscle fiber conduction velocity and between the largest muscle fiber diameter and the fastest muscle fiber conduction velocity*

	No. of Studies	R_{low}	R_{high}
All subjects	96	0.58†	0.46†
Measurements made in the same muscle	53	0.58†	0.58†
Measurements made in different muscles	43	0.60†	0.26
Myopathies	36	0.40*	0.46*
Neurogenic disorders	15	0.82†	0.27
Inflammatory myopathies	21	0.72†	0.47†

R_{low} and R_{high}, correlation coefficients for the association between the smallest muscle fiber diameter and the slowest muscle fiber conduction velocity and between the largest muscle fiber diameter and the fastest muscle fiber conduction velocity, respectively. *$P < 0.05$. †$P < 0.0005$.
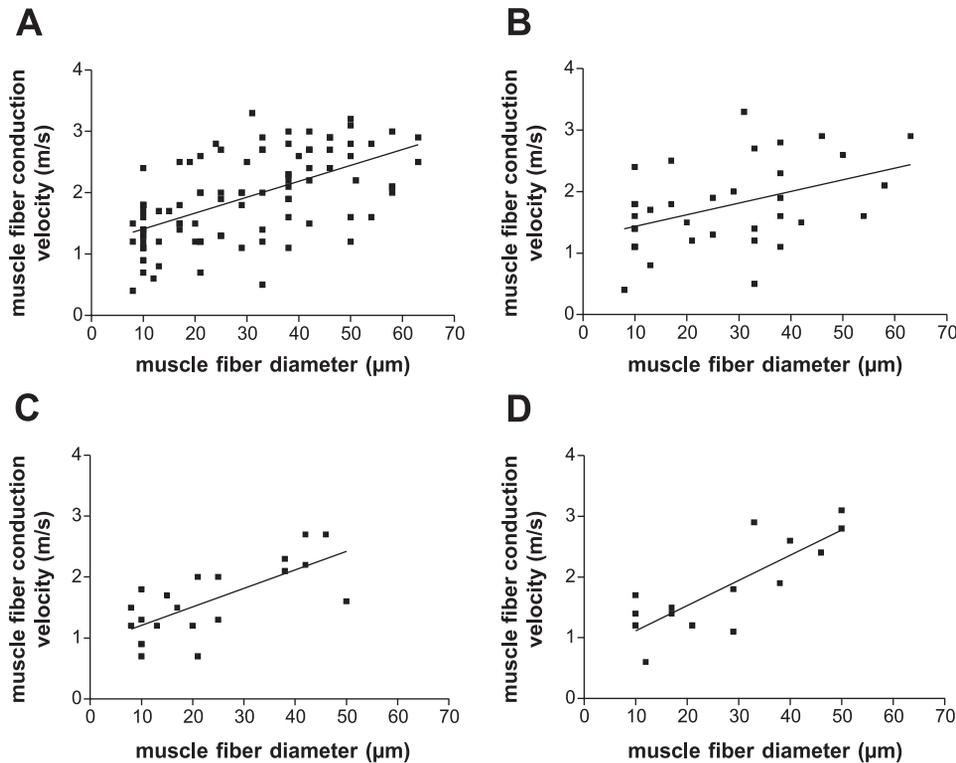
Fig. 2. Scatterplots and regression lines of the smallest muscle fiber diameter plotted against the slowest conduction velocity for all subjects (*A*; $R = 0.58$) and for the groups of patients with myopathies (*B*; $R = 0.40$), patients with inflammatory myopathies (*C*; $R = 0.72$), and patients with neurogenic disorders (*D*; $R = 0.82$), showing the correlation between diameter and conduction velocity.

significantly from linearity. The correlation between smallest MFD and slowest MFCV of measurements made in the same muscle did not differ from measurements made in different muscles (Fig. 4, *A* and *B*). The correlation between largest MFD and fastest MFCV was significant if the measurements were made in the same muscle, but not if they were made in different muscles (Fig. 4, *C* and *D*).

In the whole group, mean smallest MFD of type II fibers was significantly lower than that of type I fibers ($P < 0.001$). Mean largest MFD of type I fibers was significantly higher than that of type II fibers ($P < 0.005$). The regression model showed that the slowest MFCV was mainly dependent on smallest type II fibers, whereas the fastest MFCV was mainly dependent on largest type I fibers.

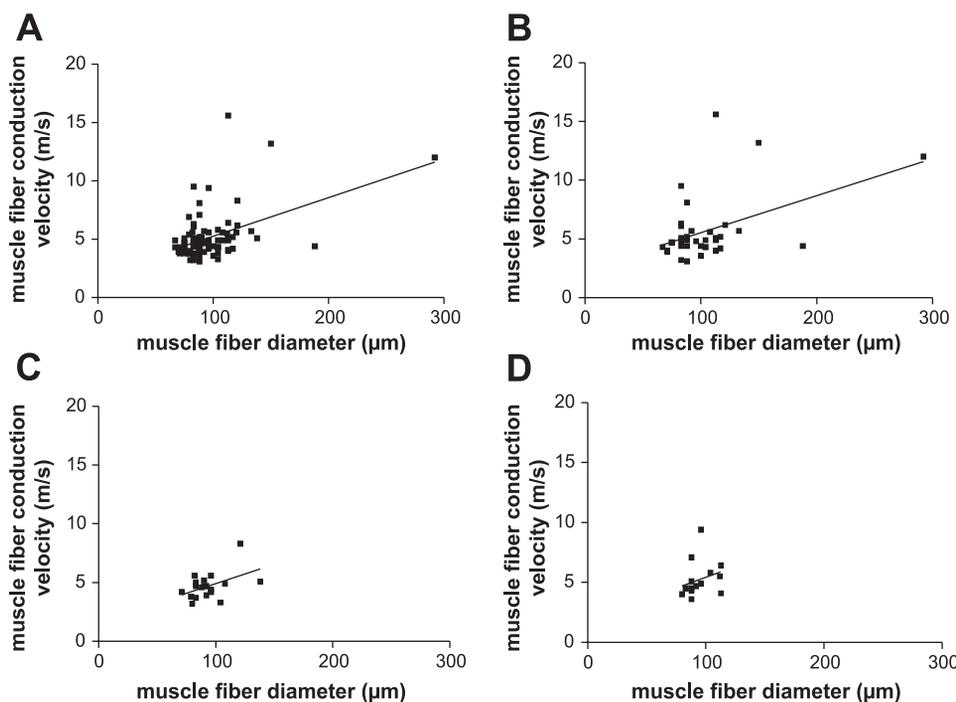Fig. 3. Scatterplot and regression line of the largest muscle fiber diameter plotted against the fastest conduction velocity for all subjects (*A*; $R = 0.46$) and for the groups of patients with myopathies (*B*; $R = 0.46$), patients with inflammatory myopathies (*C*; $R = 0.47$), and patients with neurogenic disorders (*D*; $R = 0.27$), showing the correlation between diameter and conduction velocity.

Fig. 4. Scatterplots and regression lines of the smallest muscle fiber diameter plotted against the slowest conduction velocity for samples taken in the same muscle (*A*; $R = 0.58$) and in different muscles (*B*; $R = 0.60$) and of the largest muscle fiber diameter plotted against the fastest conduction velocity for samples taken in the same muscle (*C*; $R = 0.58$) and in different muscles (*D*; $R = 0.26$).
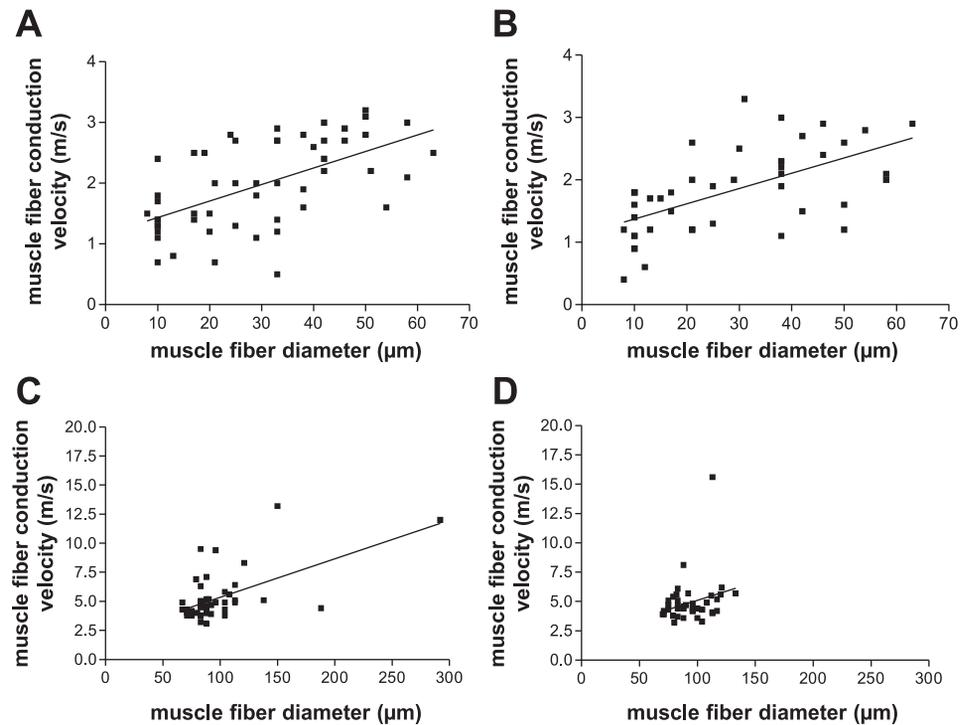
Figure 5 shows the combined data for the smallest MFD and slowest MFCV and for the largest MFD and fastest MFCV. For the whole data set, the best fitting linear equation was MFCV (m/s) $= 0.043 \cdot$ MFD (μm) $+ 0.83$. In the subgroups, this equation was MFCV $= 0.043 \cdot$ MFD $+ 0.90$ in the myopathies group, MFCV $= 0.042 \cdot$ MFD $+ 0.70$ in the inflammatory myopathies group, and MFCV $= 0.049 \cdot$ MFD $+ 0.53$ in the neurogenic group.

DISCUSSION

Our results show the validity of assuming a linear, albeit not a proportional, relation between propagation velocity and fiber
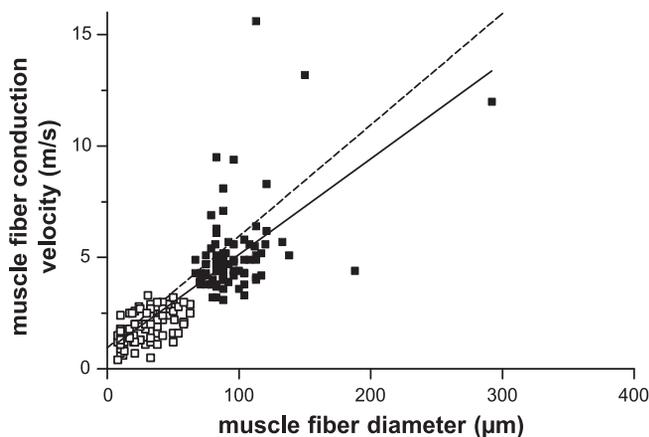

Fig. 5. Scatterplot of the associations between the smallest fiber diameter and the slowest conduction velocity ($\square$) and between the largest fiber diameter and the fastest conduction velocity ($\blacksquare$) for all patients. The solid regression line is represented by the equation of muscle fiber conduction velocity $0.043 \cdot$ muscle fiber diameter $+ 0.83$, and it was derived from our data. The dotted line represents the relation suggested for normal muscle fibers [Nandedkar and Stålberg (8); muscle fiber conduction velocity $= 0.05 \cdot$ muscle fiber diameter $+ 0.95$].

diameter in vivo in neuromuscular patients. The relation found for the whole population closely resembles the relation estimated for normal healthy muscle (7). We found only small differences between the different types of underlying neuromuscular disorder. The correlations remained significant if measurements made in two different muscles were included, suggesting a generalized disorder. The significant correlations suggest that a minimum of 25 muscle fiber potentials as a sample size is sufficient.

Relation between velocity and diameter. The "overall" relation between MFCV and MFD (MFCV $= 0.043 \cdot$ MFD $+ 0.83$) in patients with a neuromuscular disease is remarkably similar to that estimated for normal individuals (MFCV $= 0.05 \cdot$ MFD $+ 0.95$) (8). This is the case, despite the fact that the latter equation was based on MFCVs recorded from healthy subjects in combination with independently obtained fiber diameters taken from the literature, whereas our results are based on an intrasubject observation of MFCV and MFD. Despite the differences between the subgroups, discussed later, overall a relative independence on pathological factors has been found. This confirms the observations in membrane simulation studies that, both in nerve and muscle fibers, conduction velocity depends mostly on fiber diameter, more than on all the other factors implemented, even in myelinated nerve fibers (7, 15). As suggested in the introduction, the relative independence of secondary factors, next to diameter, indeed opens the use of MFCV as a predictor of fiber size, not only in patients but also in other applications where a noninvasive determination of muscle fiber diameter can be important (e.g., in the elderly or in space physiology) (11).

Myopathies vs. neurogenic disease. The correlation between the smallest MFD and the slowest MFCV was significant in the myopathies group, although significantly lower than that in the neurogenic group. The regression line was less steep, and the *y*-intercept was higher (Fig. 2, *B* and *D*). The regression

equation for the neurogenic group came closer to that for normal muscles than the equation for the myopathic group did. This indicates that, for the smallest fibers, factors other than fiber diameter have more influence on MFCV in myopathies than they have in neurogenic disease or health.

One of these factors may be a lesion of the sarcolemma, which has previously been suggested to disturb propagation (2, 4). Fiber splitting may be another contributing factor. It is conceivable that in some myopathies, especially those characterized by severe intracellular damage or extensive sarcolemmal degradation, the propagation of action potentials fails in an early stage, before fiber atrophy.

In contrast to the correlation of the smallest MFD and the slowest MFCV, the correlation between the largest MFD and the fastest MFCV was significant in both myopathies group but not in the neurogenic group. It seems that the smaller range of maximal velocities and of thickest fibers in the neurogenic group than in the other groups explains this finding. Or, from a different perspective, the higher correlation between the largest MFD and fastest MFCV in both myopathies group can be explained by the presence of outliers (Fig. 3, *B* and *C*), which were only found in the myopathies group. Although they may be outliers statistically, they are not measurement artifacts. It is remarkable that, in a specific patient, the maximal diameter was unmistakably 292 μm, and the highest MFCV was indeed 12 m/s. High MFCVs, up to 15.8 m/s, were also measured in two other subjects, also in combination with (not proportionally) hypertrophic fibers. So, high MFCVs do actually occur in the myopathic conditions, as a result of extreme fiber hypertrophy. It should be noted that stimulation of a nerve twig rather than the muscle fiber may lead to falsely high MFCVs. This is further discussed in the *Limitations of the study* section.

Type I fibers vs. type II fibers. The type II fibers showed significantly more atrophy than the type I fibers, as has been observed previously (2, 6). Fiber hypertrophy was significantly more pronounced in type I fibers, which was expected because these fibers are usually larger. This dependency of fiber type and atrophy or hypertrophy resulted in a fiber-type dependency of MFCV in the multivariance analysis. This implies that we have measured the MFCV of either the slowest or fastest fiber and that the measurement was independent of fiber type.

Influence of the site of measurements. Interestingly, the correlation between the smallest MFD and the slowest MFCV remained significant, even if the two measurements were made in different muscles. The spectrum of muscle fiber sizes in controls is quite different between the biceps (30–70 μm) and quadriceps (50–95 μm) muscles (9). Fiber atrophy, however, brings down the diameters to a small range in both muscles, thus nullifying the initial difference. The correlation coefficients between the largest MFD and the fastest MFCV were influenced by several outliers in the myopathies group, as indicated before. If these outliers were removed, both correlations were not significant. In contrast to atrophy, fiber hypertrophy may further amplify the difference between biceps and quadriceps muscle fiber diameters in the upper range.

Limitations of the study. Although we established the apparent relationship between the diameter and conduction velocity of single muscle fibers in vivo, this relationship cannot be verified on individual muscle fibers because it is technically virtually impossible to measure propagation velocity and diameter from one and the same fiber in vivo. Both MFD and MFCV measurements were affected by sampling errors because only a small proportion of all fibers were measured; unfortunately, it is not possible to determine the magnitude of this sampling error.

Another, more minor, drawback of the technique for measuring MFCV is uncertainty about the distance between the stimulation and recording electrodes, which may especially be a problem if fibers run skew with respect to the needle insertion sites. As mentioned before, another pitfall of the method is the possibility to stimulate nerve twigs instead of muscle fibers directly. This gives rise to a potential with a different morphology and preceding the direct fiber responses. It will also invariably cause a gross muscle twitch, which can be used to make the distinction with direct muscle fiber responses (13).

In conclusion, we have demonstrated that the relation between MFCV and MFD appears to be linear and that it closely resembles a relation found in normal muscle. Only small differences between myopathic and neurogenic disorders are found with respect to this relation.

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